



**Istituto Zooprofilattico Sperimentale
della Lombardia e dell'Emilia – Romagna “Bruno Ubertini”
Centro di Referenza Nazionale per la Leptospirosi**

BOLLETTINO BIBLIOGRAFICO

Edizione n. 2026/09

Settembre 2026

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia – Romagna “Bruno Ubertini”

Reparto Tecnologie Biologiche Applicate – Laboratorio Batteriologia Specializzata

Email: mario.dincau@izsler.it, crn.leptospirosi@izsler.it

Telefoni: 030 2290268, 030 2290323

Le citazioni bibliografiche presentate in questo bollettino sono state ottenute mediante la banca dati Scopus utilizzando come parole chiave "Leptospira" e "leptospirosis" ricercate nei titoli, negli abstracts e nelle parole chiave delle pubblicazioni.

Martin P.L., Koval A., Butti M.J., Warley G., Sánchez M.N., Petrakovsky J.V., Gamboa M.I.

Isolation and characterization of a virulent *Leptospira interrogans* from a dog without clinical signs in Buenos Aires, Argentina

(2026) Revista Veterinaria, 37, pp. 1 - 6

DOI: 10.30972/vet.3719486

ABSTRACT: Leptospirosis is a zoonotic disease of global importance where dogs may contribute to environmental contamination with pathogenic *Leptospira*. This study aimed to isolate and characterize a virulent *Leptospira interrogans* strain obtained from the urine of an asymptomatic dog in Argentina. Blood and urine samples were collected for hematological, biochemical, serological, and bacteriological analyses. Serology was performed using the microscopic agglutination test (MAT), and urine samples were cultured for bacterial isolation. The isolate was characterized by PCR amplification and sequencing of the *rrs* gene, followed by phylogenetic and serological analysis. Virulence was assessed using a susceptible hamster infection model. MAT revealed a low antibody titer (1:100) against *L. interrogans* serovar Canicola. *Leptospira* was successfully isolated from urine. Molecular and phylogenetic analyses confirmed that the isolate belonged to the pathogenic clade (P1) of *L. interrogans*, and serological characterization identified it as serogroup Canicola. Experimental infection in Syrian hamsters confirmed the virulence of the isolate, despite the absence of clinical signs in the dog. These findings highlight the epidemiological role of dogs as asymptomatic carriers and the importance of surveillance strategies within a One Health framework.

LANGUAGE OF ORIGINAL DOCUMENT: English

Pua'at Zuzaki M.F.M., Sundram B.M., Halim A., Mohamed N.A., Pahrol M.A., Zailani M.H., Yusoff J., Mohamad R.J., Ismail R.

Trends and climatic drivers of leptospirosis in Malaysia, 2012–2022: spatiotemporal analysis

(2026) Journal of Public Health and Emergency, 10, art. no. AB121

DOI: 10.21037/jphe-26-ab121

ABSTRACT: Background: Leptospirosis remains a significant zoonotic disease in Malaysia, where climatic and environmental factors influence transmission dynamics. However, comprehensive national analyses of spatiotemporal patterns and their relationship to climate remain limited. The objective of the study is to describe the demographic characteristics, spatiotemporal patterns, and density; identify spatiotemporal clustering and hotspots; and determine the association between climatic conditions and state-level leptospirosis incidence in Malaysia from 2012 to 2022. Methods: A retrospective ecological study was conducted using 51,564 leptospirosis cases reported across Malaysia from 2012 to 2022. Demographics and spatiotemporal patterns were presented accordingly, and density was assessed using kernel density estimates (KDE). Spatiotemporal clustering and hotspots were identified using the nearest neighbor index (NNI), spatial autocorrelation (Global Moran's I), local indicators of spatial association (LISA), and Getis-Ord G_i^* statistics. Associations between climatic factors and annual state-level incidence were determined using a generalized linear mixed model (GLMM). Results: Most cases were in males (68.1%) and Malay Bumiputera (62.6%), with a median age of 30 years. Case numbers peaked in 2014–2016 and 2022, with higher density observed in parts of Peninsular Malaysia and Sarawak. Spatial clustering was seen every year (NNI 0.19–0.28; Moran's I 0.02–0.21). LISA found persistent hotspots in rural areas of Sarawak, while others appeared intermittently. Getis-Ord G_i^* confirmed the persistence and spread of these high-risk areas. Temperature was inversely associated with leptospirosis incidence [incidence rate ratio (IRR) =0.88; 95% confidence interval (CI): 0.78–0.99], while

rainfall, humidity, and flooding were not statistically significant. Conclusions: The local climate and environment strongly shape leptospirosis in Malaysia, with certain areas consistently experiencing higher risk. Focusing prevention efforts in these hotspots, while considering ongoing changes in climate and environment, can improve disease control. These strategies may also be useful for managing leptospirosis and similar diseases in other countries with changing environments.

LANGUAGE OF ORIGINAL DOCUMENT: English

Silva da Motta Daiany E., Scofano A.S., dos Santos Loria de Melo J., Lilenbaum W.

Seroreactivity for leptospirosis in pigs with poor reproductive performance in Espírito Santo state, Brazil

(2026) Ciencia Rural, 56 (7), art. no. e20250272

DOI: 10.1590/0103-8478cr20250272

ABSTRACT: Leptospirosis is a significant cause of reproductive losses in the pig industry worldwide, affecting reproductive efficiency and resulting in substantial economic losses. In Brazil, many studies on pig infections focus on serosurveys of random populations, independent of reproductive efficiency, and many regions, such as the state of Espírito Santo, remain poorly studied. Therefore, this study conducted a leptospirosis serosurvey in sow herds with poor reproductive performance from different regions of Espírito Santo, Brazil. Blood samples for serology (Microscopic Agglutination Test - MAT) were collected from 131 sows with poor reproductive performance from 13 herds. A seroreactivity rate of 29.8% (39/131) sows were revealed, and all the tested herds had at least one seroreactive animal. The most common serogroups were Autumnalis 43.8% (17/39), Icterohaemorrhagiae 30.8% (12/39), and Australis 15.4% (6/39). Thus, the study highlighted the importance of leptospirosis for the pig industry in that region, leading to significant economic impacts due to reproductive problems, and reinforcing the importance of specific prevention and control measures.

LANGUAGE OF ORIGINAL DOCUMENT: English

Visweswara Rao P., Chiranjeevi Reddy P., Lakshmi Aishwarya N., Prasanth P., Ruakesh R., Reshita T.

A division-level leptospirosis early warning system for Fiji using lagged hydro-meteorological signals and explainable xgboost

(2026) GSEACT 2026 - 2026 IEEE Global Symposium on Emerging and Communication Technologies

DOI: 10.1109/GSEACT68539.2026.11620150

ABSTRACT: Leptospirosis is a bacterial zoonosis whose transmission and reporting dynamics are often amplified after rainfall-driven flooding and standing-water persistence. We present a practical Early Warning System (EWS) tailored for a Fiji demonstration setting that produces weekly, location-specific risk forecasts intended for operational use: calibrated probabilities are converted into alert levels and delivered through stakeholder-facing maps. The pipeline aligns surveillance data to a weekly index, merges lagged hydro-meteorological signals (rainfall-derived features and short-window flood persistence), and attaches static exposure/vulnerability proxies. We train an XGBoost classifier under rolling, time-respecting splits to emulate forward-in-time deployment and to avoid overly optimistic evaluation from temporal leakage. For transparency, we generate explainability outputs and export a reproducible run directory containing tables, figures, GeoJSON layers, and interactive HTML maps. Leptospirosis, Early Warning System (EWS), XGBoost, SHAP, climate-driven disease, rolling validation, GIS risk mapping, Fiji.

LANGUAGE OF ORIGINAL DOCUMENT: English

Khutwada S., Deepa P.M., Shyma V.H., Bipin K.C., Janus A., Rathish R.L., Vergis J.

Aptamer-functionalized silver nanoparticle pre-analytical enrichment integrated with loop-mediated isothermal amplification for improved detection of pathogenic *Leptospira interrogans*

(2026) Journal of Microbiological Methods, 249, art. no. 107677

DOI: 10.1016/j.mimet.2026.107677

ABSTRACT: Early diagnosis of leptospirosis remains challenging because the low and transient leptospiraemia during the acute phase often results in bacterial DNA concentrations below the analytical detection limit of conventional molecular assays. This proof-of-concept study evaluated the feasibility of integrating aptamer-functionalised silver nanoparticles (AgNPs) as a pre-analytical enrichment step with loop-mediated isothermal amplification (LAMP) to improve the molecular detection of pathogenic *Leptospira interrogans*. Unlike previously reported aptamer–nanoparticle systems that primarily function as biosensors, the proposed approach employs aptamer-functionalised AgNPs as an upstream enrichment platform prior to nucleic acid amplification. Silver nanoparticles were synthesised by sodium borohydride reduction and characterised using UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and transmission electron microscopy (TEM), while aptamer conjugation was supported by a characteristic surface plasmon resonance shift from 390 to 404 nm. Compared with unfunctionalised AgNPs and unenriched controls, aptamer-functionalised AgNP enrichment resulted in significantly greater DNA recovery (21.6% versus 7.3% at 103 CFU/mL; $p = 0.018$). Enrichment-assisted LAMP achieved an analytical limit of detection of 10 CFU/mL, representing a 100-fold improvement over conventional LAMP (103 CFU/mL), and demonstrated improved amplification performance in plasma samples containing low bacterial concentrations. Preliminary evaluation using 52 clinically suspected canine samples yielded a diagnostic sensitivity of 88.5% and specificity of 100%. Although additional mechanistic studies, comprehensive nanoparticle characterisation and larger multicentre clinical validation are required, these findings demonstrate the feasibility of aptamer-functionalised AgNP enrichment as a promising pre-analytical strategy for improving molecular detection of low-abundance *Leptospira* in resource-limited laboratory settings.

LANGUAGE OF ORIGINAL DOCUMENT: English

Diefenthaler Madeira B., Deluca Antunes A., Seki Manami J., Duarte Tavares M.A., da Silva Gregory V., Borges Giacomet S., Gomes Ferreira R., Rigatto M.H.

Floods in Rio Grande do Sul State, Brazil, in 2024 and their effects on the epidemiology of leptospirosis

(2026) Cadernos de Saude Publica, 42, art. no. e00111125

DOI: 10.1590/0102-311XPT111125

ABSTRACT: Extreme climate events favor the spread of waterborne diseases. This study analyzes the impact of the historic floods occurred in Rio Grande do Sul State, Brazil, in 2024 on leptospirosis incidence. This is a descriptive cross-sectional study based on secondary data from official health information systems, comparing confirmed cases, hospitalizations, and deaths between April and July 2023 and the same period in 2024. Monthly trends, sociodemographic characteristics, and spatial distribution of cases were examined. From April to July, the number of cases increased from 117 in 2023 to 1,007 in 2024, a 7.6-fold rise, with a peak in May 2024 (516 cases). Hospitalizations increased from 89 to 228, with June showing a marked rise (76 in 2024 and 15 in 2023), and deaths rose from seven to 35, with the sharpest increase in May (one in 2023 and 20 in 2024). Despite the absolute increase, mortality rates declined from 6% in 2023 to 3.5% in 2024. In 2024, the most affected groups remained young white men, consistent with the 2023 profile. Over 200 municipalities

reported cases in 2024, particularly Porto Alegre, Alvorada, Canoas, and São Leopoldo (all located in the Porto Alegre Metropolitan Area), which together accounted for nearly half of cases. The findings suggest that floods contributed to intensified transmission, increased hospital burden, and higher leptospirosis-related mortality. These results underscore the need for integrated epidemiological surveillance, resilient urban infrastructure, and preventive strategies to reduce population vulnerability to future climate-related disasters.

LANGUAGE OF ORIGINAL DOCUMENT: Portuguese

França D.A., Mateus-Vargas R.H., Fischer I., Menozzi B.D., Fornazari F., Langoni H.

High occurrence of pathogenic *Leptospira* in vampire bats (*Desmodus rotundus*) in Brazil

(2026) Research in Veterinary Science, 211, art. no. 106363

DOI: 10.1016/j.rvsc.2026.106363

ABSTRACT: This study investigated the occurrence of pathogenic *Leptospira* in urban and rural bats from São Paulo State, Brazil. Kidney samples from 203 bats representing 13 species were screened by real-time PCR targeting the lipL32 gene. Positive samples were further characterized by conventional PCR targeting the lipL32 gene followed by DNA sequencing. Spatial analyses were performed to evaluate possible associations between *Leptospira* detection, cattle density, and land-use characteristics. *Leptospira* DNA was detected in 42.6% (43/101) of rural vampire bats (*Desmodus rotundus*), whereas all 102 urban non-hematophagous bats tested negative. DNA sequencing identified *Leptospira borgpetersenii* and *Leptospira interrogans* in 14 positive samples. No significant associations were observed between infection rates and the evaluated environmental variables. The high frequency of pathogenic *Leptospira* detected in vampire bats highlights their potential ecological association in rural ecosystems and supports further investigations into their role in the ecology and transmission dynamics of leptospirosis.

