

Unravelling of Leptospirosis in the Humid Subtropical Climate of the Eastern Part of Northern India

Manoj Kumar^{1#}, Deepak Kumar^{1#}, Sudhir Kumar Singh¹, Alka Shukla¹, Mayank Gangwar², Ranjeet Kumar Vishwakarma³, Ashish R Singh¹, Sonam Rastogi¹, Akshay Kumar⁴, Pradyot Prakash¹, Gopal Nath^{1*}

¹Viral Research and Diagnostic Laboratory, Department of Microbiology, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

²Scientist D, Clinical Studies and Trial Unit, Division of Development Research, Indian Council of Medical Research, Department of Health Research, Ministry of Health and Family Welfare, Government of India, New Delhi, India.

³Department of Physiology, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

⁴Department of Microbiology, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

*Correspondence:

Gopal Nath, Viral Research and Diagnostic Laboratory, Department of Microbiology, Faculty of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Received: 04 Jun 2025; Accepted: 02 Aug 2025; Published: 11 Aug 2025

Citation: Manoj Kumar, Deepak Kumar, Sudhir Kumar Singh, et al. Unravelling of Leptospirosis in the Humid Subtropical Climate of the Eastern Part of Northern India. *Int J Res Virol.* 2025; 1(2): 1-6.

ABSTRACT

Leptospirosis, a neglected zoonotic disease caused by Leptospira spirochetes, poses a significant health burden, particularly in the tropical climate of most states of India. This study examines the occurrence and epidemiological distribution of leptospirosis in the eastern region of Northern India, specifically Eastern Uttar Pradesh. Out of the total 2430 suspected cases, 550 samples (22.6%) tested positive using the Leptospira-specific IgM ELISA.

Demographic analysis revealed a similar distribution of leptospirosis cases between males (49.4%) and females (50.5%), with age-specific prevalence in the 11–20 years age group among males and 21–40 years in females. Seasonal trends revealed a peak for males in October and for females in November. The highest infection rates occurred in the 11–30 age group, due to more outdoor exposure. Housewives, farmers, and students had high positivity rates, highlighting both domestic and agricultural risk factors. A strong correlation with the monsoon and post-monsoon seasons indicates the need for targeted surveillance and prevention strategies. Epidemiologically, cases were distributed across 16 districts, with the highest positivity reported in Deoria (33.3%), Prayagraj (31.2%), and Azamgarh (23.6%). It is intriguing to note that in Varanasi city, the subjects with the highest prevalence of leptospira antibodies were residing along the bank of the River Ganga.

This study highlights the endemic nature of leptospirosis in eastern UP and calls for enhanced suspicion index, diagnostic facilities, and preventive strategies in this region of a subtropical climate.

Keywords

Endemic diseases, Epidemiology, Leptospirosis, Seroprevalence, Tropical diseases, Zoonotic disease.

Introduction

India's diverse agro-ecosystems support the livelihoods of billions, yet these regions are increasingly vulnerable to environmental disturbances that carry significant public health

implications. One notable consequence is the rise in zoonotic diseases, particularly leptospirosis—a bacterial infection caused by pathogenic spirochetes of the genus *Leptospira* [1]. The disease has an incubation period ranging from 2 to 20 days [2]. The disease presents a wide clinical spectrum, ranging from asymptomatic or mild febrile illness to severe, life-threatening infections involving organ failure. Up to 30% of cases may also lead to long-term health complications [6]. The World Health Organization

(WHO) estimates the global incidence of human leptospirosis at approximately 5 cases per 100,000 people annually in endemic areas, rising to 14 per 100,000 during outbreaks [3]. However, more recent estimates indicate a significantly higher global burden of leptospirosis, with approximately 1 million cases and 60,000 deaths each year [4]. Notably, 13% of all reported outbreaks originate from South Asia, positioning leptospirosis as one of the leading zoonotic causes of morbidity and mortality worldwide [5].

