Choudhary et al | Article 8984
J Pure Appl Microbiol. 2023;17(4):1968-1977. doi: 10.22207/JPAM.17.4.44
Received: 09 September 2023 | Accepted: 14 October 2023
Published Online: 30 November 2023

P-ISSN: 0973-7510; E-ISSN: 2581-690X

REVIEW ARTICLE OPEN ACCESS

Epidemiological Status of Leptospirosis in India

Shanti Choudhary[1], Ratan Kumar Choudhary[1], Manish Kumar[2], Satparkash Singh[1] and Yashpal Singh Malik[1]*

[1]College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.
[2]Department of Bioscience and Bioengineering, Indian Institute of Technology, Guwahati, Assam, India.

Abstract

A corkscrew-shaped spirochaete named *Leptospira interrogans* causes the infectious disease called leptospirosis. Leptospirosis, a growing public health concern worldwide, from subclinical infections to potentially lethal pulmonary hemorrhage. The disease caused by the pathogenic Leptospira, poses a threat to both humans and animals, and its transmission occurs through contact with infected animals, contaminated water, and soil. The prevalence of leptospirosis is influenced by various factors, including climate, urban development, and animal-rearing practices. It can manifest with severe symptoms in humans, making early diagnosis crucial. Diagnostic methods like microscopic agglutination test (MAT) and enzyme-linked immunoassay (ELISA) are widely used for the screening of leptospira infection. Molecular technique like PCR and qPCR offering higher sensitivity and rapidity. This paper meta-analyses the incidence of leptospirosis in various animals based on the literature published from 2005 to 2023 and provides prevalence of the disease in various animal including humans. Results suggest the significance prevalence of the disease in humans and various animal species, namely buffalo, rodents, and dogs. Coastal regions in India were particularly vulnerable to the disease. Efforts to control leptospirosis include surveillance programs and public health initiatives. Understanding the epidemiology and prevalence of leptospirosis, as highlighted in this paper, is essential for implementing effective preventive measures. Finally, a continued research, diagnostic advancements, public awareness campaigns and addressing research gaps in epidemiology of the disease are critical in mitigating the impact on human and animal health. This review provides important data for public health authorities, veterinarians, scientists and for the public, in general.

Keywords: Leptospirosis, Risk Factor, Prevalence, Diagnosis, Zoonotic Disease

***Correspondence:** malikyps@gmail.com

Citation: Choudhary S, Choudhary RK, Kumar M, Singh S, Malik YS. Epidemiological status of Leptospirosis in India. *J Pure Appl Microbiol.* 2023;17(4):1968-1977. doi: 10.22207/JPAM.17.4.44

INTRODUCTION

Leptospirosis is becoming a significant concern for public health, causing subclinical infections to lethal pulmonary hemorrhage.[1] The disease is widely distributed and occurs both in developed and developing countries in suitable habitat where the conditions are suitable for the growth of causative organisms.[2] Incidences of the presence of pathogenic leptospires have been reported in a variety of domestic or pet animals, such as dogs, cattle, horses, rats, and pigs[3-6] and also in several wildlife animals including coyotes, sea lions, raccoons, opossums, bats, white-tailed deer and surprisingly in frogs and caimans.[7,8] Rats, canines, buffaloes, and cattle are the hosts for leptospirosis.[9]

Humans or animals are infected accidentally by direct or indirect contact with leptospira infected animals or contaminated water or urine.[10,11] Infection is also possible by other methods, like handling clinically infected animal tissues, inhalation of droplets of urine,[12] and consuming contaminated food and water. Rodents, especially the common rat (*Rattus norvegicus*) is the most common host. Certain serovars are linked with particular animal hosts e.g., Icterohemorrhagiae with rats, canicola in dogs, and Hardjo in cattle.[13,14]

In humans, the early stages of leptospirosis are characterized by high fever, headaches, jaundice, and mucosal hemorrhages.[15] Other essential factors responsible for the rise in cases are natural calamities like heavy rainfall, unhygienic cattle-rearing practices, and unplanned development of cities.[10,16] Pathogenic strains of leptospires can survive in soil and water, indicating their survivability outside the host.[17,18] For the survival of the leptospires, moisture is an essential key factor in the environment.[10] Recent estimates show the annual incidence of leptospirosis worldwide is around one million cases and contributes to around 60,000 deaths.[19,20] MAT and ELISA are widely used tests, of which the microscopic agglutination test (MAT) is considered the most authentic.[21] For the clinical detection of leptospirosis, molecular diagnostic methods are more popular as they are more sensitive and rapid than other methods, such as culture.[22] Also, these techniques are less time-consuming and are more accurate than the conventional serological test, making the detection of organisms right in the samples in a short time.[9] Among molecular biology techniques, real-time polymerase chain reaction (qPCR) is advantageous. It is more sensitive in diagnosing various infectious diseases compared to conventional PCR assays.

Etiology

The causative agent of leptospirosis is the spirochaete, which belongs to the order *Leptospirales*, family *Leptospiraceae,* and genus *Leptospira* containing 82 species (https://lpsn.dsmz.de/search?word=Leptospira). Based on their pathogenicity, the genus is divided into pathogenic and non-pathogenic strains. The differentiation of various serovars is due to the difference in agglutination reaction in MAT. Furthermore, 60 serovars for *L. biflexa* and above 300 serovars for *L. interrogan*s have been reported Routray *et al.*[23]

Leptospires are aerobic and motile, and they can sustain alkaline pH but cannot survive in the hot and dry environment. The bacteria are Gram-negative. In addition to Gram staining, silver impregnation staining and immunofluorescence are used, followed by visualization using a dark field microscope. Ellinghausen-McCullough-Johnson-Harris (EMJH) medium is the most popular medium for the culture of *Leptospira*.