LANGUAGE OF ORIGINAL DOCUMENT: English

Miswan N., Rosli N., Hussin N.A.M., Beng E.O.B., Ali M.R.M., Ahmed S.A., Ismail N., Othman N., Rahumatullah A.B., Singham G.V., Lau N.-S.

Evaluation of novel primers for rapid single-step detection of pathogenic *Leptospira* spp. using duplex polymerase chain reaction (PCR) assay

(2026) Tropical Life Sciences Research, 37 (2), pp. 53 - 74

DOI: 10.21315/tlsr2026.37.2.3

ABSTRACT: Pathogenic *Leptospira* spp. infections cause leptospirosis, an endemic zoonotic disease characterised by flu-like and febrile symptoms that may progress to severe and potentially fatal complications if antibiotic treatment is delayed. However, the current gold-standard diagnostic method, being used, the microscopic agglutination test (MAT), detects antibodies only approximately one week after symptoms and requires both live cultures and skilled personnel. In this study, two primer sets targeting the rrs and lipL32 genes were designed for the simple, rapid, and multiplex molecular detection of *Leptospira* spp. The duplex PCR assay was evaluated using 15 *Leptospira* strains comprising both pathogenic and non-pathogenic species and demonstrated simultaneous detection and clear differentiation of pathogenic *Leptospira* spp, within a single PCR run. In a duplex PCR setting, the assay showed 100% sensitivity for both urine-spiked *Leptospira interrogans* serovar Copenhageni and its genomic DNA (gDNA), with detection limits of 100 bacteria/mL for urine and 30 fg/uL for gDNA samples. The results were comparable to those obtained using previously adopted primers targeting lfb1 or secYIV genes in a singleplex PCR setting. The duplex PCR assay

showed 100% specificity in detecting *Leptospira* spp. by amplifying the highly conserved *rrs* gene in *Leptospira* spp. and pathogenicity differentiation through the highly conserved lipoprotein encoding *lipL32* gene in pathogenic strains. In contrast, the specificity of the singleplex PCR assay targeting *lfb1* or *secYIV* genes for pathogenic strain differentiation was 89% and 78%, respectively.

LANGUAGE OF ORIGINAL DOCUMENT: English

Azhar A., Ali I., Azhar M., Govinda N., Wang H.

A cog in the Weil: a case report of severe leptospirosis

(2026) Infectious Diseases and Immunity, pp. 1 - 3

DOI: 10.1097/ID9.0000000000000218

ABSTRACT: Abstract – The nonspecific presentation of leptospirosis and delays in serologic confirmation often make its diagnosis challenging. The presence of comorbidities or potential drug toxicities, which can mimic or obscure clinical findings, further adds to this diagnostic difficulty. Here, we present the case of a 46-year-old male with well-controlled HIV infection on antiretroviral therapy, who presented with a four-day history of fever, malaise, and jaundice. The patient is a pilot with recent travel to the United Kingdom and prior travel throughout North America, Central America, and the Caribbean. On admission, he was febrile and tachycardic, with laboratory studies demonstrating elevated creatinine and transaminases. Broad-spectrum antibiotics were initiated and subsequently de-escalated to doxycycline, while antiretroviral therapy was temporarily held. A renal biopsy demonstrated acute tubulointerstitial nephritis. The patient improved clinically with doxycycline, with normalization of liver and kidney function by the time of discharge. Post-discharge serologic testing returned positive for *Leptospira* IgM antibodies, confirming the diagnosis of Weil's disease. This case underscores the diagnostic challenges of leptospirosis, particularly in patients without clear exposure histories, and highlights the importance of early empiric therapy when clinical suspicion is high.

LANGUAGE OF ORIGINAL DOCUMENT: English

Loong S.K., Majid M.A.A., Sha'ahuri N.S.A., Hanapi I.R.M., Hassan M., Ali M.R.M., Ya'cob Z., Teoh B.-T., Zain S.N.M., Kamaruzaman I.N.A., Abubakar S., Sahimin N.

Molecular detection of pathogenic *Leptospira* among refugee and asylum-seeker communities in the Klang Valley, Malaysia

(2026) Southeast Asian Journal of Tropical Medicine and Public Health, 57 (4), pp. 602 - 619

ABSTRACT: Malaysia has been a destination of choice for refugees for the past 45 years, and as of the end of February 2026, there were approximately 215,600 refugees and asylum-seekers in Malaysia. Unlike other countries, refugees residing in Malaysia are not confined to designated camps but are scattered throughout the country. Many of them live in squatter homes and overcrowded flats with poor waste management and sanitation, an ideal environment for rodents to thrive in. Consequently, there is a high risk of transmission of rodent-borne zoonotic infections such as leptospirosis, which is endemic in the Asia-Pacific region, wherever poor socioeconomic and behavioral conditions exist. A wellness program was conducted to determine the prevalence of leptospirosis among the refugee communities in the Klang Valley, Malaysia. A semi-nested PCR amplifying a fragment of the *Leptospira* *lipL32* gene identified 18/312 positive individuals, all from Myanmar. Successful nucleotide sequencing of eight (235 bp) amplicons revealed them as *L. interrogans*, but microscopic agglutination tests of sera from PCR-positive cases were negative against a panel of live reference and local *Leptospira* sp. The substantial prevalence of leptospirosis among refugees warrants improving

hygiene practices and enhancing knowledge on disease transmission through community engagement activities.

LANGUAGE OF ORIGINAL DOCUMENT: English

Fasching E., Kuchling S., Waldner K.

Serological survey of *Leptospira* antibodies in cattle from Lower Austria and Vorarlberg

(2026) Wiener Tierärztliche Monatsschrift, 113, art. no. doc11

ABSTRACT: Introduction Leptospirosis is a globally distributed zoonotic disease caused by pathogenic *Leptospira* species, which can infect a wide range of mammals, including humans. Transmission occurs primarily through contact with urine from infected animals. In cattle, leptospirosis leads to significant economic losses due to reproductive disorders such as abortions, stillbirths, birth of weak calves, and reduced milk yield. For Austria, there are so far hardly any comprehensive data from systematic studies on the seroprevalence in cattle. Environmental factors, including climate change and management practices like pasture grazing, may influence pathogen survival and transmission dynamics. Collecting prevalence data is essential to improve diagnostic strategies, monitor herd health, and reduce zoonotic risk. This study aims to determine the seroprevalence of *Leptospira* antibodies in cattle from Lower Austria and Vorarlberg and to identify potential risk factors associated with infection in these regions. Materials and Methods Serum samples from cattle in Lower Austria and Vorarlberg were analysed using the MAT according to the WOAHP guidelines. The analysis panel contained the following *Leptospira* serovars: Australis, Canicola, Grippotyphosa, Copenhageni, Pomona, Hardjo, Saxkoebing and Tarassovi. Farm-level data (herd size, alpine grazing history, age, season, sampling year) were obtained from the Austrian VIS database. Statistical analyses were conducted in R using Bayesian models to estimate true prevalence (accounting for test sensitivity and specificity) and GEE modeling to identify risk factors. Results A total of 2,339 cattle serum samples were analysed. In Lower Austria, an estimated mean true seroprevalence of 4.8 % (95 % CI: 0.3–11.1) was detected. The most frequent serovars were Saxkoebing and Hardjo. In Vorarlberg the estimated mean true seroprevalence was 63.6 % (95 % CI: 52.3–76.1), with Serovars Saxkoebing, Hardjo, and Tarassovi prevailing. GEE modelling identified alpine grazing, herd size and age category as significant risk factors for seropositivity. Conclusion This study reveals marked regional differences in *Leptospira* antibody prevalence in Austrian cattle, with significantly higher rates in Vorarlberg compared to Lower Austria. Key risk factors include alpine grazing, herd size, and age. These findings highlight the need for targeted surveillance and integrated One Health strategies to mitigate zoonotic risks.

LANGUAGE OF ORIGINAL DOCUMENT: German

Agustar H.K., Shamsusah N.A., Noor M.M., Firdaus-Raih M., Amran F., Hod R., Hassan M.R.

Pathological effects of *Leptospira yasudae* strain BJ3 infection in Experimental Golden Syrian Hamster isolated from Perak, Malaysia

(2026) Sains Malaysiana, 55 (8), pp. 1296 - 1309

DOI: 10.17576/jsm-2026-5508-06

ABSTRACT: Leptospirosis is a zoonotic disease caused by helical bacteria of the genus *Leptospira* that affects animals and humans. Recently, a newly identified isolate called *Leptospira yasudae* strain BJ3 has been discovered in soil samples obtained from a wildlife conservation center located in Malaysia. Currently, data on the virulence of *L. yasudae* in susceptible hosts is scarce. Hence, this study was conducted to identify the virulence of *L. yasudae* strain BJ3 based on the histopathological effects due to leptospirosis infection towards

susceptible hosts. The strain was inoculated intraperitoneally in hamsters at different doses (1×10^8 cells, 2×10^8 cells, and 3×10^8 cells). Histopathological effects of hamsters infected with *L. yasudae* strain BJ3 were compared with negative control (uninfected) and positive control (infected with *L. interrogans* serovar Lai strain Langkawi) hamsters. The results demonstrated that infection with 1×10^8 *L. yasudae* strain BJ3 led to noticeable weight loss. Microscopically, pulmonary edema in the lung, sparse focal inflammatory cells infiltration but with normal hepatocytes architecture in the liver and mild tubular degeneration in the kidney were observed. More vital pathological changes occurred at higher dose (3×10^8 cells). The gross examination showed crucial weight and fur loss. Microscopic examination showed the presence of interstitial pneumonia and lung lesions, hepatocyte expansion, hypertrophy and hyperemia with severe lesions in kidney cells. In conclusion, infection caused by *L. yasudae* strain BJ3 may represent a noteworthy emerging local agent of leptospirosis due to its ability to induce virulence and cause histopathological alterations in the kidneys of susceptible hosts. To the best of our knowledge, this is the first report demonstrating that a novel *Leptospira* species isolated from soil can infect hamsters and induce histopathological changes in organs.

LANGUAGE OF ORIGINAL DOCUMENT: English

Ivakhiv O.L., Sverstiuk A.S., Vyshnevskaya N.Yu., Vyshnevskaya Y.A.

Diagnosis, risk factors, and multifactorial model for predicting severe leptospirosis

(2026) Odesa Medical Journal, 2026 (1), pp. 20 - 26

DOI: 10.32782/2226-2008-2026-1-3

ABSTRACT: Introduction. Leptospirosis is a common zoonotic infection with high mortality. Despite increasing incidence, diagnosis and severity assessment remain inadequate due to varied clinical signs and limitations of current diagnostic methods. The aim of this study was to stratify the risk coefficient for an unfavorable course of leptospirosis based on a developed mathematical prediction model. Materials and methods. Clinical and laboratory data from 60 leptospirosis patients were analyzed using multifactorial regression to identify independent predictors of severe disease. Results. Significant predictors of severe leptospirosis included: seasonality, initial diagnosis, vomiting, calf pain, serovar type, age, delayed antibiotic therapy, hepatosplenomegaly, neutrophil count, ESR, and urinary changes (diuresis, erythrocytes, protein). The regression model showed high prognostic value for early detection of patients at risk of severe disease. Conclusions. The proposed algorithm and model enable reliable early assessment of severe leptospirosis risk, support timely treatment, and help prevent complications and reduce mortality.

LANGUAGE OF ORIGINAL DOCUMENT: English

San Martin F., Brady M.R., Fule L., Nouchikian L., Rodriguez A., Duchateau M., Mondino S., Larrieux N., Wunder E.A., Jr, Ko A.I., Rey M., Chamot-Rooke J., Duran R., Trajtenberg F., Picardeau M., Sindelar C.V., Buschiazzi A.

Core-sheath coupling controls flagellar curvature and motility in *Leptospira*

(2026) Nature Communications , 17 (1), art. no. 9075

DOI: 10.1038/s41467-026-75995-6

ABSTRACT: Spirochaete pathogens are among the most invasive bacteria known, causing syphilis, Lyme disease, and leptospirosis. Their tissue penetration depends on periplasmic flagellar filaments that, unlike other bacterial flagella, are encased in a spirochaete-specific multi-protein sheath and deform the cell body into motile waves. How these filaments achieve the mechanical properties needed for invasive motility has

remained unclear. Here we determine complete atomic structures of the *Leptospira* endoflagellar filament, revealing an elaborate sheath of 9 to 12 distinct asymmetrically arranged proteins. We show that the flagellin variant forming the filament core determines sheath composition, producing curvatures ranging from $\sim 3.5 \mu\text{m}^{-1}$ to $\sim 5.6 \mu\text{m}^{-1}$. The lower-curvature architecture, employed by pathogenic *Leptospira interrogans*, proves essential for motility in viscous environments and during infection. Thus, *Leptospira* achieves environment-specific motility through modular core–sheath coupling, linking atomic-scale structural plasticity to large-scale changes in swimming behaviour. Conservation of key sheath components suggests this mechanism may extend across spirochaetes.