The prevalence and transmission dynamics of leptospirosis are closely associated with geographic and climatic factors, with over 75% of cases occurring in tropical regions worldwide [4,7]. The disease thrives in the areas characterized by high humidity, heavy rainfall, frequent flooding, and inadequate sanitation—all of which are prominent in many parts of India, especially during the monsoon season [8]. These reservoirs are often contaminated with the urine of infected animals, particularly rodents, which act as primary carriers, as well as domestic animals such as cattle, pigs, and dogs [2,9]. Humans, typically incidental hosts, acquire the infection either through direct contact with infected animals or indirectly through exposure to contaminated water or soil, with numerous outbreaks documented following floods, storms, and heavy rainfall events [10,11].

This environmental mode of transmission makes leptospirosis a significant occupational hazard for people who work closely with animals or water- and soil-based systems—such as livestock handlers, veterinarians, sewage and waste workers, construction workers, military personnel, aquaculture workers, and farmers [6]. It is also an emerging risk for outdoor recreationalists, including swimmers, kayakers, and fishermen [12]. Moreover, the disease disproportionately affects low-income communities, where poor sanitation infrastructure and limited access to safe drinking water amplify the risk of exposure. Numerous studies have documented significantly higher transmission rates of leptospirosis in socioeconomically disadvantaged populations [13,14]. The majority of the data available in India is from coastal areas with a typical tropical climate that remains almost uniform throughout the year. Contrary to this, the climate in the eastern part of Northern India has a humid subtropical climate, characterized by hot, humid summers and cool, dry winters. There is limited data on leptospirosis from this region, as despite the life-threatening nature of *Leptospira* infections, they often remain undiagnosed or misdiagnosed due to their non-specific symptoms and low suspicion index among clinicians, who may mistake them for diseases such as dengue, hepatitis, and meningitis. Given the strong correlation between climatic factors and disease transmission, timely clinical suspicion prompting for early diagnosis, and prompt initiation of treatment are crucial for avoiding mortality and curbing outbreaks and its endemicity.

Materials and Methods

Study Period and Location

This study was conducted from January to December of the year 2024 at the Viral Research and Diagnostic Laboratory (VRDL), Institute of Medical Sciences (IMS), Banaras Hindu University

(BHU), Varanasi, India.

Study Population

A cross-sectional study design was employed, involving 2,430 patients presenting at Sir Sunder Lal Hospital, BHU, with febrile illness lasting three or more days and clinical suspicion of leptospirosis. Inclusion criteria comprised patients with symptoms such as severe myalgia, jaundice, haemorrhagic manifestations, or aseptic meningitis, including signs like neck rigidity, seizures, or altered mental status. Detailed clinical histories were recorded for all participants. Blood samples were collected and processed at VRDL, IMS, BHU. Serum samples were screened for leptospiral IgM antibodies using ELISA. The total count of acute febrile illness (AFI) cases was retrieved from the laboratory's electronic database.

Enzyme-Linked Immunosorbent Assay (ELISA)

Detection of IgM antibodies against *Leptospira* was carried out using a commercial ELISA kit (Lepto IgM MICROLISA, J. Mitra & Co. Pvt. Ltd, India) according to the manufacturer's standard operating procedure. This qualitative in vitro diagnostic assay is designed to detect *Leptospira*-specific IgM antibodies in human serum or plasma.

Molecular confirmation by using *Leptospira*-specific 16S rRNA gene sequences

Isolation of *Leptospira* DNA from blood samples and its quantification using Qubit fluorometer

A few representative samples ($n = 22$) were selected for DNA isolation using the QiAamp DNA Mini Kit, as per the manufacturer's instructions. All the DNA samples were quantified for the DNA quantity using QUBIT and the results are presented in terms of nanogram/millilitre (ng/mL). All the samples reported with a high quantity of DNA range from 400-1200 ng/mL.