As the pathogenic and non-pathogenic strains cannot be differentiated based on their morphology, a few tests, like testing *in vivo* pathogenicity and detection of outer membrane proteins, are done. Outer membrane proteins and other virulence factors have been characterized well in the past.[24-28]

Epidemiology

Leptospirosis outbreaks have been reported globally. Human infections are rampant in tropical regions with high rainfall, where the human population gets exposed to water contaminated with the urine of infected animals.[29] People who live in under developed nations with poor sanitation have a significant risk of contracting an infection through polluted water or soil. Leptospirosis is a serious issue in low-lying, densely inhabited parts of India that experience

frequent flooding or water logging during the monsoon season. Farmers, sewage workers, butchers, and other occupational risk groups are most vulnerable to getting the illness. Swimming, sailing, and other water sports are among the leisure activities that are thought to increase the risk of contracting leptospirosis.

A recent meta-analysis in humans found that exposure to rain was a significant risk factor for leptospirosis and that the majority of cases were reported from the coastal zone and among farmers. 97% of patients had fever, and 35% of them had conjunctival suffusion (red conjunctiva). In 34% and 35% of the patients, the liver and kidney, respectively, were affected. The combined mortality from multiple studies was estimated to be 11%.[30]

Research gaps in epidemiology

In India, several research gaps exists that hampers understanding of epidemiology of leptospirosis in human and animals. Some of the key research gaps are;

- Zoonotic disease servillance: Limited servillance and data collection on zoonotic diseases that are infection of human and animals and vice versa.
- Antimicrobial resistance (AMR): Understanding of transmission dynamics of AMR in leptospira in human and animals are limited.
- Emerging infectious disease: Pathway of spillover from animal to human and factors that facilitate the events are poorly understood.
- Data Accessibility: Lack of data sharing facility and transpiracy hinders collaborative research and policy formulations.
- One health approach: Insufficient research on One Healthy Approach due to insufficient interdisciplinary research programs and efforts.

Addressing these research gaps in the epidemiology of diseases in India is essential for developing evidence-based strategies for disease prevention, control, and mitigation of leptospirosis in human and animals.

Clinical Manifestations

The symptoms of leptospirosis include fever, renal and hepatic insufficiency, pulmonary signs, and reproductive failure across human beings and domestic animals, primarily dogs, cattle, and pigs. Fever, jaundice, vomiting, diarrhea, intravascular disseminated coagulation, uremia followed by renal failure, hemorrhages, and death are possible symptoms of typical canine leptospirosis. Leptospirosis symptoms in cattle and pigs include reproductive failures, such as abortion, fetal mummification, weak new born, and agalactia.[31] Humans may experience symptoms ranging from a mild, influenza-like illness to a severe infection with respiratory distress and renal and hepatic failure (Weil's disease).

Risk factors and transmission

The cases of leptospirosis are higher in the tropical regions than in the temperate parts because leptospires can survive longer in warm and humid conditions. Countries where there are chances of exposure of the human population to infected animals have greater chances of infection in humans. The host range of *Leptospira* spp is diverse because of its capability to infect and survive in different hosts. It occurs in a wide variety of wild, domestic, and marine animals. Rodents are a primary reservoir host, while other animals like cattle, pigs, and dogs may also act as carriers and sources of infection to other animals or humans.[32] Controlling rat infestation on farms and fencing to keep stray dogs out of the farm can be effective prevention strategies.[33] In humans and animals, infections can occur by coming in contact with infected water and soil.[34,35]

Transmission of leptospirosis is either by direct contact with an infected animal or by indirect contact with soil or water contaminated with the urine of infected animals.[17] Human-to-human transmission is rarely reported. Infected urine is the source of infection in humans and animals coming in contact. In humans, the risk factors associated with leptospirosis are mainly occupational groups, i.e., farmers, abattoir workers, veterinarians, rice field workers, and animal handlers, or recreational activities like swimming and hunting. Hence, it is also known as paddy field worker disease, mud fever, or sewer worker disease. For animals, the important risk factors include shared grazing with common water resources, purchase or introduction of infected

cattle, rodents in the farm, level of hygiene in milking and status of leptospiral vaccination, presence of other animals in the farms like dogs, sheep and goats, horse, pigs, and others (Figure 1). Leptospirosis can also be transmitted through the semen of infected animals.[36]

METHODOLOGY

Data source and study selection

The published data on leptospirosis was retrived from all animals (including dogs, cows, buffalo, pigs, and goats) reported from India using PubMed and Google search engines. Terms like "leptospira", 'leptospirosis" and "dogs" or "cows", or "buffaloes", or "pigs" or "goats" were searched. We included data from 2005 to 2023, along with a list of authors, year of publication, geographic location of Indian states, sample type, sample size, methods of detection, and prevalence (any kind, including culture/sero/molecular/others) of *Leptospira* spp. During the initial screening of titles and abstracts, criteria were determined to include based on their suitability. Inclusion criteria for detail analysis was defined for each species. Otherwise, review articles, metanalysis, and case reports were excluded from this analysis. The full-text documents were assessed, and data were retrieved from the table or the abstract. Publications in other languages were excluded from this study. Microsoft Excel using was used with "Data → Data Analysis" tool functions for the data analysis.