LANGUAGE OF ORIGINAL DOCUMENT: English

Bothammal P., Michelraj S., Verma A., Venkatachalam G., Natarajaseenivasan K.

Polyaniline-gold nanoparticle-based biosensor targeting miR-21-5p for early diagnosis of leptospirosis

(2026) Analytical and Bioanalytical Chemistry

DOI: 10.1007/s00216-026-06754-1

ABSTRACT: Blood-based disease diagnosis facilitates the early-stage detection of leptospirosis because leptospires were present in the blood in the first 5 to 10 days after exposure. We present a sensor employing polyaniline (PANI) and gold nanoparticles (AuNPs) for the specific detection of miR-21-5p in individuals with leptospirosis. The electropolymerization of PANI on glassy carbon electrodes (GCEs) creates a porous and durable coating that remains stable over extended periods without mechanical damage. To enhance the biosensor's performance, we functionalized the sensor surface with gold nanoparticles through electrodeposition, which facilitated an electroactive surface and selective binding of the thiolated probe. Our data confirmed that the thiolated DNA probe (anti-miRNA-21) exhibits a higher affinity for the target miRNA-21 and serum samples from individuals with leptospirosis compared to non-complementary sequences and healthy serum samples. The biosensor demonstrated remarkable sensitivity with a detection range spanning from 1 aM to 50 nM, boasting a limit of detection (LOD) of 1 aM and a limit of quantification (LOQ) of 4.59 aM. This represents an unprecedented achievement in ultra-low-level miRNA-based biosensors for diagnosing leptospirosis. Early-stage disease detection is crucial for effective disease management and timely treatment. Such an approach holds promise for point-of-care testing in remote settings to facilitate the early diagnosis of leptospirosis.

LANGUAGE OF ORIGINAL DOCUMENT: English

Costarelli S., D'Incau M., Cruciani D., Panzieri C., Fiorucci A., Scorcelletti S., Nunzi A., Brancaleoni M.G., Latini L., Catalano A.

A leptospirosis outbreak in a riding stable in Central Italy

(2026) Veterinary Sciences, 13 (8), art. no. 750

DOI: 10.3390/vetsci13080750

ABSTRACT: Leptospirosis is a widespread zoonotic disease of major concern for both veterinary and public health. In horses, it is associated with equine recurrent uveitis, a painful condition that may lead to blindness and affects approximately 10% of the global horse population. In May 2025, following the occurrence of three uveitis cases in a riding stable in Central Italy, 94 equine blood samples were collected and tested using the microscopic agglutination test (MAT) for *Leptospira* spp. Antibody titres against serogroup Icterohaemorrhagiae were detected in 68 horses (72.4%). An on-site investigation was conducted, and control

measures were implemented, including movement restrictions, clinical monitoring of horses, and antibiotic treatment. Follow-up serological testing showed no significant variation in antibody titres, no additional clinical cases were observed in animals, and no symptoms were reported among individuals exposed to the facility. These findings suggest widespread exposure to *Leptospira* spp. without evidence of ongoing transmission or public health impact. This study highlights the diagnostic and management challenges of equine leptospirosis, together with the importance of surveillance within a One Health framework.

LANGUAGE OF ORIGINAL DOCUMENT: English

Peiró-Mestres A., Franco S., Pastor V., Rodríguez C., Barahona L., Escudero-Pérez B., Petit R., Martínez M.J., Montalvo T.

***Leptospira interrogans* in Norway rats from Barcelona, Spain**

(2026) Heliyon, 12 (14), art. no. e45318

DOI: 10.1016/j.heliyon.2026.e45318

ABSTRACT: Background Leptospirosis is an under-diagnosed zoonotic emerging disease caused by pathogenic *Leptospira* and is primarily maintained in the environment by rodents, which serve as its main reservoir in urban settings. Information of *Leptospira* prevalence in urban rats in Spain is scarce. We aimed to investigate the presence and prevalence of pathogenic *Leptospira* in rats of the sewer system and parks of Barcelona at two different time-points. Methods *Rattus norvegicus* were captured over two different sampling periods in ten different Barcelona districts. Kidney and bladder samples were tested to detect pathogenic *Leptospira* by real-time PCR and sequenced to determine *Leptospira* species. Results A total of 82 rats were sampled in 2017 whereas 136 rats were sampled in 2021-2022. In the first sampling period we found 12% of rats were positive for *L. interrogans* whereas in the second sampling period *L. interrogans* was found in 4% of the tested rats. *L. interrogans* infected rats were present in seven Barcelona districts. In our study, *L. interrogans* was detected in both kidney and bladder tissues. Conclusions Our results demonstrate the presence of *L. interrogans* in urban rats, supporting the need to promote One Health surveillance programs to detect pathogenic *Leptospira* in Barcelona.

LANGUAGE OF ORIGINAL DOCUMENT: English

Castillo-Sánchez L.O., de la Peña-Moctezuma A., Bautista-Trujillo G.U., Tapia-Mendoza E., Romo-Pérez A., Martínez-González S., Avila-Ramos F., Carmona-Gasca C.A.

Identification of quorum sensing molecules of N-acyl-homoserine lactone in *Leptospira* strains supernatants

(2026) Microorganisms, 14 (8), art. no. 1806

DOI: 10.3390/microorganisms14081806

ABSTRACT: The bacterial Quorum Sensing system refers to the recognition of signaling molecules called autoinducers produced by bacteria when a certain cell density is reached in the environment. Those cell-density-dependent autoinducers regulate and coordinate diverse functional processes, such as bioluminescence, biofilm production, sporulation, and even the expression of some virulence factors, among others. There is a wide variety of autoinducers, and for Gram-negative bacteria, the canonical autoinducers are the N-acyl-homoserine lactones (AI-1). Presently, the production of autoinducers in *Leptospira* has not been described; therefore, the objective of this study was to detect and identify autoinducers in this bacterial genus. We report here the expression of AI-1 in cultures $\geq 2.4 \times 10^8$ of *Leptospira meyeri*. Ethyl acetate extracts

of *Leptospira* culture supernatants were capable of activating the β -galactosidase system in the biosensor *Agrobacterium tumefaciens* strain NTL4. Partial identification of the leptospiral supernatant extracts was done by thin-layer chromatography (TLC), showing a similar retention factor to the synthetic standard N-Octanoyl-DL-homoserine lactone (C8-AHL) in the *Leptospira* supernatant extracts. In addition, infrared spectroscopy (IR) analysis showed peaks corresponding to the lactone and amide groups in both the C8-AHL standard and the *Leptospira meyeri* culture extracts. Moreover, High-Performance Liquid Chromatography–Mass Spectrometry (HPLC-MS/MS) confirmed the same retention time (10.7 ± 0.1 min) in both the *Leptospira meyeri* supernatant extracts and the C8-AHL standard. These results show that *Leptospira meyeri* synthesizes N-acyl homoserine lactone family autoinducers, particularly the N-Octanoyl-DL-homoserine lactone, and lay the groundwork for future research on Quorum Sensing systems in *Leptospira*.

LANGUAGE OF ORIGINAL DOCUMENT: English

Sivapornnukul P., Limothai U., Tachaboon S., Dinhuzen J., Jitvaropas R., Thanasarnthungcharoen C., Chitcharoen S., Sawaswong V., Chanchaem P., Payungporn S., Srisawat N.

Microbial profiles in non-leptospirosis acute undifferentiated febrile illness: associations with clinical parameters and implications for diagnosis

(2026) Journal of Microbiology, Immunology and Infection

DOI: 10.1016/j.jmii.2026.08.001

ABSTRACT: Background: Acute undifferentiated febrile illness (AUF) remains a challenge in medical diagnosis. This study aimed to investigate the microbial profiles of non-leptospirosis AUF patients from Sisaket province, an endemic region for leptospirosis, and to elucidate their associations with clinical parameters. Methods: Blood samples from 78 non-leptospirosis AUF patients were confirmed negative for leptospirosis by bacterial culture, lipL32-qPCR, and the microscopic agglutination test. 16S rDNA and ITS sequencing were used to identify bacterial and fungal communities. Patients were categorized into Only Bacteria (OB), Only Fungi (OF), and Both Bacteria and Fungi (BBF) groups. Microbial differences were assessed using Principal Coordinate Analysis (PCoA) and associations with clinical variables analyzed using Generalized Linear Models (GLM). Results: *Stenotrophomonas* emerged as the predominant bacterial genus in both OB and BBF groups, while *Aspergillus* and *Candida* dominated the fungal profiles in OF and BBF groups. Bacterial compositions differed significantly between OB and BBF groups ($P = 0.002$), indicating that concurrent bacterial–fungal presence alters bacterial community structure, whereas fungal profiles remained stable. GLM analysis revealed significant associations between specific microbial taxa and clinical parameters, including blood pressure, temperature, hematological indices, and liver function tests. Conclusion: Our study sheds light on the microbial landscape in non-leptospirosis AUF patients, establishing connections with clinical parameters. This holistic approach enriches our understanding of AUF's etiology, presenting avenues for targeted diagnostic and therapeutic strategies in systemic infections.

LANGUAGE OF ORIGINAL DOCUMENT: English

Nguyen V.D., Dang-Xuan S., Le Dinh Ha T., Steele S., Firestone S., Stevenson M.

Prevalence of and risk factors for leptospirosis exposure in cattle in Ho Chi Minh City, Viet Nam, 2022

(2026) Preventive Veterinary Medicine, 255, art. no. 106979

DOI: 10.1016/j.prevetmed.2026.106979

ABSTRACT: A cross-sectional study was conducted in 2022 to estimate the true prevalence of *Leptospira* spp. exposed cattle herds in Ho Chi Minh City, Viet Nam and to identify characteristics associated with herd level *Leptospira* spp. exposure. Herd managers completed a questionnaire about herd management and whole blood samples were collected from their cattle and tested for *Leptospira* spp. serovars using the microscopic agglutination test (MAT). Multivariable logistic regression analyses were used to identify characteristics associated with seropositive herd status. Sixty of 99 dairy herds and 28 of 61 beef herds had at least one animal with *Leptospira* spp. antibodies detected using the MAT. For the total of 160 herds, the estimated true prevalence was 27 (95% CI 5–47) *Leptospira* spp. exposure positive herds per 100 herds at risk. The odds of being *Leptospira* spp. exposure positive for dairy herds with a median cow age of greater than 3 years was 3.8 (95% CrI 1.7–15) times that of dairy herds with a median cow age of less than or equal to 3 years. Given the high herd-level prevalence of *Leptospira* spp. exposure we conclude that leptospirosis is a risk not only to animal health and productivity, but also to herd managers and those who work with their stock in this area of Viet Nam. Action to minimise water pooling in the farm environment and to implement rodent control and cattle leptospirosis vaccination programs are strongly recommended as key measures to reduce infection pressure.

LANGUAGE OF ORIGINAL DOCUMENT: English

Fernandes G.S.C., Azevedo B.O.P., Damiano D.K., Lima M.V.R., Macena P.P., Teixeira A.F., Barazzone G.C., Nascimento A.L.T.O., Lopes A.P.Y.

The epidemiological scenario of human leptospirosis in Brazil from 2015 to 2024

(2026) Epidemiologia, 7 (4), art. no. 114

DOI: 10.3390/epidemiologia7040114

ABSTRACT: Background: Leptospirosis is a neglected tropical disease with substantial public health impact in Brazil, closely associated with socio-environmental vulnerabilities and climatic extremes. This study analyzed the epidemiological profile and spatiotemporal distribution of leptospirosis incidence and lethality rates in Brazil from 2015 to 2024. Methods: An ecological time-series study was conducted using secondary data from the Notifiable Diseases Information System (SINAN). Variables included geographic region, probable infection environment, and occupational and educational level (ISCED-2011). Results: A total of 31,397 cases were notified, with an annual average of 3140 cases. The South and North regions exhibited the highest incidence rates, while the Northeast and Southeast presented lethality rates above the national average (9.20%). A marked reduction in notifications occurred during the COVID-19 pandemic. Infections occurred predominantly in the domiciliary environment (64%). Rural workers (27.45%) and civil construction workers (18.63%) were the most affected occupational groups, with a higher incidence rate among illiterate and low-education populations. Conclusions: The dynamics of leptospirosis in Brazil are complex and multifactorial, influenced by climatic variations and driven by deficits in basic sanitation and education level. Mitigating the disease burden requires sustained, region-specific public health strategies, targeted infrastructure improvements, and enhanced epidemiological surveillance to address underreporting.

LANGUAGE OF ORIGINAL DOCUMENT: English

de Jesus Silva M., de Almeida Martins M.E., Mariúba Morais L.A.