To confirm ELISA findings, molecular testing was performed on selected ELISA-positive cases. DNA was extracted from EDTA-anticoagulated blood samples and quantified using a Qubit fluorometer, with DNA concentrations ranging from 400 to 1200 ng/mL. Amplification of the *Leptospira*-specific 16S rRNA gene (with primers Forward-A 5'-GGCGGCGCGTCTTAAACATG-3', Reverse-B 5'-TTCCCCCATTGAGCAAGATT-3' and Nested PCR Forward-A 5'-TGCAAGTCAAGCGGAGTAGC-3' and Reverse-B 5'-TTCTTAACTGCTGCCTCCCC-3') was performed using conventional nested polymerase chain reaction (PCR) with modifications, utilizing the BIO-RAD C1000 Touch™ thermal cycler.

Data analysis:

Statistical analyses were performed using SPSS 25 (trial version) and GraphPad Prism 8.0.2. Associations between categorical variables were assessed using Pearson chi-square, likelihood ratio, and linear-by-linear association tests ($p < 0.05$ considered significant). Tukey's multiple comparisons test was used for post-hoc analysis following ANOVA. All p -values were two-tailed, with significance defined as $p < 0.05$. Data visualization was performed

in Microsoft Excel, using bar charts. Appropriate statistical tests verified the validity by checking expected cell counts. Results are presented as percentages (%). Geospatial micro-mapping of the positive cases was performed using the Google Maps application to visualize their distribution.

Results

Molecular characterization and confirmation of *Leptospira* using conventional PCR followed by gel electrophoresis

For molecular testing, all the extracted DNA samples were amplified using conventional PCR for both the primary and nested cycles. Eight out of 22 (36.4%) were found positive for *Leptospira spp.* On gel electrophoresis after nested PCR, the DNA bands found were approximately 300 bp amplicons (Figure 1). The majority of PCR-positive cases had a fever of ≤ 5 days' duration, which signifies the leptospiremia phase of illness.

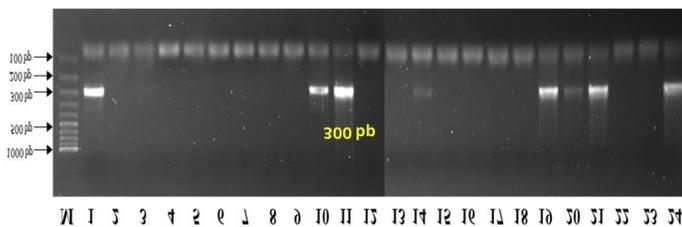


Figure 1: Gel electrophoresis of amplified *Leptospira* DNA after nested Polymerase Chain Reaction (PCR).

Age and Gender-wise distribution of Leptospirosis cases in Eastern Uttar Pradesh

All cases were analyzed using the case report forms received in the laboratory. Of the 550 ELISA-positive leptospirosis cases, 272 (49.4%) were males and 278 (50.5%) were females (Figure 2A). Figure 2B depicts that in terms of age distribution, the highest numbers of positive cases were observed in the 11–20 (24.5%) and 21–30 (23.6%) age groups, with a gradual decline in cases on either side of these ranges. The lowest numbers of cases were recorded in the age groups 71–80 years (1.1%), 81–90 years (0.5%), and 91–100 years (0.2%). Figure 3 depicts the gender-wise distribution of *Leptospira*-positive cases across defined age groups.

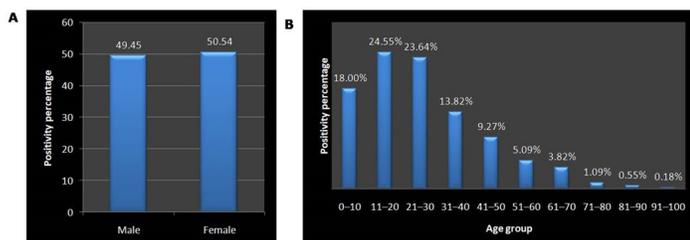


Figure 2: Leptospirosis positivity distribution A: Gender-wise distribution, B Age-wise distribution.