RESULTS

A meta-analysis of published data from India included in this study suggested the highest

Table. Prevalence (%) of species-wise livestock leptospirosis in India (total data of 2005- 2023)

Species	Sum of # of samples	Sum of Favorable	Prevalence %
Buffalo	1176	538	45.75
Cow	8398	2038	24.26
Dog	1039	271	26.08
Goat	2900	563	19.42
Human	4394	416	9.47
Pig	177	28	15.80
Rodents	60	24	40.00

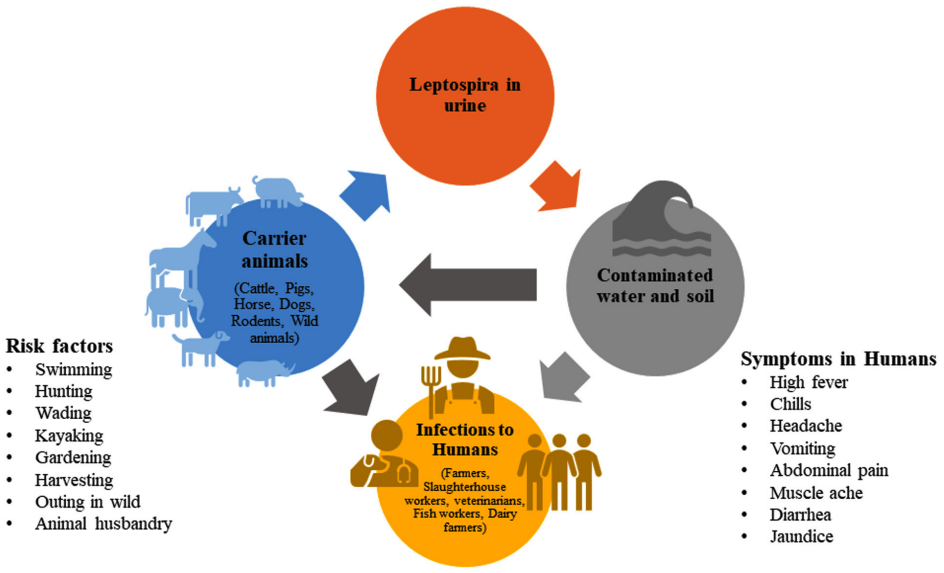

Figure 1. A flow diagram of transmission of *Leptospira* and organisms in humans and animals showing the zoonotic importance of the disease. Bacteria are shed into the water and soil from the mammalian reservoir host from where humans and animals (cattle, dogs, pigs, horses, and others) pick infection, and further shed organisms in the urine. Pathogen-containing urine further contaminates water and soil. Leptospirosis is considered an occupational health hazard because the farmers, personnel working in the slaughterhouse, veterinarians and other workers coming in close proximity to infected animals are prone to get the disease.

prevalence of leptospirosis in buffalo (45.75%), followed by rodents (40%), dogs (26%), cows (24.26%), goats (19.42%), and pigs (15.8%) (Table). Apart from the analysis of retrieved data, some of the recent reports are mentioned below.

A study was conducted using animal samples from several districts in the south Gujarat region that were tested for leptospirosis. A total of 151 (or around 11%) of the 1406 animal samplesof goats, cows, buffalo, and bullocks tested positive. In cows, buffaloes, bullocks, and goats, the most prevalent serovars were Icterohemorrhagiae (22%), Patoc (58%), Hardjo (50%), and Autumonalis (50%), respectively.[37]

A recent study was conducted on a total of 300 cow samples from cases of mastitis, miscarriage, and recurrent breeding from both organized and unorganized farms in Jabalpur to check for leptospirosis. According to the findings, leptospirosis seroprevalence among suspected cases was 48.67%, and it was notably higher in the organized sector and with high seropositivity in older cattle.[38]

In another recent study conducted on canines in Gujarat, 45 of 410 sera were found to be positive, showing a seroprevalence of 10.98%, with serovarPyrogenes being the predominate serotype. According to MAT, ImmunoComb, and PCR, the total prevalence of leptospirosis was 16.59%.[39]

On the basis of our meta-analysis, the prevalence of leptospirosis was found to be