Advances in the development of a vaccine for the prevention of human leptospirosis: a review

(2026) Revista do Instituto de Medicina Tropical de Sao Paulo, 68, art. no. e55

DOI: 10.1590/S1678-9946202668055

ABSTRACT: Leptospirosis is a zoonosis with global distribution caused by spirochetes of the genus *Leptospira*. In regions with poor sanitation, leptospirosis is considered a neglected disease, resulting in approximately one million cases and thousands of deaths annually. Transmission occurs mainly through contact with the urine of infected rodents, and clinical manifestations range from mild symptoms to the severe form known as Weil's disease. Although licensed vaccines are available, they are targeted at specific risk groups. Bacterins are composed of specific serovars, induce a short-term immune response, and may cause adverse reactions. Advances in molecular biology—including sequencing of the *Leptospira* genome—have spurred the development of new vaccination strategies, particularly protein subunit vaccines. These vaccines are based on proteins located in the outer membrane of pathogenic *Leptospira* species. These antigens are expressed in prokaryotic hosts, exhibit high immunogenicity, and, because they are directly exposed to the host's immune system, are considered promising targets for the development of safer and more effective vaccines. Among the main antigens studied, the most notable are LipL32, LipL41, OmpL1, OmpL37, Loa22, LigA, and LigB. However, the identification of antigens capable of inducing an effective immune response while containing conserved regions across different serovars remains a major challenge in developing a universal vaccine against leptospirosis. This review presents the principal outer membrane proteins of pathogenic *Leptospira* that are considered promising candidates for new vaccine formulations, as well as their role in inducing the immune response in the human host.

LANGUAGE OF ORIGINAL DOCUMENT: English

Goarant C., Girault D., Thibeaux R., Soupé-Gilbert M.-E.

Isolation and culture of *Leptospira* from clinical and environmental samples

(2026) *Methods in Molecular Biology*, 3068, pp. 1 - 8

DOI: 10.1007/978-1-0716-5500-9_1

ABSTRACT: Leptospire, the etiological agents of leptospirosis, are fastidious, slow-growing organisms. Here, we describe the isolation and routine maintenance of leptospire from clinical (blood, urine, or tissue) and environmental (water or soil) samples. Using combinations of filtration, agar plating, and selective agents, leptospire can be isolated in pure cultures even from complex contaminated sources in standard EMJH culture medium.

LANGUAGE OF ORIGINAL DOCUMENT: English

Gaspar Cuba R., Menozzi Donizete B., Henrique Pizzaia A.L., Angeluci Gismelli G., Carvalho Ribeiro N.O., Jaggi K., dos Santos Rocha G., Alaniz T.G., Góes Bentim L.G., Marinho M., Langoni H., Fornazari F.

Molecular investigation of coronavirus and *Leptospira* spp. in bats (*Chiroptera*, *Mammalia*) from Brazil

(2026) *Veterinary Research Communications*, 50 (6), art. no. 554

DOI: 10.1007/s11259-026-11496-7

ABSTRACT: The emergence of zoonotic diseases highlights the need for continuous surveillance of wildlife, particularly bats, known as natural reservoirs of several pathogens. This study aimed to investigate the presence of coronaviruses (CoV) and *Leptospira* spp. in non-hematophagous bats from the northwestern region of São Paulo State, Brazil, between 2019 and 2024. Samples of intestine and kidney from 98 specimens from 18 species belonging to three families (Molossidae, Phyllostomidae, and Vespertilionidae) were collected in 8 municipalities of the Araçatuba Intermediate Geographic Region. Coronaviruses detection was performed by pan-CoV nested RT-PCR, targeting fragments of 602 and 440 bp of the RNA-dependent RNA polymerase

(RdRp) gene. Investigation of *Leptospira* spp. was conducted by real-time PCR, aiming the amplification of a fragment of 331 bp of the 16 S rRNA gene. The analyses yielded no amplification in any of the intestinal and renal samples. Due to geographic bias and the underrepresentation of phyllostomids – a central taxon in CoV epidemiology in Brazil -, the lack of detection reflects sampling limitations rather than the real absence of these pathogens in the local fauna. Continuous surveillance, integrated with comprehensive and standardized sampling in this under-studied region, remains essential for the early detection of emerging zoonotic pathogens and for characterizing local host-pathogen ecology.

LANGUAGE OF ORIGINAL DOCUMENT: English

Stone N.E., Sahl J.W.

AUTHOR FULL NAMES: Stone, Nathan E. (55104679900); Sahl, Jason W. (16175997500)

55104679900; 16175997500

High resolution genotyping of *Leptospira* direct from clinical samples using Multiplexed Amplicon Sequencing

(2026) Methods in Molecular Biology, 3068, pp. 29 - 47

DOI: 10.1007/978-1-0716-5500-9_4

ABSTRACT: Amplicon sequencing (AmpSeq) is a high-throughput, PCR-based sequencing approach for high-resolution and inexpensive genotyping. We designed and validated a 42 locus AmpSeq system for strain-level *Leptospira* genotyping directly from complex clinical samples such as urine, blood, or animal tissues.

LANGUAGE OF ORIGINAL DOCUMENT: English

Kiuno K., Koizumi N.

Laboratory diagnosis of leptospirosis

(2026) Methods in Molecular Biology, 3068, pp. 361 - 371

DOI: 10.1007/978-1-0716-5500-9_30

ABSTRACT: Accurate laboratory diagnosis of leptospirosis is essential because the clinical manifestations are nonspecific and overlap with those of many other infectious diseases. The optimal diagnostic approach depends on the stage of illness, with nucleic acid amplification tests (NAATs) being most useful during the acute phase and serological methods becoming more informative after antibody production. This chapter reviews current laboratory diagnostic methods for leptospirosis, including culture, NAATs, serological assays, and antigen detection methods. The advantages and limitations of each approach are discussed, together with recent advances in molecular and serological diagnostics. Appropriate selection and combination of diagnostic methods according to the stage of illness remain essential for accurate diagnosis. Further development of rapid and reliable diagnostic tests suitable for resource-limited settings is still urgently needed.

LANGUAGE OF ORIGINAL DOCUMENT: English

Bonhomme D., Werts C.

AUTHOR FULL NAMES: Bonhomme, Delphine (57222438048); Werts, Catherine (6602667643)

57222438048; 6602667643

Purification of lipopolysaccharides from *Leptospira*

(2026) Methods in Molecular Biology, 3068, pp. 81 - 93

DOI: 10.1007/978-1-0716-5500-9_8

ABSTRACT: *Leptospira* species are one of the few spirochetes to possess a lipopolysaccharide (LPS) embedded in their outer membrane. Two protocols are currently available to extract and/or purify the leptospiral lipopolysaccharides: the rapid proteinase K method and the classical hot water/phenol extraction. The first method allows to get a quick overview of the LPS O antigen structure, whereas the second method is fitted to study the immunological properties of the leptospiral LPS. These two methods will be detailed in this chapter. Methodologies to assess the quality of the purification, such as the modified silver staining coloration, will also be reviewed. Both advantages and limitations of the different analyses will be described.

LANGUAGE OF ORIGINAL DOCUMENT: English

Maheswari B.U., Chitra K., Kumarasamy P.S.

Comparative analysis of ELISA, MAT, and RT-PCR for early diagnosis of human leptospirosis

(2026) Natural Resources for Human Health, 6 (7S), pp. 990 - 1010

ABSTRACT: A neglected zoonotic illness that affects people all around the world is human leptospirosis. Contact with soil or water tainted by sick animals' urine is the primary way it spreads. Early identification is challenging since the symptoms frequently mimic those of other feverish diseases including dengue, malaria, or influenza. This study evaluated the effectiveness of the Enzyme-Linked Immunosorbent Assay (ELISA), Microscopic Agglutination Test (MAT), and Real-Time Polymerase Chain Reaction (RT-PCR) in a structured diagnostic process for suspected human leptospirosis. Blood samples were taken under sterile conditions and analyzed for both serological and molecular details. ELISA was used to detect IgM and IgG antibodies. MAT served as the serological standard, while RT-PCR targeted the LipL32 gene for direct detection of the pathogen. Clinical, demographic, and laboratory data were compiled into a standard database. After that, the data undergo preprocessing to ensure consistency. The study assessed diagnostic performance using Receiver Operating Characteristic (ROC) analysis. The Area under Curve (AUC) values were 0.942 for MAT, 0.904 for ELISA, and 0.981 for RT-PCR. Among these, RT-PCR showed the best diagnostic accuracy, especially within the first 7 days of illness, proving its advantage for early detection. These results highlight the supportive role of RT-PCR alongside serological tests, which aids in the quick, sensitive, and reliable diagnosis of leptospirosis.

LANGUAGE OF ORIGINAL DOCUMENT: English

Picardeau M.

***Leptospira* and leptospirosis**

(2026) Methods in Molecular Biology, 3068, pp. 355 - 359

DOI: 10.1007/978-1-0716-5500-9_29

ABSTRACT: *Leptospira* spp. are morphologically and phylogenetically unique bacteria that are ubiquitous in the environment. Pathogenic species are the agents of leptospirosis, an emerging zoonotic disease transmitted to both humans and animals from the environment contaminated with the urine of reservoir animals. The taxonomy of *Leptospira* has recently undergone extensive revisions with the use of whole-genome sequences, thus expanding the number of species considerably.

LANGUAGE OF ORIGINAL DOCUMENT: English

Abe K., Koizumi N., Takazaki H., Hirose M., Takabe K., Kato T., Nakamura S.

A periplasmic layer formed by an outer membrane lipoprotein governs the cell-envelope integrity and stiffness of *Leptospira interrogans*

(2026) Communications Biology, 9 (1), art. no. 1175

DOI: 10.1038/s42003-026-10443-1

ABSTRACT: The structural integrity and mechanical properties of the cell envelope are crucial for bacterial physiology and dynamics; however, how specific molecular components govern these properties remains poorly understood. Here, we report the structural and mechanical functions of LipL32, the most abundant outer-membrane (OM) lipoprotein in the pathogenic spirochete *Leptospira interrogans*. LipL32 accounts for approximately 75% of the total OM proteins, yet its functional role remains enigmatic. Cryo-electron microscopy reveals that the highly dense LipL32 forms a thin layer in the periplasmic space, positioned adjacent to the peptidoglycan layer. The structural analysis also demonstrates that the disruption of lipL32 by transposon insertion results in irregular deformation of OM and spatial variability in the width of the periplasmic space. Quantitative measurements by optical tweezers confirm that the lipL32::Tn mutant exhibits a significant reduction in whole-cell stiffness. Consistent with this mechanical impairment, the lipL32::Tn mutant displays a diminished ability to penetrate a heterogeneous agar mesh model that mimics the host's extracellular matrix, suggesting an important role for cell rigidity in initial infection via damaged skin. This work provides a mechanobiological insight into how lipoproteins contribute to cell-envelope integrity as a stabilizer, conferring stiffness and invasiveness on bacterial pathogens.

LANGUAGE OF ORIGINAL DOCUMENT: English

Vernel-Pauillac F., Werts C.

In vivo imaging of bioluminescent leptospires

(2026) Methods in Molecular Biology, 3068, pp. 239 - 250

DOI: 10.1007/978-1-0716-5500-9_20

ABSTRACT: The study of pathological processes is often limited to in vitro or ex vivo assays, while understanding pathogenesis of an infectious disease requires in vivo analysis. The use of pathogens, genetically modified to express with luminescent enzymes, combined to charge coupled device (CCD) cameras constitutes a major technological advance for assessing the course of infection in an intact, living host in real time and in a non-invasive way. This technology, also called bioluminescence imaging, detects the photons emitted from biological sources of light through animal tissues. Here, we describe the method we developed to monitor leptospirosis in a mouse model, by following in a spatiotemporal scale, the dissemination and spread of leptospires. These bacteria have been genetically modified to express the firefly luciferase, which produces light in the presence of the substrate D-luciferin. This useful and accessible technology facilitates the study of the kinetics of blood and tissue dissemination of live leptospires, and the pharmacological impact of treatments and host-directed therapeutics.

LANGUAGE OF ORIGINAL DOCUMENT: English

Matsunaga J.

Transposon Sequencing in *Leptospira interrogans*

(2026) Methods in Molecular Biology, 3068, pp. 127 - 137

DOI: 10.1007/978-1-0716-5500-9_11

ABSTRACT: The development of random transposon mutagenesis in *L. interrogans* has enabled the creation of large libraries of mutants, facilitating the identification of genes involved in key phenotypes such as virulence. However, phenotypic screening of individual mutants from these libraries remains time-consuming and labor-intensive. Here, we describe a transposon-sequencing protocol (Tn-Seq) that combines random transposon mutagenesis with high-throughput sequencing to simultaneously assess fitness changes across a large collection of *L. interrogans* mutants under defined conditions. Inoculating individual animals with a pooled set of mutants enables rapid identification of candidate virulence genes based on reduced fitness. This approach significantly reduces time and resource requirements compared with traditional screening techniques.

LANGUAGE OF ORIGINAL DOCUMENT: English

Grassmann A.A., Caimano M.J.