Seasonal distribution of leptospirosis cases in Eastern Uttar Pradesh

The analysis indicated that positive cases peaked during the fall and post-monsoon seasons. September (21.6%) and October (20.4%)

recorded the highest positive rates (Table 1). Figure 4 depicts the gender-wise seasonal distribution of positive cases.

Table 1: Total number of AFI suspected cases tested from January 2024 to December 2024 at the Viral Research and Diagnostic Laboratory (VRDL), Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, India.

	Total No of Cases Tested	IgM Positive Cases	Absolute Positivity
Total AFI Cases	4170		
Scrub Typhus	2482	460	18.53
<i>Leptospira</i>	2430	550	22.63
Dengue	1248	273	21.87
Chikungunya	621	298	47.98
Japanese Encephalitis	67	2	2.98
Influenza A	525	45	8.57
Influenza B	525	15	2.85
Pandemic 09	525	35	6.66
Covid-19	898	18	2.00
CMV	154	71	46.10

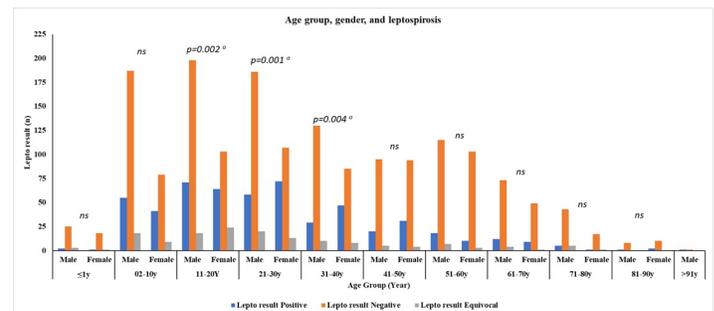


Figure 3: Age and Gender wise distribution of leptospirosis cases. The statistically significant difference among males and females were reported in the age group 11-20 Y ($p=0.002$) 21-30Y ($p=0.001$) and 31-40Y ($p=0.004$).

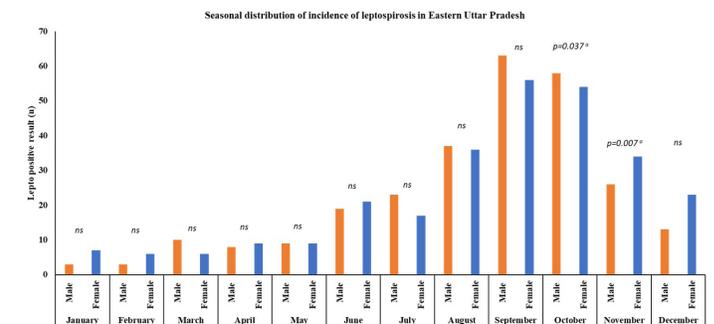


Figure 4: Gender-wise Seasonal distribution of incidence of leptospirosis in Eastern Uttar Pradesh. The statistically significant difference among males and females were reported in the month of October ($p=0.037$) and November ($p=0.007$).

Epidemiological distribution of leptospirosis cases in Eastern Uttar Pradesh

There have been reports of suspected leptospirosis cases from some UP districts. The majority of these were found to be positive in 16 districts in Eastern Uttar Pradesh (Figure 5). Table 2 clearly shows that Ambedkar Nagar reports the highest percentage of

leptospira-positive cases, but the sample size was very small. The highest percentage of individuals who tested positive was Deoria, which received 8 out of 24 cases (33.3%), followed by Prayagraj (31.2%), Azamgarh (23.6%), Balia (23.1%), Varanasi (22.71%). Basti and Kushinagar have no incidence of leptospirosis due to the very small sample size. The data also shows that the leptospira cases are evenly distributed in eastern Uttar Pradesh. As the highest number of suspected leptospira cases was received from the Varanasi district, we did micro mapping of leptospira-positive results, which are depicted in figure 6. Micro-mapping of leptospirosis in the Varanasi district revealed that most positive cases originate from the banks of the River Ganga.