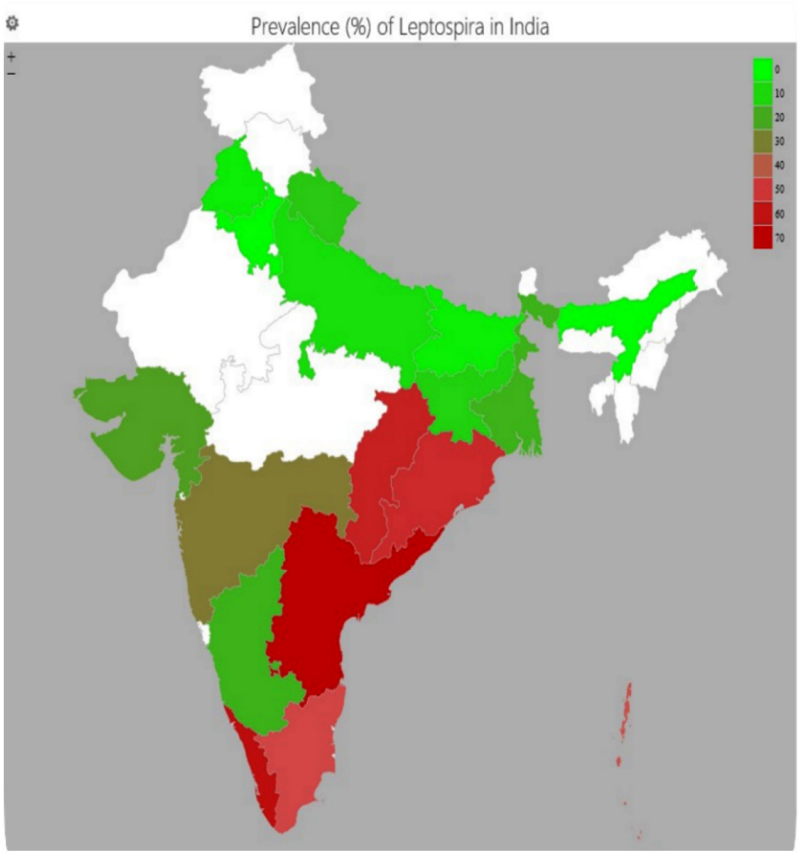

Figure 2. State wise prevalence of leptospirosis in India evidenced by the data published from 2005-2023. Data analysis showed that coastal states of India like Andhra Pradesh (64.7%), Kerala (60.6%), Orissa (52.3%), Tamil Nadu (45%), Andaman Nicobar (42.15%) have a high prevalence of leptospirosis. High to the low prevalence of the disease is indicated by the red to green spectrum of color. White color indicates insufficient or no data available for the state.

highest in Andhra Pradesh (64.71%), followed by Kerala (60.5%), Chhatisgarh (54.7%), and Odisha (52.26%). The lowest prevalence of the disease was found in Assam (0.9%) and Haryana (1.4 %) (Figure 2).

The prevalence % of leptospirosis in humans is 9.47% as seen in Table. This disease is most prevalent in coastal areas. The reported incidence of human leptospirosis state-wise in India is described below.

Kerala

Leptospirosis is common and endemic in most parts of Kerala. The Autumnalis, Australis and Icterohemorrhagiae, Canicola, Pomona, Shermani are the common serogroups. A study found that leptospirosis is also occupation-related, as it is common in oyster shell catchers (82%). There was an increase of 74 % in the monsoon and a sharp rise in cases in some areas where the disease was not found earlier, such as Kolenchery, a midland, and leptospirosis was rare before 1987.[23,40] A post-flood outbreak of human leptospirosis in Kerala claimed about 70 lives in 2018.[41]

Andaman and Nicobar Islands

Andaman and Nicobar Islands have reported leptospirosis since the early part of the 20thcentury and are endemic for leptospirosis. There have been reports of outbreaks of Andaman hemorrhagic fever (AHF) since 1988. The disease surveillance system recorded 544 cases and 93 deaths in the Andamans between 2000 and 2004, with 2002 having the highest incidence.[42] Five hundred twenty-four cases of AHF were reported from 1988-97.[23,43,44]

Maharashtra

Leptospirosis in Maharashtra has been regularly reported Karande *et al*.[45] and Karande *et al*.[46] In 2005, because of the large outbreak during monsoon season and floods, 2355 cases

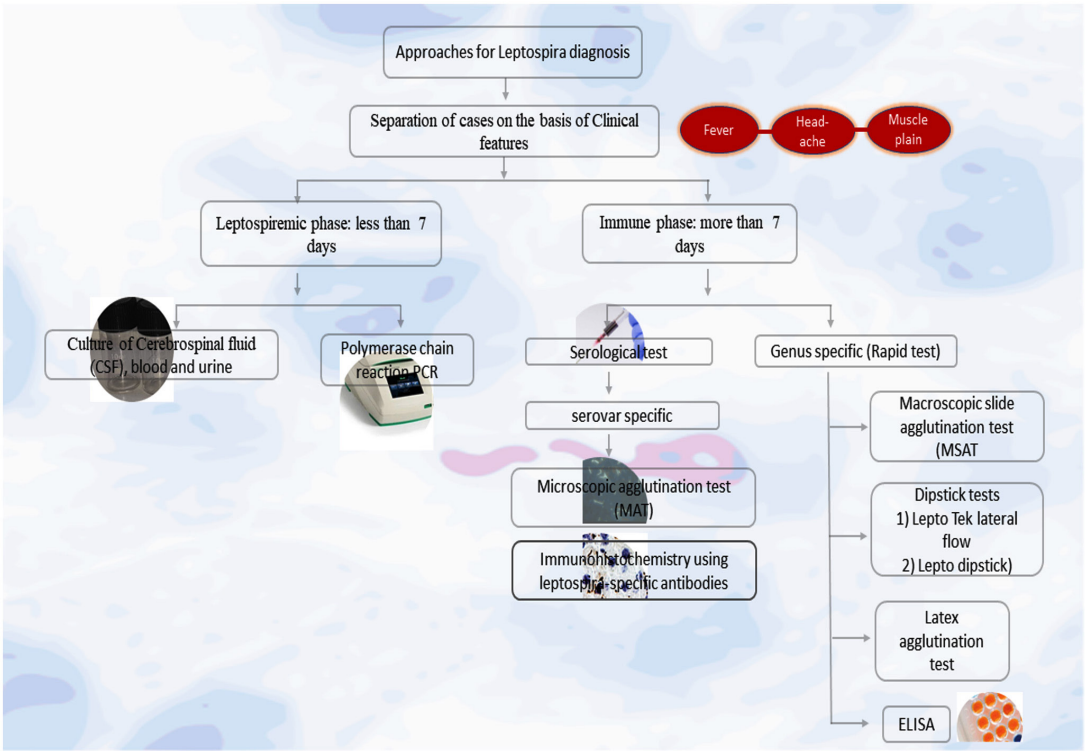

Figure 3. Various approaches to detect leptospires in the blood and urine of humans and animals. PCR is a rapid test for the detection of the nucleic acid of leptospires in blood and urine. In contrast, MAT is the standard gold method for the detection of antibodies in the serum. Isolation and identification of *Leptospira* species are done by the culture of samples (blood, urine, CSF).