Cultivation of *Leptospira interrogans* within rat peritoneal dialysis membrane chambers

(2026) *Methods in Molecular Biology*, 3068, pp. 327 - 341

DOI: 10.1007/978-1-0716-5500-9_27

ABSTRACT: To sustain its zoonotic lifecycle, leptospires must adapt to growth within the host milieu. Signals encountered within the mammal also trigger regulatory programs required by *Leptospira* for the expression of virulence-related gene products. The complex transcriptional, antigenic, and physiological changes leptospires undergo within the mammal are collectively referred to as “host-adaptation.” In this chapter, we describe procedures for the generation of host-adapted *Leptospira* spp. by cultivation within dialysis membrane chambers (DMCs) implanted in rat peritoneal cavities. In this model, *Leptospira* spp. diluted in EMJH medium are sequestered within sterile dialysis membrane tubing closed at both ends. The chamber is then surgically implanted within the peritoneal cavity of a rat and incubated for 7–10 days. During this period, leptospires are exposed to many, if not all, of the physiological and nutritional cues required for host adaptation while at the same time protected from clearance by host innate and adaptive immune defenses.

LANGUAGE OF ORIGINAL DOCUMENT: English

Waidyasuriya L.S.M., Chathurangani K.K.S., Ambillapitiya L.H.M.R.L., Wickramaratna D.D., Jayasekara J.M.K.B.

Managing leptospirosis as a public health issue: the burden of renal and hepatic involvement

(2026) *Indonesian Journal of Public Health*, 21 (2), pp. 233 - 245

DOI: 10.20473/Ijph.v21i2.2026.233-245

ABSTRACT: Introduction: Leptospirosis is a zoonotic infection that is found worldwide, mainly resulting in hepatic and renal complications. The occurrence of acute kidney injury (AKI) in leptospirosis varies from 40-60%, with a mortality rate of 22%. Aims: Identifying potential risk factors and assessing incidents of AKI and liver involvement are useful for managing comorbidities and mortality. Methods: A prospective cross-sectional study including one hundred and three clinically confirmed leptospirosis patients admitted to the Teaching Hospital, Rathnapura, Sri Lanka. An interviewer-administered questionnaire and medical records were used to collect data. Biochemical and hematological investigations were performed on admission and at discharge. Results: Farming (30.7%) and mining (31.8%) were the most common occupational exposures. The majority had cuts or bruises and had also been exposed to floodwater or contaminated water. The mean age was 45.5 ± 15.96 years, and most (94.2%) were males. AKI was observed in 26.2% (KDIGO criteria), and liver involvement in 28.16% (National Guidelines of Leptospirosis). Significant increases in hematological and

biochemical parameters were observed in AKI patients compared to non-AKI patients ($p<0.001$). Similar observations were presented in patients with liver involvement compared to those without. Conclusion: Both AKI and liver involvement were observed in 29.6% of the population. Mining and agricultural involvement were identified as the main risk factors for leptospirosis. It is recommended that individuals refrain from entering the field if they have wounds and wear protective footwear before engaging in high-risk occupations.

LANGUAGE OF ORIGINAL DOCUMENT: English

Jogunčić A., Salčinović A., Hodžić A.P., Bajramović A., Hamza Z., Pošković-Bajraktarević A., Tekin A., Amos L.
Leptospirosis outbreak in an urban setting: epidemiological and clinical characteristics of the 2025 epidemic in Sarajevo Canton, Bosnia and Herzegovina

(2026) Annali dell'Istituto Superiore di Sanita, 62 (3), pp. 224 - 231

DOI: 10.4415/ANN_26_03_05

ABSTRACT: Background. Leptospirosis is an emerging public-health concern in urban settings, but data from temperate European cities remain limited. This study describes the 2025 leptospirosis outbreak in Sarajevo Canton, Bosnia and Herzegovina. Methods. A descriptive observational study was conducted using mandatory surveillance data and a supplementary epidemiological questionnaire based on the WHO Leptospirosis Surveillance Tool. Cases were classified according to the ECDC case definition. Epidemiological, clinical, laboratory, and exposure characteristics were analysed. Results. A total of 119 cases were recorded; 63.9% were male and the mean age was 45.8 ± 13.2 years. The overall incidence was 2.84 per 10,000 population, highest in Novi Grad municipality. Common symptoms included weakness, fever, chills, myalgia, and headache. Elevated liver enzymes were the only independent predictor of hospitalisation. Conclusion. The outbreak indicates a shift toward urban leptospirosis, likely linked to environmental contamination and rodent activity rather than traditional rural or flood-related exposure.

LANGUAGE OF ORIGINAL DOCUMENT: English

Fernandez M., Klein J., Werts C.

Bacterial size analysis using high-content imaging: application to *Leptospira* strains

(2026) Methods in Molecular Biology, 3068, pp. 173 - 185

DOI: 10.1007/978-1-0716-5500-9_15

ABSTRACT: Leptospire are thin, motile spirochetes whose morphology and size can vary among species and strains. Accurate measurement of bacterial size is essential for phenotypic characterization and comparative analyses. Here, we describe a standardized protocol for labeling and measuring the size of *Leptospira* using carboxyfluorescein succinimidyl ester (CFSE), and high-content fluorescence microscopy. Leptospiral strains were cultured until the exponential phase, stained with CFSE, and plated prior to imaging. Imaging was performed using the OPERA Phenix system (Revity). ImageJ with the MicrobeJ plugin was used to analyze the images, allowing for automated detection, and measurement of individual bacteria. This method enables accurate and reproducible measurement of leptospiral size across different species. It can also be adapted to other bacterial species.

LANGUAGE OF ORIGINAL DOCUMENT: English

Khaksar E., Neshandar Y., Abdollahpour G., Hajivaliei M.M., Kazemrab M., Kakoei M.

Molecular detection of *Leptospira* spp. in urinary and vaginal samples of domestic and free-roaming cats in Tehran, Iran

(2026) Comparative Immunology, Microbiology and Infectious Diseases, 130, art. no. 102532

DOI: 10.1016/j.cimid.2026.102532

ABSTRACT: Objective Leptospirosis is a globally important zoonotic disease associated with renal and hepatic injury. Cats are frequently exposed to *Leptospira* spp., yet their epidemiological role remains unclear. This study aimed to determine whether *Leptospira* DNA could be detected in feline vaginal samples, in addition to urine, using Nested-PCR. Secondary objectives were to determine the proportion of PCR-positive cats and assess renal involvement by comparing renal parameters between PCR-positive and PCR-negative cats. Methods Seventy-six domestic and free-roaming cats were examined in Tehran, in 2025. Urine samples were collected from all cats and vaginal swabs from female cats. Nested-PCR targeting the 16S rRNA (*rrs*) gene was performed on both sample types followed by confirmatory sequencing of representative amplicons. Renal status was assessed using biochemical, hematological, and urinalysis parameters in all cats. Results *Leptospira* DNA was detected in 15/76 cats (19.7%). Positive results were obtained from urine samples in 10/76 cats (13.1%) and from vaginal swabs in 5/61 female cats (8.2%), with no concordant positive results between urine and vaginal samples (95% CIs reported) . Vaginal PCR identified *Leptospira* DNA in cats that were negative by urine PCR. No significant differences were observed in biochemical, hematological or urinalysis parameters between PCR-positive and PCR-negative cats. Conclusions *Leptospira* DNA was detected in urine and vaginal samples of both domestic and free-roaming cats, frequently in subclinical animals without observable clinical signs. The absence of concordance between urine and vaginal PCR results suggests that vaginal sampling may provide complementary molecular detection to urine testing. Further investigation is warranted to elucidate the biological significance and clinical implications of *Leptospira* DNA detection in feline vaginal samples.

LANGUAGE OF ORIGINAL DOCUMENT: English

Fernandes L.G.V., Nally J.E.

Gene Knockout in *Leptospira* spp. by CRISPR/Cas9-NHEJ (Non-Homologous End-Joining) and CRISPR-Prime Editing

(2026) Methods in Molecular Biology, 3068, pp. 139 - 156

DOI: 10.1007/978-1-0716-5500-9_12

ABSTRACT: Genetic manipulation of *Leptospira* spp. has progressed significantly in recent years. Like most prokaryotes, leptospires are unable to survive double-strand breaks (DSBs) induced by the Cas9 endonuclease, prompting the development of alternative strategies for gene knockout. We have established two systems for targeted mutagenesis of *Leptospira* spp.: CRISPR/Cas9-NHEJ and CRISPR-Prime Editing (PE). The CRISPR/Cas9-NHEJ approach involves coexpression of the CRISPR/Cas9 machinery alongside the DNA repair proteins LigD and Ku from *Mycobacterium smegmatis*, facilitating error-prone repair of DSBs that results in indel mutations. In contrast, CRISPR-PE is a DSB-free strategy that utilizes a Cas9-nickase fused to a reverse transcriptase that facilitates precise single-nucleotide edits in the genome. This expanded toolbox has placed *Leptospira* spp. at the forefront of bacterial genetic manipulation.

LANGUAGE OF ORIGINAL DOCUMENT: English

Teixeira A.F., Nascimento A.L.T.O.

Cell adhesion assays to investigate leptospiral protein interactions with ECM, cell receptors, and cultured mammalian cells: methods for understanding host-*Leptospira* interactions

(2026) *Methods in Molecular Biology*, 3068, pp. 263 - 279

DOI: 10.1007/978-1-0716-5500-9_22

ABSTRACT: Pathogenic bacteria must overcome host barriers and survive to establish infection. Adhesion to host cells and extracellular matrix (ECM) components is a critical step in this process, as it facilitates colonization and invasion. Pathogenic *Leptospira* species have been shown to bind both to host cell receptors and to ECM molecules, contributing to infection. Previous studies have mainly reported in vitro interactions of recombinant *Leptospira interrogans* proteins with ECM components. Our work extends these studies by focusing on two major classes of macromolecules that constitute the mammalian ECM: glycosaminoglycans (GAGs) and fibrous proteins such as collagens, elastin, fibronectin, and laminin. Additionally, we have investigated interactions with various integrin cell receptors and examined the binding of recombinant proteins to cultured mammalian cell. In this chapter, we describe the established protocols to investigate the interactions between putative leptospiral adhesins and host components and mammalian cells.

LANGUAGE OF ORIGINAL DOCUMENT: English

Guzmán D., Pazmiño-Zurita G., Melo S., Pearson T., Barragán V.

A systematic review of *Leptospira* and leptospirosis in wildlife: a global perspective

(2026) *One Health*, 23, art. no. 101559

DOI: 10.1016/j.onehlt.2026.101559

ABSTRACT: There are many different pathogenic species of *Leptospira* that circulate among a wide range of wild and domestic animals. Certain species and serovars can cause disease in some hosts but not in others. Although leptospirosis has been extensively studied in humans and domestic animals, with its epidemiology and economic impact being well characterized, much less is known about its ecological dynamics in wildlife. The host-pathogen associations, transmission pathways, and disease outcomes in wild animal populations remain poorly understood. This review summarizes the current evidence on *Leptospira* and leptospirosis in wildlife, identifying methodological biases and highlighting key knowledge gaps. It also compiles information on wild animal species in which exposure to the pathogen has been documented. A systematic search of the scientific literature published between 2000 and 2025 was performed across multiple databases in accordance with PRISMA guidelines. Following screening and an assessment of eligibility, data were extracted from 389 peer-reviewed articles reporting exposure to, infection with, or detection of the pathogen in wildlife species worldwide. An analysis of study locations and publication trends was also performed. The reviewed studies revealed substantial heterogeneity in diagnostic methods, sampling effort, and geographic coverage, as well as a wide taxonomic diversity of wildlife species exposed to or infected with *Leptospira*. Clinical cases were most frequently reported in the orders Carnivora and Rodentia, with mortality documented in marine mammals, large felids and primates, particularly in untreated cases. Analysis of carriage and exposure studies revealed a strong sampling bias toward mammals compared to birds and reptiles. Molecular detection was reported in 155 articles, with lipL32 being the most commonly used marker. Sequencing of amplicons enabled identification of *Leptospira* at the level of the species. Meanwhile, serological testing predominantly relied on MAT reporting.

LANGUAGE OF ORIGINAL DOCUMENT: English

Nakamura S.

Measurement of the cell-body rotation of *Leptospira*

(2026) Methods in Molecular Biology, 3068, pp. 187 - 196

DOI: 10.1007/978-1-0716-5500-9_16

ABSTRACT: *Leptospira* spp. swim in liquid and crawl on surfaces with two periplasmic flagella. The periplasmic flagella attach to the protoplasmic cylinder via basal rotary motors (flagellar motors) and transform the ends of the cell body into a spiral or hook shape. The rotations of the periplasmic flagella are thought to gyrate the cell body and rotate the protoplasmic cylinder for propelling the cell; however, the motility mechanism has not been fully elucidated. Since motility is a critical virulence factor for pathogenic leptospires, the kinematic insight is valuable to understand the mechanism of infection. This chapter describes microscopic methodologies to measure the motility of *Leptospira*, focusing on the rotation of the helical cell body.

LANGUAGE OF ORIGINAL DOCUMENT: English

Mariet J.-F., Grillová L., Picardeau M.