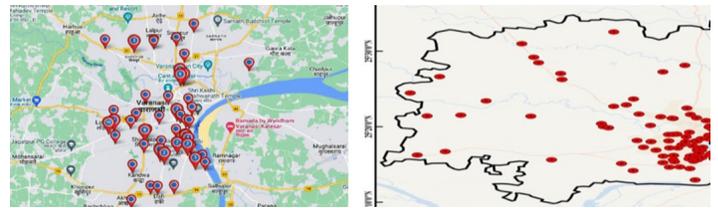


Figure 6: Micro-mapping presentation of leptospira-positive cases received from Varanasi district on the map of Uttar Pradesh, India.

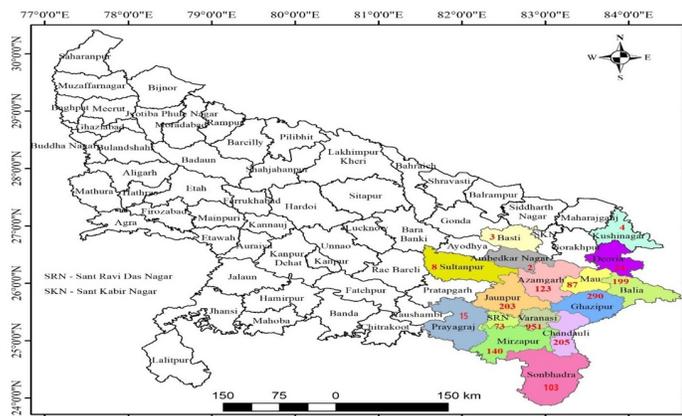


Figure 5: Pictorial representation of suspected leptospirosis cases from Eastern Uttar Pradesh on the state map of Uttar Pradesh, India.

Table 3: Epidemiological distribution of Leptospirosis cases in different districts of Eastern Uttar Pradesh, India.

Area	Positive Count (%)	Negative Count (%)	Total Count	<i>p-value</i>
Ambedkar Nagar	1 (50.0%)	1 (50.0%)	2	0.988
Azamgarh	29 (23.6%)	94 (76.4%)	123	
Balia	46 (23.1%)	153 (76.9%)	199	
Basti	0 (0.0%)	3 (100.0%)	3	
Chanduli	43 (21.0%)	162 (79%)	205	
Deoria	8 (33.3%)	16 (66.6%)	24	
Ghazipur	62 (21.4%)	228 (78.6%)	290	
Jaunpur	43 (21.2%)	160 (78.8%)	203	
Kuhsinagar	0 (0.0%)	4 (100.0%)	4	
Mau	20 (23.0%)	67 (77%)	87	
Mirzapur	37 (26.4%)	103 (73.6%)	140	
Prayagraj	5 (33.3%)	10 (66.7%)	15	
Bhadohi	14 (19.2%)	59 (80.8%)	73	
Sonbhadra	24 (23.3%)	79 (76.7%)	103	
Sultanpur	2 (25.0%)	6 (75.0%)	8	
Varanasi	216 (22.7%)	735 (77.3%)	951	
Total	550 (22.6%)	1880 (77.3%)	2 4 3 0 (100%)	

Table 2: Seasonal distribution of leptospirosis cases.