and 167 deaths were reported. In Mumbai, during the course of ten days in July 2015, the Mumbai Corporation reported 15 leptospirosis fatalities after massive floods.[47]

Gujarat

The disease is endemic in Gujarat, with the highest number of cases in Valsad, Navsari, and Surat since 1994.[48,49] Situations such as heavy rainfall, high water table, and clay soil in south Gujarat favor endemicity for Leptospirosis.[42]

Tamil Nadu

There have been reports of leptospirosis from Chennai since 1980s.[50,51] To study leptospirosis, a laboratory at the Madras Medical College was established in 1994.[42] In a study,a total of 2035 patient serum samples were tested for the presence of specific IgM antibodies using the Panbio Leptospira IgM ELISA kit to determine the seroprevalence of leptospirosis over a 10-year period from 2011 to 2021 in Chengalpattu district, Tamil Nadu, which revealed aprevalence rate of 9.14%.[52]

Karnataka

In Karnataka, 152 cases and 11 fatalities were reported in 2004 while 224 cases and 19 fatalities were reported in 2005.[42] In another study from the state, leptospirosis was shown to be substantially connected with environmental factors, proximity to an open sewer, occupational factors, the existence of skin cuts, direct contact with mud while working, and the presence of rats or rodent habitats, according to a population-based case-control study in the Kodagu district.[53]

Odisha

In Odisha, 142 patients with febrile sickness and hemorrhagic symptoms were assessed during October and November 1999, following the cyclone. Incidences of disease shoot-up during the rainy season. When tested, 28 (19.2%) were found positive for leptospirosis by MAT and 6 were positive by PCR/culture.[54]

Other States

Data from other Indian states is reviewed as follows. The evaluation of acute febrile patients in Uttar Pradesh revealed that 7% had leptospirosis (25/ 346), and 17 of the 25 patients had jaundice.[55] Similarly, in a study of 55 cases of leptospirosis in Hyderabad, 52% had renal failure, and 42% had jaundice. Apart from these, leptospirosis has also been reported in Rajasthan, Uttarakhand, Punjab, and Haryana.

Govt of India launched a pilot project on prevention and control of leptospirosis under the 11th five-year plan, which in the next five-year plan launched a scheme called "Prevention and Control of Leptospirosis."In 2019, the National Center for Disease Control (NCDC) issued an advisory notice and preparatory checklist to control leptospirosis, especially during the outbreak in affected districts (https://ncdc.gov.in/showfile.php?lid=390).

Diagnosis of leptospirosis

Laboratory support is required for the diagnosis to find the serovar responsible for causing specific infection, a possible source of infection, location, and potential reservoir. The tests performed for leptospirosis are isolated by culture and PCR, while serological tests can be used for the detection of antibodies. For the confirmation of diagnosis, isolation of bacteria is the most definite way. However, blood culture takes much time and is not suitable for an early diagnosis, but it is considered valuable in patients who are at risk of losing life in the first week before developing antibodies. An advanced technique like PCR is promising, and it is sensitive and specific.[56,57]

The serological microscopic agglutination test (MAT) is the gold standard for the diagnosis. The main advantage of MAT is identifying serovar of epidemiological importance.[58] Leptospires are cultured, and an agglutination reaction is carried out in laboratories to detect the antibodies. MAT is laborious and is not an economical method, and needs live bacterial antigen for the test. Because of these factors, MAT is conducted only in particularly designated labs.[59] Various approaches to detect leptospirosis are presented in Figure 3.

Leptospirosis research at IIT Guwahati

Molecular characterization for a comprehensive understanding of the CRISPR/ Cas system of *Leptospira* is being carried out at the Indian Institute of Technology, Guwahati. The long-term goal of the lab is to utilize the outer membrane proteins (OMPs) of the spirochete

to develop a rapid diagnostic assay and vaccine to control the loss incurred by the disease. The overall goal is to elucidate the role of Cas proteins (Cas 6/7/8) in CRISPR adaptive immunity of the spirochetes and develop a CRISPR-Cas, an emerging microbial adaptive immune defense system of microbes (including spirochetes) against the foreign genetic elements that were being transferred horizontally from viruses or plasmid. Recent studies (from the lab of Manish Kumar, the 3rd author) include transcriptional analysis of CRISPR 1-B array of *L. interrogans*,[60] assembly of Cas7 subunit of *Leptospira*,[61] and Cas2 protein of subtype I-C.[62]

Leptospirosis research at the vet versity

The Guru Angad Dev Veterinary and Animal Sciences University started working on leptospirosis in 2018, collaborating with IIT Guwahati, a joint collaborative project funded by the Department of Biotechnology- North East Region (DBT-NER).

In 2018, DBT launched a DBT-Canine Research Center and Network, and univeristy is a collaborating institution of this mega project. The overall goal of this mega project was focused on the development of canine diagnostics and vaccines, under which a lab on leptospirosis has been established at the College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University. Under this project, a dedicated Bio-safety lab for diagnosing leptospirosis has been set up for serological diagnosis. Detection of anti-leptospiral antibodies using the Microscopic agglutination test (MAT) and molecular detection by PCR is carried out, in which about 10 percent seropositivity was found in animals using microscopic agglutination test.[63] The samples from various parts of the state are coming for the diagnosis of leptospirosis.