Core Genome Multi-Locus Sequence Typing analyses of *Leptospira* spp. using the bacterial isolate genome sequence database

(2026) Methods in Molecular Biology, 3068, pp. 49 - 57

DOI: 10.1007/978-1-0716-5500-9_5

ABSTRACT: With the advent of whole-genome sequencing (WGS), comparative genomic analyses have enabled the development of core genome multilocus sequence typing (cgMLST) schemes for high-resolution, reproducible typing of bacterial isolates. In cgMLST, hundreds of loci are used for gene-by-gene comparisons of assembled genomes for studying the genetic diversity of isolates, including clinically important pathogens. Combination of the cgMLST data and metadata of the isolates is useful for epidemiological investigations. Here we present a cgMLST scheme for the high-resolution typing of isolates from the whole *Leptospira* genus, enabling identification at the level of species, clonal groups, and sequence types. We show several examples of how the cgMLST *Leptospira* database, which is a publicly available web-based database, can be used for the analyses of WGS data of *Leptospira* isolates. This effort was undertaken to facilitate international collaborations and support the global surveillance of leptospirosis.

LANGUAGE OF ORIGINAL DOCUMENT: English

Couto Alves Petit C., Ferreira Corrêa L., Ribeiro A.J., Silva Alves K., Resende Ananias A.C., Gandra Braga I., Barcelos Dos Santos I.C., Pereira S.P., Rodrigues Machado Camargo R., de Oliveira Silva L., Goveia Freitas F.A., Balassiano Teruszkin I., Giunchetti Cordeiro R., Ferraz Coelho E.A., de-Ávila Machado R.A., Chávez-Fumagalli M.A., Cassiano Souza C.S., Belo Silva V., Maia Gonçalves A.A., Da Paz Campos M., Santos Avelar K.E., Galdino Sobreira A.

Recombinant proteins, multiepitope constructs, and synthetic peptides for ELISA-based diagnosis of leptospirosis: a comprehensive review

(2026) Current Medicinal Chemistry

DOI: 10.2174/0109298673473711260706145941

ABSTRACT: Leptospirosis, caused by pathogenic species of the gram-negative spirochete called *Leptospira* spp., is one of the most serious zoonotic diseases in the world. This disease commonly affects impoverished or underdeveloped populations with no proper sanitary conditions and is especially endemic in Latin American

and tropical countries. Currently, the standard test for diagnosing leptospirosis, recommended by the World Health Organization (WHO), is the microscopic agglutination test (MAT). However, this test can be considered inefficient due to several technical and logistical constraints. For this reason, enzyme-linked immunosorbent assays (ELISAs) using recombinant or synthetic antigens have emerged as promising alternatives for serodiagnosis. Facing the need to improve the diagnosis of leptospirosis, and with the promising applications of recombinant and synthetic antigens, we performed a comprehensive narrative review of the use of recombinant proteins (RP), recombinant multiepitope proteins (RMP), and synthetic peptides (SP), applied in ELISAs for leptospirosis diagnosis. It was possible to observe some promising results using recombinant and synthetic antigens, which represent powerful means of optimizing the ELISA-based diagnosis of leptospirosis. Among the studies reviewed, RPs, the type of antigen most widely studied, showed outstanding diagnostic performance. Although RMPs and SPs are not studied as widely, all these antigens showed diagnostic potential with great values for sensitivity and specificity. Despite these good results, it remains necessary to improve the studies under development to find quicker, more accurate, and widely accessible diagnostic tools for leptospirosis, which may provide a better prognosis for patients. 2026, Bentham Science Publishers
LANGUAGE OF ORIGINAL DOCUMENT: English

Saito M.

Hamster model of leptospira infection

(2026) *Methods in Molecular Biology*, 3068, pp. 319 - 325

DOI: 10.1007/978-1-0716-5500-9_26

ABSTRACT: Leptospirosis is a globally important zoonotic disease with a high mortality rate in severe cases. The Syrian hamster (*Mesocricetus auratus*) is highly susceptible to *Leptospira* and is commonly used as a lethal acute model that mimics human leptospirosis. This chapter describes procedures for establishing various infection models, including the intraperitoneal, subcutaneous, transdermal, oral, and respiratory routes. Additionally, it provides detailed methods for collecting samples and evaluating the pathology of infected hamsters.

LANGUAGE OF ORIGINAL DOCUMENT: English

Davignon G., Kainiu M., Thibeaux R.

Protocols for standard and advanced visualization and quantification of *Leptospira* biofilms

(2026) *Methods in Molecular Biology*, 3068, pp. 301 - 317

DOI: 10.1007/978-1-0716-5500-9_25

ABSTRACT: The ability of *Leptospira* spp. to form biofilms is increasingly recognized as a key factor in their environmental persistence and possibly in host colonization. In this chapter, we present a set of protocols for the *in vitro* growth, quantification, and structural characterization of *Leptospira* biofilms. We detail standard high-throughput approaches, such as crystal violet (CV) staining in 96-well microtiter plates, as well as an alternative based on phase contrast microscopy and image analysis. To probe biofilm architecture and composition, we describe advanced imaging methods including confocal laser scanning microscopy (CLSM) with fluorescent probes targeting viability or matrix components and scanning electron microscopy (SEM) for high-resolution surface imaging. We also introduce a method for growing *Leptospira* biofilms on polycarbonate membranes, facilitating downstream structural analyses without disturbing their spatial organization. Together,

these protocols provide a robust and adaptable toolbox for investigating the development and complexity of *Leptospira* biofilms.

LANGUAGE OF ORIGINAL DOCUMENT: English

Schiettekatte O., Bourhy P.

Isolation, purification, and characterization of leptophages

(2026) *Methods in Molecular Biology*, 3068, pp. 95 - 103

DOI: 10.1007/978-1-0716-5500-9_9

ABSTRACT: Only the three leptophages LE1, LE3, and LE4 or bacteriophages of leptospires, are known to date. However, numerous prophages have been found in this genus, particularly in the genomes of pathogenic species. Consequently, some laboratories attempt to isolate leptophage particles from environmental samples or from bacterial cultures following mitomycin C induction. Here, we present several procedures for isolating, purifying, and characterizing bacteriophages based on the leptophages characterization protocols.

LANGUAGE OF ORIGINAL DOCUMENT: English

Machado Dahlke C., Kmetiuk Bach L., Sibim A.C., Biondo Meneguelli L., Chiba de Castro Wagner W., Biondo Welker A.

Occupation as an associated risk factor for leptospirosis

(2026) *Frontiers in Public Health*, 14, art. no. 1810977

DOI: 10.3389/fpubh.2026.1810977

ABSTRACT: Introduction – Although several studies have described occupation as an associated risk factor for leptospirosis, most have reported absolute case counts without normalization by the general population, service attendance, or notification capacity. Methods – Accordingly, this study assessed the Brazilian National Surveillance System (SINAN) from 2007 to 2022, examining gathered leptospirosis cases analyzed by occupation as an associated risk factor within the working-age population. To ensure homogeneous attendance and notification capacity, we focused our analysis on the 100 largest Brazilian cities, utilizing censitary working occupation data per region as the denominator for normalization. Non-active populations (students and retirees) were excluded from the denominators to prevent bias dilution. Occupational groups were categorized into low, medium, and high risk for leptospirosis. Results – Overall, the occupations with the highest absolute frequency included farmers (12.0%), construction workers (5.8%), and recycling material collectors (4.6%), with the work environment (75.5%) identified as the most common site of infection. The highest normalized occurrence of leptospirosis was reported in southern Brazil, and high-risk occupational groups showed significantly higher infection rates compared to lower-risk categories ($p < 0.001$). Furthermore, professional groups with higher exposure loads presented greater frequencies of severe clinical outcomes, including renal failure, jaundice, and higher case fatality rates. The present study suggests occupation as an important predictor of leptospirosis, varying across regions according to local professional frequencies. In this scenario, the Brazilian national surveillance system serves as an effective indicator for public health actions and policies, enabling the dynamic monitoring of occupational exposure over time. Finally, occupation must systematically be analyzed as a predictor after normalizing for professional frequency, ensuring equitable access to healthcare assistance and standardized institutional notification. Copyright

LANGUAGE OF ORIGINAL DOCUMENT: English

Hamond C., Wunder E.A.

EMEM and DMEM as an alternative media for leptospiral culture, diagnostic, and pathogenesis studies

(2026) Methods in Molecular Biology, 3068, pp. 21 - 28

DOI: 10.1007/978-1-0716-5500-9_3

ABSTRACT: Leptospire require specific conditions and nutrition to survive and thrive, essential for its ability to multiply or adapt to in vitro culture. This constraint on laboratory cultures limits the applicability of in vitro studies to the understanding of even simple pathogenic processes and diagnostics. Culture of leptospire in a laboratory setting using DMEM and EMEM media, common for culture of mammalian cells, is an alternative that mimics host environmental conditions, less complex, with high performance, and lower cost, which are imperative characteristics to improve routine laboratory activities and studies of leptospirosis.

LANGUAGE OF ORIGINAL DOCUMENT: English

Kawamoto A.

In situ structural analysis of *Leptospira* spp. by electron cryotomography

(2026) Methods in Molecular Biology, 3068, pp. 165 - 172

DOI: 10.1007/978-1-0716-5500-9_14

ABSTRACT: Spirochetes such as *Treponema*, *Borrelia*, and *Leptospira* species can rotate their bodies to swim in liquid environments by rotating periplasmic flagella or endoflagella, which are present inside the cell. Electron cryotomography (ECT) is an imaging technique that directly provides three-dimensional (3D) structures of cells and molecular complexes in their cellular environment at nanometer resolution. Here, I present a general protocol of ECT that covers the sample preparation, data collection, tilt series alignment and tomographic reconstruction for visualization of intact periplasmic flagella in *Leptospira* spp. This protocol is capable of determining protein structures at resolutions high enough to visualize their individual domains and secondary structures in their cellular environment.

LANGUAGE OF ORIGINAL DOCUMENT: English

Pappas C.J., Xu H., Motaleb M.A.

Creating a library of random transposon mutants in *Leptospira*

(2026) Methods in Molecular Biology, 3068, pp. 105 - 125

DOI: 10.1007/978-1-0716-5500-9_10

ABSTRACT: Generation of a random transposon mutant library is advantageous in *Leptospira* as site-directed mutagenesis remains a challenge, especially in pathogenic species. This procedure is typically completed by transformation of *Leptospira* with a Himar1-containing plasmid via conjugation with *Escherichia coli* as a donor cell. Here, we describe the methodology to generate random transposon mutants in the saprophyte *Leptospira biflexa* via conjugation of plasmid pSW29T-TKS2 harbored in *E. coli* β 2163. Determination of transposon insertion site by semi-random nested PCR will also be described. A similar methodology may be employed to generate Tn mutants of pathogenic *Leptospira* species.

LANGUAGE OF ORIGINAL DOCUMENT: English

Takabe K., Koizumi N.

Machine learning-based quantification of motility parameters in individual *Leptospira* cells

(2026) Methods in Molecular Biology, 3068, pp. 209 - 217

DOI: 10.1007/978-1-0716-5500-9_18

ABSTRACT: *Leptospira* spp. are characterized by highly active motility, exhibiting swimming in liquid environments and crawling on solid surfaces, including host cells. Because motility of pathogenic *Leptospira* spp. is closely associated with successful infection, a deeper understanding of the underlying mechanism of motility and cellular behavior in response to environmental changes is essential for advancing countermeasures against leptospirosis. However, the precise role of motility as a virulence factor remains incompletely understood. One major obstacle has been the technical difficulty of quantitative analyzing complex motility patterns in a high-throughput manner. This chapter describes methodologies for automatic measurement of motility parameters, such as swimming and crawling speeds, using machine learning-based recognition and tracking of individual leptospiral cells.

LANGUAGE OF ORIGINAL DOCUMENT: English

Hamond C., Fernandes L.G.V., Nally J.E.

Preparation of HAN medium for the isolation of *Leptospira* directly from infected hosts

(2026) *Methods in Molecular Biology*, 3068, pp. 9 - 20

DOI: 10.1007/978-1-0716-5500-9_2

ABSTRACT: Here, we describe the formulation and application of Hornsby-Alt-Nally (HAN) growth medium for the isolation and propagation of *Leptospira* species and serovars from host tissues. Cultures can be isolated directly from host samples by incubation at 29°C or 37°C using HAN liquid, semi-solid, or solid agar media.

LANGUAGE OF ORIGINAL DOCUMENT: English

Oyamada Y., Ozuru R.