Months		Positive Count (%)	Negative Count (%)	Total Count	<i>p-value</i>
January- March	Count	35	237	272	0.000
	% within quarter	12.9%	87.1%	100.0%	
April- June	Count	75	339	414	
	% within quarter	18.1%	81.9%	100.0%	
July- September (Monsoon)	Count	232	630	862	
	% within quarter	26.9%	73.1%	100.0%	
October- December (Post-Monsoon)	Count	208	674	882	
	% within quarter	23.6%	76.4%	100.0%	
Total	Count	550	1880	2430	
	% within quarter	22.6%	77.4%	100.0%	

Occupational Distribution of Leptospirosis Positivity

The highest leptospirosis positivity rate was observed among housewives (44%), followed by farmers and students (both at 42%), and businessmen (40%), with no statistically significant difference in positivity rates between these occupational groups (Table 3).

Discussion

Leptospirosis is a significant yet under-reported public health concern in India and is classified as one of the neglected tropical diseases. The disease demands urgent attention due to its reported case fatality rate (CFR), which ranges from 7.7% to over 25% across various states of India [15]. The present study, one of the few conducted in Eastern Uttar Pradesh, provides a comprehensive analysis of the clinical, molecular, and epidemiological aspects of leptospirosis in this region.

Out of 4,170 individuals presenting with acute febrile illness (AFI), 2,430 were identified as suspected cases and subjected to leptospirosis testing. A total of 550 individuals (22.3%) tested positive for leptospirosis using the IgM ELISA method based on early humoral immune response. This detection rate is notably higher than the previous study's 7% positivity rate for Uttar Pradesh, nearly two decades ago [16]. However, the present positivity rate aligns closely with recent studies showing a 26.6% positivity rate [17], which was conducted in central Uttar Pradesh, with a similar subtropical climate to Eastern Uttar Pradesh. Contrary to this, there is a very wide range in positivity rate from 0.9% to 64.7% [18]. This range reflects the extreme variations in climatic conditions in India, as it represents a subcontinent rather than a single small country. Leptospirosis was found to be geographically widespread

across Eastern Uttar Pradesh, with confirmed cases reported from 16 districts in the region. While leptospirosis positivity rates varied across districts in Eastern Uttar Pradesh, with notably higher values in Deoria (33.3%), Prayagraj (33.3%), Azamgarh (23.6%), and Ballia (23.1%), statistical analysis indicated that these differences were insignificant ($p = 0.9$). This apparent variation in proportions could be due to random sample size rather than true geographic disparities in disease burden. We did not consider the scenario in Ambedkar Nagar, where the positivity rate appeared to be the highest, but the sample size was too small. In Varanasi, the district reporting the highest number of suspected leptospirosis cases and thus positivity rate, micro-mapping revealed a spatial cluster of confirmed cases near the bank of the River Ganga. This spatial distribution suggests a potential association between local transmission and environmental risk factors, including proximity to contaminated water bodies such as the Assi Nala drainage stream and stagnant ponds, combined with high population density and suboptimal sanitation infrastructure. Furthermore, it has been observed that the riverbank area is heavily infested with rodents. The strategy to tackle the rodent problem, if linked to the high positivity rate, must be further developed in the future.

Additionally, earlier underreporting and underdiagnosis of such an alarmingly high positivity rate (22.2%), largely stemming from limited awareness of leptospirosis and inadequate laboratory diagnostic infrastructure, may have contributed to previously underestimated prevalence figures [15].

Although northern India generally receives less annual rainfall (100 cm) than the southern and coastal regions (average 1000 to 3000 cm), during the monsoon season (July–October), it often experiences episodic flooding. The predominantly agrarian communities in this region frequently engage in high-risk behaviours, including paddy cropping, handling livestock, working barefoot in waterlogged fields, and being exposed to other contaminated water bodies that are conducive to *Leptospira* transmission [19].

Demographic analysis revealed no significant gender-based difference in overall susceptibility to the disease. This contrasts with earlier studies that reported a higher prevalence among males, likely due to gender-specific occupational or environmental exposure patterns [21–23]. It means the entire population of eastern Uttar Pradesh is exposed equally to *Leptospira* infection, irrespective of gender and occupation. However, the 11–20 years age group of males might be more engaged in outdoor activities, resulting in significantly higher positivity. The higher positivity in females of the 21–40 age group indicates that the source of infection is not limited to outdoor activities. It might be contaminated drinking water due to a faulty water supply system. This speculation is further supported by the observation that occupation does not affect the positivity rates [25].