Only a few referral labs maintain the leptospiral serovars because there is no long-term preservation method, and it has to be continuously sub-cultured every week. There is a high risk of contamination and loss of culture. We maintain reference cultures obtained from ICAR-NIVEDI and seven reference serovars that are commonly prevalent in domestic species in India, i.e., Australis, Autumnalis, Canicola, Hardjo, Icterohaemorrhagiae, Pomona, and Pyrogenes are being maintained. These reference cultures are used as antigen panels in the MAT and positive control in the molecular detection of leptospires in samples by PCR.

CONCLUSION AND FUTURE PROSPECTIVE

Due to the wide distribution of the disease in domestic and wild animals, prevention and control of leptospirosis are difficult. Control of leptospirosis includes identification of infected and carrier animals, immunization through vaccination, suitable drainage systems, and disinfection of the contaminated source of water. In the future, the adoption of prevention strategies, immunization of susceptible populations using current or next-generation vaccines, and timely reporting of outbreaks could mitigate disease incidences.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This study was supported by the Department of Biotechnology, New Delhi, India, (Ref no. No. BT/PR25083/NER/95/1002/2017) awarded to MK & RKC and the establishment of the Leptospira lab under the Canine Research Network Project at GADVASU, Ludhiana, India.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Rao RS, Gupta N, Bhalla P, Agarwal SK. Leptospirosis in India and the rest of the world. *Braz J Infect*

Dis. 2003;7(3):178-193. doi: 10.1590/S1413-86702003000300003

2. Narita M, Fujitani S, Haake DA, Paterson DL. Leptospirosis after recreational exposure to water in the Yaeyama Islands, Japan. *Am J Trop Med Hyg.* 2005;73(4):652-656. doi: 10.4269/ajtmh.2005.73.652
3. Barragan V, Nieto N, Keim P, Pearson T. Meta-analysis to estimate the load of Leptospira excreted in urine: beyond rats as important sources of transmission in low-income rural communities. *BMC Research Notes*. 2017;10(1):71. doi: 10.1186/s13104-017-2384-4
4. Goldstein RE. Canine Leptospirosis. *Vet Clin North Am Small Anim Pract*. 2010;40(6):1091-101. doi: 10.1016/j.cvsm.2010.07.008
5. Costa F, Wunder E A, De Oliveira D, et al. Patterns in Leptospira shedding in Norway rats (*Rattus norvegicus*) from Brazilian slum communities at high risk of disease transmission. *PLOS Negl Trop Dis*. 2015;9(6):e0003819. doi: 10.1371/journal.pntd.0003819
6. Khurana SK, Dhama K, Minakshi P, Gulati B, Malik YS, Karthik K. Leptospirosis in horses: special reference to equine recurrent uveitis. *J Exp Biol Agric Sci.* 2016;4(Spl 4-EHIDZ):s124-s131. doi: 10.18006/2016.4(Spl-4-EHIDZ).S123.S131
7. Norman SA, DiGiacomo RF, Gulland FM, Meschke JS, Lowry MS. Risk factors for an outbreak of Leptospirosis in California sea lions (*Zalophus californianus*) in California. *Journal of Wildlife Diseases*. 2008;44(4):837-844. doi: 10.7589/0090-3558-44.4.837
8. Rossetti CA, Uhart M, Romero GN, Prado W. Detection of leptospiral antibodies in caimans from the Argentinian Chaco. *Vet Record*. 2003;153(20):632-623. doi: 10.1136/vr.153.20.632
9. Rohilla P, Khurana R, Kumar A, Batra K, Gupta R. Detection of Leptospira in urine of apparently healthy dogs by quantitative polymerase chain reaction in Haryana, India. *Vet World*. 2020;13(11):2411-2415. doi: 10.14202/vetworld.2020.2411-2415
10. Bhatia M, Kumar P, Gupta P, Gupta PK, Dhar M, Kalita D. Serological evidence of human leptospirosis in patients with acute undifferentiated febrile illness from Uttarakhand, India: A pilot study. *J Lab Physicians.* 2019;11(1):11-16. doi: 10.4103/JLP.JLP_121_18