Instance segmentation as a foundation for quantitative image analysis in *Leptospira* research

(2026) *Methods in Molecular Biology*, 3068, pp. 219 - 237

DOI: 10.1007/978-1-0716-5500-9_19

ABSTRACT: The microscopic agglutination test (MAT) is the gold standard for leptospirosis serodiagnosis, but its reliance on subjective visual assessment leads to interobserver variability. Artificial intelligence (AI) offers a powerful pathway to overcome this limitation, enabling objective and reproducible quantification. The core feature of this technique lies in its ability to pinpoint the precise location and morphology of individual bacteria through instance segmentation. By identifying each bacterium at the pixel level, researchers can not only calculate agglutination rates in MAT based on objective physical definitions but also unlock diverse applications such as morphological profiling and motility analysis when integrated with object tracking techniques. This allows for more granular assessments of drug effects and fundamental bacterial behavior. This chapter provides a comprehensive, end-to-end guide for implementing advanced AI-driven analysis of *Leptospira* images (including MAT samples), covering the entire workflow from foundational data preparation to the deployment of sophisticated deep learning models.

LANGUAGE OF ORIGINAL DOCUMENT: English

Wunder E.A., Jr., Karovic E.

Cell monolayer translocation assay

(2026) *Methods in Molecular Biology*, 3068, pp. 251 - 262

DOI: 10.1007/978-1-0716-5500-9_21

ABSTRACT: An essential property associated with leptospiral virulence is the pathogen's ability to translocate across host cells, enabling *Leptospira* to evade the host immune response, disseminate, and establish infection. The cell monolayer translocation assay allows for the quantification of *Leptospira* strain's competence to cross cell barriers while measuring the integrity of the polarized eukaryotic cell monolayer during this process.

LANGUAGE OF ORIGINAL DOCUMENT: English

Nakamura S., Abe K.

Machine learning-based, label-free motion tracking of *Leptospira* spp. on cultured cells

(2026) *Methods in Molecular Biology*, 3068, pp. 197 - 207

DOI: 10.1007/978-1-0716-5500-9_17

ABSTRACT: *Leptospira* spp. swim in liquid and crawl on surfaces with two periplasmic flagella. The *Leptospira* motility is a crucial virulence factor, especially crawling motility, which is possibly associated with the host-dependent severity of leptospirosis. In vitro infection assay using monolayers of cultured host cells provides basic knowledge on host-pathogen interaction in leptospirosis; however, it is difficult to observe leptospires on the host cells due to their similarity in refractive index. Though fluorescent labeling could solve the problem in general, it could negatively affect the leptospiral physiology. Here, we introduce a label-free observation technique for *Leptospira* spp. using machine learning. The machine-learning-based "background subtraction" automatically detects bacteria moving over cultured animal cells, allowing researchers to analyze bacterial motion trajectories, velocity, and diffusivity. Assay without any labeling procedures, including genetic manipulations, removes the restriction of available serovars and strains of *Leptospira*.

LANGUAGE OF ORIGINAL DOCUMENT: English

Girault D., Kainiu M., Barsac E., Thibeaux R., Goarant C.

Use of MALDI-TOF mass spectrometry for identification of *Leptospira*

(2026) *Methods in Molecular Biology*, 3068, pp. 59 - 65

DOI: 10.1007/978-1-0716-5500-9_6

ABSTRACT: Medical microbiology has used phenotypical and metabolic criteria to identify bacterial pathogens for decades. However, no such criteria have been applied to identify leptospires at the species level. In the recent years, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-ToF) mass spectrometry (MS) has emerged as new tool for the identification of bacterial species in the medical microbiology laboratory. This technology has rapidly gained more and more popularity. Actually, this technique is sensitive and economic, saving both labor and bench costs, but also rapid, significantly reducing turnaround time from isolation to identification. MALDI-ToF MS provides an unprecedented tool for the rapid identification of *Leptospira* at the species level.

LANGUAGE OF ORIGINAL DOCUMENT: English

Lambert A.

***Leptospira* spp. toolbox for chemotaxis assay**

(2026) *Methods in Molecular Biology*, 3068, pp. 157 - 164

DOI: 10.1007/978-1-0716-5500-9_13

ABSTRACT: A toolbox for chemotaxis assay adapted to *Leptospira* spp. has emerged in the recent years: soft agar assay, capillary assay, and videomicroscopy tracking. Those methods allow to demonstrate chemotaxis defect, identify diverse chemoattractant, or decipher motile behavior quantitatively. These experiments have demonstrated a role of motility and potentially chemotaxis in leptospirosis pathogenesis. We describe extensively the methods and provide the key steps to use this toolbox.

LANGUAGE OF ORIGINAL DOCUMENT: English

Coullin K., Zavala-Alvarado C., Benaroudj N.

The single-step method of RNA purification applied to *Leptospira*

(2026) *Methods in Molecular Biology*, 3068, pp. 67 - 80

DOI: 10.1007/978-1-0716-5500-9_7

ABSTRACT: Establishing a rapid method to obtain pure and intact RNA molecules has revolutionized the field of RNA biology, enabling laboratories to routinely perform RNA analysis such as Northern blot, reverse transcriptase quantitative PCR, and RNA sequencing. Here, we describe an application of the effective single-step method of RNA extraction (or guanidinium thiocyanate–phenol–chloroform extraction) applied to *Leptospira* species. This method is based on the powerful ability of guanidinium thiocyanate to inactivate RNases and on the different solubility of RNA and DNA in acidic phenol. This method allows one to reproducibly obtain total RNAs with high yield and integrity, as determined by capillary electrophoresis, suitable for RNA sequencing technology.

LANGUAGE OF ORIGINAL DOCUMENT: English

Palanisamy B., Faisal S.M.

***Leptospira*–host interactions: advancing next-generation vaccines and diagnostics**

(2026) *Clinical Microbiology Reviews*, 39 (3)

DOI: 10.1128/cmr.00373-25

ABSTRACT: Leptospirosis, a widespread zoonotic disease caused by pathogenic *Leptospira* species, remains a major public health challenge, particularly in tropical and subtropical regions. Despite advances in understanding *Leptospira* biology and pathogenesis, effective disease control continues to be limited by the lack of rapid, early diagnostics, and broadly protective vaccines. This review comprehensively examines recent progress in deciphering *Leptospira*–host interactions, with emphasis on key virulence factors, immune-evasion mechanisms, and host immune responses that influence disease outcomes. Particular focus is placed on the molecular and cellular basis of adhesion, invasion, immune modulation, and persistent colonization. We further discuss the limitations of current vaccines and diagnostic approaches, and highlight how emerging technologies, including pan-genomics, proteomics, reverse vaccinology, immunoinformatics, and omics-based antigen discovery, are facilitating the development of next-generation vaccines and diagnostics. Finally, we outline major translational challenges and future perspectives for improving clinical management, surveillance, and prevention of leptospirosis. The concepts discussed in this review may also provide broader insights into vaccine and diagnostic development for other zoonotic bacterial infections.

LANGUAGE OF ORIGINAL DOCUMENT: English

Wongbutdee J., Jittimanee J., Saengnill W.

Spatial determinants of pathogenic *Leptospira* viability in paddy field surface waters: mechanistic insights from machine learning and geographically weighted logistic regression

(2026) ACS ES and T Water, 6 (9), pp. 5697 - 5709

DOI: 10.1021/acsestwater.6c00208

ABSTRACT: Pathogenic *Leptospira* spp. persist in surface waters, forming environmental reservoirs that sustain leptospirosis transmission in agricultural landscapes. However, the physicochemical controls governing environmental viability and their spatial variability remain poorly resolved. This study integrated controlled laboratory experiments with machine learning and geographically weighted logistic regression (GWLR) to quantify determinants of *Leptospira* viability in paddy field surface waters from northeastern Thailand. Surface water samples (n = 126) were analyzed for pH, calcium, magnesium, and iron, and *Leptospira* viability was experimentally assessed over 20 days. Median viability across days 5–20 was used to classify samples into high- and low-persistence outcomes. Machine learning classifiers achieved moderate discrimination in independent testing (AUC = 0.64–0.70), with a histogram-based gradient boosting machine (HistGBM) providing the strongest performance (AUC = 0.70, 95% CI: 0.493–0.888). SHAP analysis identified pH as the dominant predictor, followed by magnesium and calcium, with iron contributing more weakly. GWLR revealed significant spatial nonstationarity in physicochemical effects and identified a characteristic interaction scale of approximately 2700–3000 m. Together, these results demonstrate that *Leptospira* persistence is governed by spatially heterogeneous water chemistry gradients and highlight the value of integrating interpretable machine learning with spatially adaptive regression for environmental pathogen risk assessment. LANGUAGE OF ORIGINAL DOCUMENT: English

Nukhonthod N., Wirasit I., Bangdee D., Sriden N.

Molecular epidemiology of pathogenic *Leptospira* spp. in Thailand during 2024-2025, with detection of *Leptospira weilii* in human clinical specimens

(2026) PLoS neglected tropical diseases, 20 (9), pp. e0014720

DOI: 10.1371/journal.pntd.0014720

ABSTRACT: BACKGROUND: Leptospirosis is a significant zoonotic disease in Thailand and globally. The causative agents are the spirochete bacteria in the genus *Leptospira*. An updated assessment of molecular epidemiology in Thailand is needed. **METHODOLOGY/PRINCIPAL FINDINGS:** Remnant EDTA blood specimens from suspected leptospirosis cases collected between June 2024 and July 2025 from the Central, Northern, Northeastern, and Southern regions of Thailand, were referred to the National Institute of Health. Demographic data were collected. Confirmed cases were diagnosed using TaqMan real-time PCR targeting LipL32 with a positive threshold of Ct < 37. Partial 16S rDNA (approximately 500 bp) was sequenced using Oxford Nanopore (Oxford, UK) and analyzed by BLASTn. Among 1,063 suspected cases, 34 (3.2%) were real-time PCR confirmed. The majority of the cases were male (58.8%), primarily residing in Nan province (55.9%), and most positives were from specimens collected during the rainy season (76.5%). Twenty-six of 34 sequences (76.5%) clustered with *L. interrogans*, and 8 of 34 (23.5%) clustered with *L. weilii* within the primary pathogenic (P1) clade. Re-examination with published Thai and regional datasets showed broad circulation of *L. interrogans* and a Northern Thailand-Lao People Democratic Republic (PDR) focus for *L. weilii*. To our knowledge, this is the first evidence of *L. weilii* detected in human clinical specimens in Thailand. **CONCLUSION/SIGNIFICANCE:** Pathogenic *L. interrogans* and *L. weilii* caused human Leptospirosis in

Thailand during 2024-2025. These findings update the epidemiological status of *Leptospira* spp. in Thailand and can guide surveillance and control.

LANGUAGE OF ORIGINAL DOCUMENT: English

Yasir A., Tarr G.A.M., Munoz-Zanzi C.

Genomic and ecological systems-thinking framework for pathogenic *Leptospira* in Puerto Rico

(2026) *Frontiers in Public Health*, 14, art. no. 1838089

DOI: 10.3389/fpubh.2026.1838089

ABSTRACT: Introduction – Leptospirosis is a complex zoonotic disease requiring high-resolution surveillance. A systems-thinking framework was used to connect genomic and ecological data and map the geographic and host-based structuring of co-circulating pathogenic *Leptospira* lineages in Puerto Rico. Methods – Forty-four core genomes of *L. interrogans*, *L. borgpetersenii*, and *L. kirschneri* from human, domestic, and wildlife hosts were analyzed. Spatiotemporal and landscape metadata were integrated using root-to-tip regression, isolation-by-distance profiling and calibrated single-nucleotide polymorphism (SNP) thresholds (≤ 1 , ≤ 5 , and ≤ 10 SNPs) to define transmission clusters. Results – *Leptospira* species exhibited distinct ecological pathways partitioned by geography, explaining 56% of genomic variance for *L. interrogans* and 91% for *L. borgpetersenii* (PERMANOVA). *L. interrogans* displayed high landscape connectivity across multiple hosts, forming localized networks (≤ 1 to ≤ 10 SNPs) that capture active spillovers (human-to-rat linkages at ≤ 1 SNP) and resolved into rodent host-specific lineages ($R^2 = 0.34$). Conversely, *L. borgpetersenii* showed spatial and temporal genomic homogeneity and a lack of host-associated structure within an unpartitioned transmission pool dominated by *Mus musculus*. As a result, fixed genomic thresholds yielded disparate outcomes: *L. interrogans* resolved into 4 to 5 discrete, expanding clusters, whereas *L. borgpetersenii* grouped into a single uniform population at the ≤ 10 -SNP threshold. Conclusion – Co-circulating pathogenic leptospires occupy distinct ecological niches shaped by varying host restriction and environmental persistence. Fixed genomic thresholds lack universal applicability; effective genomic epidemiological surveillance must employ species-specific threshold calibration to accurately map transmission pathways.

LANGUAGE OF ORIGINAL DOCUMENT: English

Mulyani G.T., Primatika R.A., Widiastih D.A.