Seasonal analysis revealed a strong correlation between leptospirosis incidence and climatic patterns, with a statistically significant variation in monthly prevalence ($p < 0.001$) (Table 4). The highest number of cases occurred during the monsoon (26.9%)

and post-monsoon (23.6%) seasons. This pattern is consistent with the well-documented association between leptospirosis and environmental conditions such as heavy rainfall, flooding, and waterlogging, which facilitate the persistence and dissemination of *Leptospira* spp. in contaminated water and soil [18,26]. The observed post-monsoon surge underscores the critical need for intensified surveillance, timely diagnosis, and strengthened public health interventions during and following the monsoon period to mitigate the risk of outbreaks.

Occupation	Lepto Positive	Lepto Negative	Total	p-value
Business	18 (40%)	27 (60%)	45 (100%)	0.698
Farmer	28 (41.79%)	39 (58.21%)	67 (100%)	
Unknown	55 (50%)	55 (50%)	110 (100%)	
Housewife	49 (44.14%)	62 (55.86%)	111 (100%)	
Student	66 (42.31%)	90 (57.69%)	156 (100%)	
Total	216	273	489	

As far as diagnostic modalities are concerned, IgM ELISA, a widely used serological method, effectively identifies early-phase leptospirosis but may be limited during the initial leptospiremic phase due to a delayed antibody response [20]. To overcome this, conventional nested PCR was performed on 22 ELISA-positive samples, detecting *Leptospira* DNA in 8 cases (36.4%), indicating active infection. Most PCR-positive patients had fever for five days or less, highlighting the utility of combining molecular and serological tests for accurate early diagnosis as well as confirmation.

This study highlights the endemicity of leptospirosis in Eastern Uttar Pradesh and emphasizes the importance of integrating both serological and molecular diagnostic approaches for accurate detection and surveillance. The findings demonstrate the significant influence of environmental, seasonal, occupational, and demographic factors on disease prevalence—particularly in flood-prone and river-adjacent areas—underscoring the complex epidemiological profile of the disease. Given its nonspecific presentation, leptospirosis should be considered a differential diagnosis in cases of acute febrile illness (AFI) within endemic regions.

Mitigating the burden of leptospirosis requires a comprehensive approach encompassing enhanced surveillance, public awareness, early diagnosis, timely treatment, and improved sanitation. State governments can implement targeted interventions in alignment with national initiatives, such as the “Prevention and Control of Leptospirosis” scheme. In 2019, the National Centre for Disease Control (NCDC) issued an advisory and preparedness checklist to support outbreak management in affected districts [18].

Acknowledgment

Authors gratefully acknowledge the support provided by the Department of Health Research under the Ministry of Health & Family Welfare (Government of India), New Delhi, India and the Indian Council of Medical Research (ICMR) in the form of establishment of State Level Viral Research and Diagnostic