11. Dhanze H, Suman MK, Mane BG. Epidemiology of Leptospirosis: An Indian perspective. *Journal of Foodborne Zoonotic Disease.* 2013;1(1):6-13.
12. WHO. Leptospirosis Fact Sheet. World Health Organization. http://www.searo.who.int/. 2018
13. Dutta TK, Christopher M. Leptospirosis-an overview. *J Assoc Physicians India.* 2005;53:545-51.
14. Andre-Fontaine G. Canine leptospirosis-do we have a problem? *Vet Microbiol*. 2006;117(1):19-24. doi: 10.1016/j.vetmic.2006.04.005
15. Bharti AR, Nally JE, Ricaldi JN, et al. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis.* 2003;3(12):757-771. doi: 10.1016/S1473-3099(03)00830-2
16. Sethi S, Sharma N, Kakkar N, Taneja J, Chatterjee SS, Banga SS. Increasing trends of Leptospirosis in Northern India: A clinico epidemiological study. *PLOS Negl Trop Dis.* 2010;4(1):e579. doi: 10.1371/journal.pntd.0000579
17. Haake DA, Levett PN. Leptospirosis in humans. *Curr Top Microbiol Immunol.* 2015;387:65-97 doi: 10.1007/978-3-662-45059-8_5
18. Lau CL, Smythe LD, Craig SB, Weinstein P. Climate change, flooding, urbanization and leptospirosis: fuelling the fire? *Trans R Soc Trop Med Hyg*. 2010;104(10):631-638 doi: 10.1016/j.trstmh.2010.07.002
19. Rosa MI, Reis MFD, Simon C, et al. IgM ELISA for leptospirosis diagnosis: a systematic review and meta-analysis. *Cien Saude Colet.* 2017;22(12):4001-4012. doi: 10.1590/1413-812320172212.14112016
20. Hagan JE, Costa J, Calcagno M, et al. Global morbidity and mortality of leptospi¬rosis: a systematic review. *PLOS Negl Trop Dis*. 2015;9(9):e0003898 doi: 10.1371/journal.pntd.0003898
21. Cheema PS, Srivastava SK, Chaudhuri P, Singh S, Amutha R, Singh H. Application of outer membrane protein antigen based elisa for diagnosis of leptospirosis. *Indian Vet J.* 2007;84(5):445-448.
22. Cheema PS, Srivastava SK, Amutha R, Singh S, Singh H, Sandey M. Detection of pathogenic leptospires in animals by PCR based on lipL21 and lipL32 genes. *Indian J Exp Biol.* 2007b;45(6):568-573.
23. Routray A, Panigrahi S, Swain K, Das M, Ganguly S. Leptospirosis- A review on its zoonosis and related aspects. *Int J Livest Res.* 2018;8(11):29-37. doi: 10.5455/ijlr.20171020111742
24. Cheema PS, Srivastava SK, Chaudhuri P, et al Cloning and Sequencing of a 21kDa Outer Membrane Protein Gene of Leptospira interrogans serovar Canicola strain Hond Utrecht IV. *Vet Res Commun.* 2007;31(5):521-527. doi: 10.1007/s11259-007-3538-y
25. Ghosh KK, Prakash A, Shrivastav P, Balamurugan V, Kumar M. Evaluation of a novel outer membrane surface-exposed protein, LIC13341 of *Leptospira*, as an adhesin and serodiagnostic candidate marker for leptospirosis. *Microbiology*. 2018;164(8):1023-1037. doi: 10.1099/mic.0.000685
26. Ghosh KK, Prakash A, Dhara A, et al. Role of supramolecule ErpY-like lipoprotein of *Leptospira* in thrombin-catalyzed fibrin clot inhibition and binding to complement factors H and I, and its diagnostic potential. *Infect Immun.* 2019;87(12):e00536-19. doi: 10.1128/IAI.00536-19
27. Hota S, Hussain MS, Kumar M. ErpY-like Lipoprotein of Leptospira Outsmarts Host Complement Regulation by Acquiring Complement Regulators, Activating Alternative Pathways, and Intervening in the Membrane Attack Complex. *ACS Infect Dis*. 2022;8(5):982-997. doi: 10.1021/acsinfecdis.1c00602
28. Prakash A, Kumar M. Transcriptional analysis of CRISPR I-B arrays of *Leptospira interrogans* serovar Lai and its processing by Cas6. *Front Microbiol*. 2022;13(960559):1-13. doi: 10.3389/fmicb.2022.960559
29. Everard J, Everard C. Leptospirosis in the Caribbean. *Rev Med Microbiol*. 1993;4(2):114. doi: 10.1097/00013542-199304000-00007
30. Gupta N, Wilson W, Ravindra P. Leptospirosis in India: a systematic review and meta-analysis of clinical profile, treatment and outcomes. *Le Infezioni in Medicina*. 2023;31(3):290.