AUTHOR FULL NAMES: Mulyani, Guntari Titik (57189466186); Primatika, Roza Azizah (57190940555); Widiastih, Dyah Ayu (6506266977)

57189466186; 57190940555; 6506266977

Serological prevalence, risk factors and spatial distribution of feline *Leptospira* exposure in Sleman, Indonesia

(2026) *International Journal of Veterinary Science*, 15 (4), pp. 1076 - 1081

DOI: 10.47278/journal.ijvs/2026.051

ABSTRACT: Leptospirosis is a widespread zoonosis caused by pathogenic *Leptospira* spp. and is typically acquired after contact with environments contaminated by urine from infected animals; the burden is especially high in tropical countries such as Indonesia. Information on feline leptospirosis and its epidemiological drivers in densely populated tropical settings remains scarce. This cross-sectional study estimated seroprevalence, examined associated risk factors, and assessed the spatial distribution of feline leptospirosis in Sleman District, Special Region of Yogyakarta, Indonesia. A total of 116 pubertal cats underwent clinical examination and blood sampling. Sera were tested by the Microscopic Agglutination Test (MAT) using a cutoff titer of 1:100 against

14 *Leptospira* serovars. Seroprevalence was calculated from MAT-positive results, while questionnaire-derived variables were evaluated using chi-square testing and odds ratio analysis. Geographic patterns of seropositive cases were explored using GIS-based mapping. Overall, 12/116 cats (10.34 %) were seropositive, with serovar Bataviae as the predominant reaction. Seropositivity was associated with several factors, including residence in urban/rural, presence of other animals, ownership of multiple cats, and kidney disease. Given the relatively small sample size, these findings should be interpreted with caution, but they suggest potential environmental and clinical correlates of exposure. These findings demonstrate feline exposure to pathogenic *Leptospira* in Sleman District and indicate that environmental setting and renal health are important correlates of infection risk. Notably, this work integrates serological, spatial, and clinical determinants to provide new epidemiological evidence relevant to One Health surveillance in an urban tropical endemic area. LANGUAGE OF ORIGINAL DOCUMENT: English

Wong Z., Teh A.-H., Sanada E., Watanabe N., Ong E.B.B.

Molecular characterisation of a putative virulence factor LIC10280 from *Leptospira interrogans* in the budding yeast *Saccharomyces cerevisiae*

(2026) Microbial Pathogenesis, 220, art. no. 108826

DOI: 10.1016/j.micpath.2026.108826

ABSTRACT: Leptospirosis, a widespread zoonotic disease caused by *Leptospira interrogans*, poses a major public health challenge, particularly in tropical regions. Despite progress in genomic studies, many putative virulence factors (VFs) remain uncharacterised. This study focuses on LIC10280, a conserved hypothetical protein identified as a potential VF through yeast-based screening. In silico analysis predicted that LIC10280 possesses a cleavable signal peptide and a β -barrel fold in its mature region, features common to secreted bacterial proteins. BlastP analysis showed that LIC10280 is conserved in pathogenic *Leptospira* but absent in saprophytic species, suggesting a role in pathogenesis. Growth assay in budding yeast, *Saccharomyces cerevisiae* revealed that only the full-length LIC10280 inhibited yeast growth and reduced cell viability as confirmed in FUN-1 staining. Fluorescence microscopy study revealed that mature part of LIC10280 was involved in localisation to yeast nucleus without vesicular trafficking. A yeast genetic screen showed that deletion of SCH9, a component of the TORC1 signalling pathway, partially suppressed LIC10280-induced toxicity, implicating a possible link to nutrient-sensing or stress-response pathways. Together, these findings provide new insights into the potential function of LIC10280 and highlight the value of yeast as a model system for studying putative virulence factors of *L. interrogans*.

LANGUAGE OF ORIGINAL DOCUMENT: English

Ullah N., Pimpunchat B., Shah K., Abdeljawad T.

Numerical and bifurcation analysis with sensitivity assessment of a deterministic leptospirosis hantavirus co-infection model

(2027) Journal of Applied Analysis and Computation, 17 (1), pp. 432 - 452

DOI: 10.11948/20260054

ABSTRACT: In this paper, a deterministic compartmental model is developed in order to study the dynamics of the transmission of leptospirosis and hantavirus co-infection with particular emphasis on the human population. The model includes compartments of susceptible individuals, singly infected classes, co-infected individuals, and recovered individuals, as well as important pathogen exposure and disease progression

mechanisms. Analytical features of the proposed model are addressed, such as positivity, boundedness, the existence of equilibrium points, and bifurcation analysis. The next-generation matrix approach is used to obtain the dominant transmission threshold, denoted by, R_{c0} which determine the qualitative behavior of the system. The transmission-free equilibrium of the model is locally asymptotically stable when $R_{c0} < 1$ and unstable when $R_{c0} > 1$, indicating disease persistence in the population. In addition, bifurcation analysis is carried out to examine the system behavior near the critical threshold. The analysis confirm the occurrence of a backward transcritical bifurcation. The validity of the analytical results is confirmed through numerical simulations. In particular, the roles of transmission rates, recovery rates, and co-infection dynamics are studied in detail. Sensitivity analysis shows that parameters associated with infection transmission and recovery have the strongest influence on disease burden, with co-infection outcomes being especially responsive to changes in recovery rates. The results indicate that improving treatment efficiency and limiting transmission pathways can reduce both single infections and co-infection occurrences. Overall, this study highlights the importance of targeted control strategies for leptospirosis hantavirus co-infection and emphasizes the need for effective intervention measures to reduce public health risks.

LANGUAGE OF ORIGINAL DOCUMENT: English

Xiao J., Wu Y., Ding Z.

Case Report: Isolated diffuse alveolar haemorrhage as the sole severe manifestation of atypical leptospirosis without jaundice or acute kidney injury and the diagnostic value of BALF metagenomic next-generation sequencing

(2026) *Frontiers in Medicine*, 13

DOI: 10.3389/fmed.2026.1906337

ABSTRACT: Background – Leptospirosis is a global zoonotic infection. Severe leptospirosis typically manifests as Weil's syndrome with jaundice and acute kidney injury, while isolated diffuse alveolar hemorrhage (DAH) without jaundice or acute kidney injury (the classic features of Weil's syndrome) is rare and easily misdiagnosed. Traditional diagnostic methods have notable limitations in the acute phase of infection, and the diagnostic value of metagenomic next-generation sequencing (mNGS) for atypical leptospirosis remains insufficiently characterized. This report highlights the clinical novelty of this rare phenotype and the diagnostic value of mNGS. Case presentation – A 60-year-old male farmer was admitted with 6 days of fever and 2 days of progressive chest tightness, with a history of contaminated water exposure via broken skin before symptom onset. Initial chest computed tomography showed bilateral pulmonary infiltrates, and he was misdiagnosed with severe community-acquired pneumonia and acute respiratory failure. Despite empiric broad-spectrum anti-infective therapy, his condition progressed rapidly, requiring invasive mechanical ventilation. Bronchoalveolar lavage fluid mNGS identified *Leptospira* spp., and the diagnosis was revised to leptospirosis with isolated DAH. After targeted anti-infective therapy combined with methylprednisolone pulse therapy, his respiratory function improved rapidly. He was weaned from mechanical ventilation on day 7 of admission, discharged with complete symptom resolution on day 19, and had no sequelae at 25-day follow-up. Conclusion – This case highlights that leptospirosis can present with isolated DAH as the sole severe manifestation without classic hepatorenal impairment, requiring high clinical suspicion in endemic areas. Bronchoalveolar lavage fluid mNGS may be a valuable tool for rapid diagnosis of atypical leptospirosis, which may reduce misdiagnosis and contribute to improved clinical outcomes.

LANGUAGE OF ORIGINAL DOCUMENT: English

Li M., Huang R., Liao Y., Cai Q., Li J., Wang J., You J.

Investigation on distribution and pathogen carriage of small mammals in Ganzhou City, Jiangxi Province from 2022 to 2024

(2026) China Tropical Medicine, 26 (7), pp. 1007 - 1012

DOI: 10.13604/j.cnki.46-1064/r.2025-1425

ABSTRACT: Objective To investigate the distribution of small mammals and the prevalence of Hantavirus, pathogenic *Leptospira interrogans*, *Orientia tsutsugamushi*, and Dabie bandavirus in small mammals in Ganzhou City, Jiangxi Province, from 2022 to 2024, providing a scientific basis for risk assessment and early warning of local zoonotic diseases. Methods Small mammals were captured using the trap-night and cage-night method with fried dough sticks or peanuts as bait in various habitats, including urban and rural residential areas, farmland, shrublands, and key industrial zones (such as catering, food production and sales, construction sites, slaughterhouses, and breweries) in Yudu County, Shangyou County, and Longnan City of Ganzhou City. Liver, spleen, kidney, and lung tissues were aseptically collected, and DNA and RNA were extracted separately. TaqMan probe real-time fluorescent quantitative PCR was used to detect four pathogens: Hantavirus, *Leptospira interrogans*, *Orientia tsutsugamushi*, and Dabie bandavirus. Differences in pathogen detection rates among different small mammals and habitats were compared. Results From 2022 to 2024, a total of 12 086 effective snap traps and cage traps were deployed, and 640 small mammals of 7 species were captured, with an average capture rate of 5.30%. The average capture rate was 5.65% (225/3 983) in Yudu County, 3.97% (203/5 117) in Shangyou County, and 7.10% (212/2 986) in Longnan City, showing a statistically significant difference among different regions ($\chi^2=27.99$, $P<0.05$). *Rattus norvegicus* was the dominant species, accounting for 42.66% (273/640) of the total captures. Hantavirus, Pathogenic *Leptospira interrogans* and *Orientia tsutsugamushi* were detected in the captured small mammals, while Dabie bandavirus was not detected. Hantavirus was detected in *Rattus norvegicus* and *Mus musculus*, with a positive rate of 2.03% (13/640); pathogenic *Leptospira interrogans* was detected in *Rattus norvegicus*, *Rattus flavipectus*, *Mus musculus*, *Rattus losea*, *Apodemus agrarius*, and *Suncus murinus*, with a positive rate of 15.31% (98/640); *Orientia tsutsugamushi* was detected in *Rattus norvegicus*, *Mus musculus*, *Rattus flavipectus*, and *Apodemus agrarius*, with a positive rate of 1.56% (10/640). The differences in detection rates among different pathogens were statistically significant ($\chi^2 =133.36$, $P<0.05$). Pathogenic *Leptospira interrogans* was detected in urban residential areas, rural residential areas, farmland, shrublands, and key industries. The detection rate was highest in urban residential areas at 22.71% (67/295), which was significantly different from other habitats ($\chi^2 =32.26$, $P<0.05$). Conclusion Hantavirus, pathogenic *Leptospira interrogans* and *Orientia tsutsugamushi* were detected in small mammals in Yudu County, Shangyou County, and Longnan City of Ganzhou City, which are potentially pathogenic to humans. Local government should strengthen the monitoring of zoonotic diseases and formulate corresponding prevention and control measures.

LANGUAGE OF ORIGINAL DOCUMENT: Chinese

Joseph G., Kulavalli S., B  lard S., Saravu K.

Leptospirosis and its co-infections in India: prevalence, geographic distribution, and clinical implications

(2026) BMC Infectious Diseases, 26 (1), art. no. 1653

DOI: 10.1186/s12879-026-13642-7

ABSTRACT: Background: Leptospirosis is a zoonotic disease prevalent in low and middle-income countries and tropical regions. It can be mimicked by endemic infectious diseases that present simultaneously, like dengue, malaria, melioidosis, or scrub typhus. However, there is a dearth of documented evidence about the frequency of coinfection, as well as related symptoms and mortality. The study aims to determine the frequency and geographical extent of coinfections in leptospirosis patients in India. Methodology: We systematically searched databases, including Scopus, PubMed, Embase, and Web of Science, for articles published between January 2010 and August 2024 assessing coinfections in leptospirosis patients in India. The eligible articles were evaluated for full text data extraction using a standardized methodology. Results: Of 804 articles screened 39 studies were included; overall these reported on 1565 leptospirosis cases with 236 (15.1%) cases with coinfections. Of these, seven observational studies assessed coinfections in 1318 leptospirosis-positive cases, identifying 113 (8.6%) coinfections: most commonly dengue (79; 69.9%), followed by hepatitis E (15; 13.2%), typhoid (9; 7.9%), and malaria (9; 7.9%). The most coinfection cases were reported from New Delhi (29.6%), followed by Kerala (21.6%), Karnataka (14.4%), Uttar Pradesh (12.2%), and Tamil Nadu (5.5%). Common symptoms included fever, vomiting, icterus, headache, thrombocytopenia, and transaminitis. Cases with coinfection had poorer outcomes with increased mortality compared to cases with isolated infection. Conclusion: In India, leptospirosis can present with co-infections such as dengue, hepatitis E, malaria, typhoid, and scrub typhus, complicating diagnosis due to overlapping clinical features. These coinfections are associated with unfavorable outcomes, including multi-organ dysfunction and increased mortality. Early diagnostic testing in febrile patients and timely, appropriate treatment are essential to improve patient outcomes. Trial registration: Clinical trial number: not applicable. Review protocol registration (Open Science Framework): DOI <https://doi.org/10.17605/OSF.IO/AS9W5>.

LANGUAGE OF ORIGINAL DOCUMENT: English