References

1. Mohammed H, Nozha C, Hakim K, et al. *Leptospira*: morphology, classification and pathogenesis. *J Bacteriol Parasitol*. 2011; 2: 6.
2. Budihal SV, Perwez K. Leptospirosis diagnosis: competency of various laboratory tests. *J Clin Diagn Res*. 2013; 8: 199-202.
3. World Health Organization. Report of the Second Meeting of the Leptospirosis Burden Epidemiology Reference Group; World Health Organization: Geneva, Switzerland. 2011.
4. Costa F, Hagan JE, Calcagno J, et al. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLoS Negl Trop Dis*. 2015; 9: 0003898.
5. Munoz Zanzi C, Groene E, Morawski BM, et al. A systematic literature review of leptospirosis outbreaks worldwide, 1970–2012. *Rev Panam Salud Pública*. 2020; 44: 78.
6. Bradley EA, Lockaby G. Leptospirosis and the environment: A review and future directions. *Pathogens*. 2023; 12: 1167.
7. Dubey S, Singh R, Gupta B, et al. *Leptospira*: An emerging zoonotic pathogen of climate change, global warming and unplanned urbanization: A review. *Architecture*. 2021; 10: 54.
8. Choudhary S, Choudhary RK, Kumar M, et al. Epidemiological Status of Leptospirosis in India. *Journal of Pure & Applied Microbiology*. 2023; 17: 1968-1977.
9. Cordonin C, Turpin M, Bringart M, et al. Pathogenic *Leptospira* and their animal reservoirs: testing host specificity through experimental infection. *Scientific Reports*. 2020; 10: 7239.
10. Bierque E, Thibeaux R, Girault D, et al. A Systematic Review of *Leptospira* in Water and Soil Environments. *PLoS ONE*. 2020; 15: 0227055.
11. Maskey M, Shastri J, Saraswathi K, et al. Leptospirosis in Mumbai: Post-Deluge Outbreak 2005. *Indian J Med Microbiol*. 2006; 24: 337-338.
12. Narita M, Fujitani S, Haake DA, et al. Leptospirosis After Recreational Exposure to Water in the Yaeyama Islands, Japan. *Am J Trop Med Hyg*. 2005; 73: 652-656.
13. Bacallao J, Schneider MC, Najera P, et al. Socioeconomic Factors and Vulnerability to Outbreaks of Leptospirosis in Nicaragua. *Int J Environ Res Public Health*. 2014; 11: 8301-8318.
14. Zhao J, Liao J, Huang X, et al. Mapping Risk of Leptospirosis in China Using Environmental and Socioeconomic Data. *BMC Infect Dis*. 2016; 16: 343.
15. Shivakumar S. Leptospirosis-current scenario in India. *Medicine update*. 2008.
16. Manocha H, Ghoshal U, Singh SK, et al. Frequency of leptospirosis in patients of acute febrile illness in Uttar Pradesh. *J Assoc Physicians India*. 2004; 52: 623-625.
17. Shukla S, Mittal V, Karoli R, et al. Leptospirosis in central & eastern Uttar Pradesh, an underreported disease: A prospective cross-sectional study. *Indian Journal of Medical Research*. 2022; 155: 66-72.
18. Choudhary S, Choudhary RK, Kumar M, et al. Epidemiological Status of Leptospirosis in India. *Journal of Pure & Applied Microbiology*. 2023; 17: 1968-1977.
19. Sehgal SC. Epidemiological patterns in leptospirosis. *Indian J Med Microbiol*. 2006; 24: 310-311.
20. Niloofa R, Fernando N, de Silva NL, et al. Diagnosis of leptospirosis: comparison between microscopic agglutination test, IgM-ELISA and IgM rapid immunochromatography test. *PLoS one*. 2015; 10: 0129236.
21. A Sethi S, Sharma N, Kakkar N, et al. Increasing trends of leptospirosis in northern India: a clinico-epidemiological study. *PLoS neglected tropical diseases*. 2010; 4: 579.
22. Costa F, Hagan JE, Calcagno J, et al. Global morbidity and mortality of leptospirosis: a systematic review. *PLoS neglected tropical diseases*. 2015; 9: 0003898.
23. Puca E, Pipero P, Harxhi A, et al. The role of gender in the prevalence of human leptospirosis in Albania. *The Journal of Infection in Developing Countries*. 2018; 12: 150-155.
24. 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: 290-305.
25. Desai KT, Patel F, Patel PB, et al. A case-control study of epidemiological factors associated with leptospirosis in South Gujarat region. *J Postgrad Med*. 2016; 62: 223-227.
26. Bradley EA, Lockaby G. Leptospirosis and the environment: A review and future directions. *Pathogens*. 2023; 12: 1167.