31. Mori M, Bakinahe R, Vannoorenberghe P, et al. Reproductive Disorders and Leptospirosis: A Case Study in a Mixed-Species Farm (Cattle and Swine). *Vet Sci*.2017;4(4):64. doi: 10.3390/vetsci4040064
32. Jain L, Kumar V. Leptospirosis a neglected re-emerging zoonoses in India: An overview. *J Anim Res*. 2020;10(6):853-858. doi: 10.30954/2277-940X.06.2020.1
33. Singh S and Malik YS. Monograph on Leptospirosis. ISBN: 978-93-91208-72-1. 2021.
34. Abhilash R, Sumitra P, Krutanjali S, Malay D and Subha G. Leptospirosis: A Review on Zoonosis in Indian Scenario. *Int J Livest Res*. 2018;8(11): 29-37.
35. Singh S and Malik YS. 2022. Policy Paper on Leptospirosis, NAVS. ISBN no. 978-93-94490-41-3.
36. Ellis WA. Animal leptospirosis. *Curr Top Microbiol Immunol.* 2015;387:99-137. doi: 10.1007/978-3-662-45059-8_6
37. Panwala T, Mistry Y, Mullan S. Analysis of Prevalent Leptospira Serovars in Different Animals of South Gujarat Region during Year of 2020. *J Res Appl Basic Med Sci.* 2022;8(1):15-18. doi: 10.52547/rabms.8.1.15
38. Sharma S, Gupta D, Tiwari A, et al. Seroprevalence of Bovine Leptospirosis using Indirect ELISA. *Indian J Anim Res*. 2022;1:4. doi: 10.18805/IJAR.B-4951
39. Kanthala S, Patel DR, Balamurugan V, et al. Seroprevalence and Molecular Detection of Canine Leptospirosis in and around Navsari, South Gujarat, India. *Indian J Vet Sci Biotechnol.* 2023;19(3):58-64.
40. Kuriakose M, Eapen CK, Paul R. Leptospirosis in Kolenchery, Kerala, India: Epidemiology, Prevalent local serogroups and serovars and a new serovar. *Eur J Epidemiol*.1997;13(6):691-697. doi: 10.1023/A:1007300729615
41. James S, Sathian B, Teijlingen EV, Asim M. Outbreak of Leptospirosis in Kerala. *Nepal J Epidemiol*. 2018;8(4):745-747. doi: 10.3126/nje.v8i4.23876
42. Shivakumar S. Leptospirosis- Current scenario in India. In: Medicine Update. 2008;18: 799-809.
43. Sehgal SC, Sugunan AP, Vijayachari P. Outbreak of leptospirosis after the cyclone in Orissa. *The Natl Med J India*. 2002;15(1):22-23.
44. Singh SS, Vijayachari P, Sinha A, Sugunan AP, Rasheed MA, Sehgal SC. Clinical-epidemiological study of hospitalized cases of severe leptospirosis. *Indian J Med Res.* 1999;109: 94-99.
45. Karande S, Kulkarni H, Kulkarni M, De A, Varaiya A. Leptospirosis in children in Mumbai slums. *Indian J Pediatr.* 2002;69(10):855-858. doi: 10.1007/BF02723705
46. Karande S, Bhatt M, Kelkar A, Kulkarni M, De A, Varaiya A. An observational study to detect Leptospirosis in Mumbai, India. *Arch Dis Child.* 2003;88(12):1070-1075. doi: 10.1136/adc.88.12.1070
47. Akhilanand C. Leptospirosis- An Indian Experience. *Air and Water Borne Diseases*. 2016;S1:e001.
48. Clerke A M, Leuva A C, Joshi C, Trivedi, SV. Clinical profile of leptospirosis in south Gujarat. *J Postgrad Med.* 2002;48(2):117-118.
49. Patel BK, Gandhi SJ, Desai DC. Clinico-epidemiological aspects of leptospirosis in south Gujarat. *Indian J Med Microbiol*. 2006;24(4):322-326. doi: 10.4103/0255-0857.29409
50. Ratnam S, Sundararaj T, Thiagarajan SP, Rao RS, Madangopalan N, Subramanian S. Serological evidence of Leptospirosis in Jaundice and Pyrexia of unknown origin. *Indian J Med Microbiol.* 1983;77:427-430.
51. Ratnam S, Suramaniam S, Madanagopalan T, Sundararaj T, Jayanthi V. Isolation of Leptospirosis and demonstration of antibodies in Human Leptospirosis in Madras, India. *Trans R Soc Trop Med Hyg*.1983;77(4):455-458. doi: 10.1016/0035-9203(83)90111-6
52. Sathyakamala R, Shanmugam P, Selvabai APR. Changing Trends in the Seroprevalence of Leptospirosis in Kelambakkam: A Ten-Year Retrospective Study. *J Commun Dis*.2022;54(2):19-27. doi: 10.24321/0019.5138.202266
53. Udayar SE, Chengalarayappa NB, Madeshan A, Shivanna M, Marella K. Clinico Epidemiological Study of Human Leptospirosis in Hilly Area of South India-A Population Based Case Control Study. *Indian J Commun Med.* 2023;48(2):316-320. doi: 10.4103/ijcm.ijcm_316_22
54. Sehgal S, Sugunan A, Vijayachari P. Leptospirosis disease burden estimation and surveillance networking in India. *Southeast Asian J Trop Med Public Health.* 2003;34:170-177.
55. Manocha H, Ghoshal U, Singh SK, Kishore J, Ayyagari A. Frequency of leptospirosis in patients of acute febrile illness in Uttar Pradesh. *J Assoc Physicians India*. 2004;52:623-625.
56. Baburaj P, Nandakumar VS, Khanna L. Polymerase chain reaction in the diagnosis of leptospiral infection. *J Assoc Physicians India*. 2006;54:339-340.
57. Martin PL, Stanchi NO, Brihuega BF, Bonzo E, Galli L, Arauz MS. Diagnosis of canine lepto¬spirosis: Evaluation of two PCR assays in comparison with the microagglutination test. *Braz J Vet Res*. 2019;39:4. doi: 10.1590/1678-5150-pvb-5868
58. Shivakumar S, Krishnakumar B. Diagnosis of Leptospirosis- Role of MAT. *Assoc Physicians India*. 2006;54:338-339.
59. Picardeau M, Bertherat E, Jancloes M, Skouloudis AN, Durski K, Hartskeerl RA. Rapid tests for diagnosis of Leptospirosis: Current tools and emerging technologies. *Diagn Microbiol Infect Dis*. 2014;78(1):1-8. doi: 10.1016/j.diagmicrobio.2013.09.012
60. Prakash A, Kumar M. Characterizing the transcripts of Leptospira CRISPR IB array and its processing with endoribonuclease LinCas6. *Int J Biol Macromol*. 2021;182:785-795. doi: 10.1016/j.ijbiomac.2021.04.066
61. Hussain MS, Kumar M. Assembly of Cas7 subunits of Leptospira on the mature crRNA of CRISPR-Cas I-B is modulated by divalent ions. *Gene*. 2022;818:146244. doi: 10.1016/j.gene.2022.146244
62. Anand V, Harshini SP, Gogoi P, Kanaujia SP, Kumar M. Structural and functional characterization of Cas2 of CRISPR-Cas subtype I-C lacking the CRISPR component. *Front Mol Biosci*. 2022;9:988569. doi: 10.3389/fmolb.2022.988569
63. Project Report. DBT Project on Establishment of a laboratory for diagnosis of leptospirosis (unpublished work). 2021.