

# Seroprevalence and Distribution of Leptospirosis Infection among Patients with Acute Febrile Illness in Central Uttar Pradesh: A Retrospective Observational Analysis

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## ABSTRACT

**Introduction:** Leptospirosis is an anthroponozoonotic disease. The wide spectrum of clinical manifestations of leptospirosis ranges from mild febrile illness without complications to severe disease with Multiorgan dysfunction. In the central and eastern parts of Uttar Pradesh, India, it is one of the major causes of acute febrile illness.

**Aim:** To determine the seroprevalence, clinical features and geographical distribution of leptospirosis in Central Uttar Pradesh, India.

**Materials and Methods:** This current retrospective observational study was conducted in the Serology Laboratory, Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Uttar Pradesh, India, from January 2022 to December 2022 and data were analysed from January 2023 to March 2023. A total of 625 patients with fever lasting >7 days attending the Outpatient Department (OPD) were included in the study. Serum samples of these patients were tested for *Leptospira* Immunoglobulin M (IgM) by Enzyme-linked Immunosorbent Assay (ELISA). Clinical, laboratory findings and demographic data were also collected from the hospital software. The patients were also tested for other infectious aetiologies such as malaria, typhoid,

dengue and scrub typhus. Data were presented as percentages, and the Odds Ratio (OR), 95% CI, and p-value were used to test the level of significance.

**Results:** Leptospirosis was found to be positive in 63 (10.08%) patients. The patients with leptospirosis belonged to the age group of 21-40 years with a male predominance. The maximum number of cases was from Lucknow district with 23.8% prevalence, followed by Prayagraj (11.11%). The common presenting symptoms were weakness, epigastric pain, myalgia and jaundice. Co-infection with scrub typhus was seen in 11 (17.4%) patients with leptospirosis. None of the other infections showed co-infection with leptospirosis. Coagulopathy and hepatic dysfunction were found in the majority of the patients with leptospirosis. Mortality was seen in five patients with Multiple Organ Dysfunction Syndrome (MODS).

**Conclusion:** The seroprevalence of leptospirosis was found to be 10.08%. To reduce the morbidity and mortality linked to leptospirosis, it is necessary to improve diagnostic skills and the knowledge of physicians for screening the disease in patients with acute febrile illnesses, as it involves multiple systems and has overlapping common presenting symptoms.

**Keywords:** Anthroponozoonotic, Coagulopathy, Hepatic dysfunction, Mortality

## INTRODUCTION

Human leptospirosis is an anthroponozoonotic disease caused by a variety of leptospiral serotypes [1,2]. It is a neglected disease, occurring worldwide, especially in tropical and subtropical areas with heavy rainfall, and affecting vulnerable populations in rural settings and semi-urban slums [1,3,4]. It is estimated that leptospirosis causes around 58,900 deaths worldwide [5]. The disease is most prevalent in tropical and subtropical countries [6]. Cases of leptospirosis have been documented in India since 1931. Leptospirosis is quickly becoming a major public health concern throughout the country. Seroprevalence rates reported in various studies from different regions of the country range from 6.4% to 37.7% [7,8].

Environmental factors such as urbanisation, poverty and occupational circumstances may contribute to the re-emergence of the disease globally. In urban areas, the risk of human infection is significantly increased by poor housing conditions, lack of basic sanitation and limited access to healthcare and education, which are common problems in developing countries [9]. In the central and eastern parts of Uttar Pradesh, India, leptospirosis is one of the major causes of acute febrile illness [10].

Clinical signs of leptospirosis range from mild febrile illness with no sequelae to severe disease with Multiorgan dysfunction [10]. The liver, kidneys, lungs and central nervous system are most commonly affected by clinical symptoms and complications. Timely diagnosis and treatment can reduce the severity of the illness and, consequently, reduce mortality [2,10]. The clinical symptoms of leptospirosis are similar to those of many other prevalent infectious diseases in tropical contexts, including rickettsial infections, dengue, malaria, typhoid and viral hepatitis [4,11]. Studying the changing trends in patterns of leptospirosis could be of great help to clinicians. Therefore, the aim of the present study was to determine the seroprevalence, clinical pattern, and geographical distribution of leptospirosis in Central Uttar Pradesh, India. The objective of the study was to determine the prevalence of co-infection with scrub typhus among suspected cases of leptospirosis attending the referral tertiary care hospital in Uttar Pradesh.

## MATERIALS AND METHODS

The current retrospective observational study was conducted in the Serology Laboratory, Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences in Uttar Pradesh, India, from January 2022 to December 2022. The data were analysed from January 2023 to March 2023.

The study was approved by the Institutional Ethics Committee (IEC code: 2021-109-IMP-EXP-38). Since, it was a retrospective study, all consecutive patients who met the inclusion criteria and were available during the study duration were included.

A total of 625 consecutive patients with clinically suspected leptospirosis who attended outpatient clinics or were admitted to various departments such as General Medicine, Gastroenterology, Paediatrics and Hepatology in the hospital were included.

**Inclusion criteria:** Patients who were clinically suspected of having leptospirosis and presented with a history of fever lasting >7 days, accompanied by any of the following manifestations such as severe headache, severe myalgia, uveitis, arthralgia, rash, hepatosplenomegaly, evidence of haemorrhage, renal failure and icterus, were included in the study.

**Exclusion criteria:** Patients with fever <7 days and those with positive blood cultures or urine cultures were excluded from the study.

## Study Procedure

The clinical, demographical and laboratory details of these patients were extracted from the hospital information software and entered into Microsoft Excel software. Blood samples from the study population were collected, and serum was separated. The samples were tested for the detection of IgM antibodies for leptospirosis using the PanBio Leptospira IgM ELISA kit. The test was carried out according to the manufacturer's instructions, and the results were interpreted as follows: Panbio units; <9 units=negative; 9-11 units=equivocal; >11 units=positive [12]. Modified Faine's criteria were used for the diagnosis of presumptive and possible leptospirosis [13].

A presumptive diagnosis of leptospirosis may be made, if:

- Score of Part A+Part B=26 or more (Part C laboratory report is usually not available before the fifth day of illness; thus it is mainly a clinical and epidemiological diagnosis during the early part of the disease) or Part A+Part B+Part C  $\geq$ 25.
- A score between 20 and 25: Suggests a possible but unconfirmed diagnosis of leptospirosis.

These patients were also evaluated for other causes of acute febrile illness. The tests included Dengue NS1 antigen ELISA (Panbio Dengue Early ELISA kit), Dengue IgM ELISA (Panbio Dengue IgM Capture ELISA), Rapid diagnostic test for detection of *Plasmodium falciparum* HRP2 (Abbott Bioline Malaria Ag P.f./Pan), widal test for typhoid (Tydal-Widal Antigen Set/antigens for Slide Agglutination for Slide and Tube Tests), and Scrub typhus IgM ELISA (InBios Scrub Typhus IgM ELISA).

## STATISTICAL ANALYSIS

Data were entered into Microsoft Excel sheets. The data were analysed using Statistical Package for the Social sciences (SPSS) software. Normally distributed numerical data were presented as mean and range. Categorical data were presented as percentages. The OR, 95% Confidence Interval (95% CI), and p-value were used to test the level of significance. A p-value of <0.05 was considered statistically significant.

## RESULTS

Of the 625 patients included in the study, the majority of the population belonged to the age group of 21-40 years, 213 (34.08%), followed by the 41-60 years, 185 (29.6%) age groups [Table/Fig-1]. The age range and mean age of the population were 1-90 years and 39 years, respectively. The male-to-female ratio in the study population was 1.9:1. There was a male predominance in the study, with 409 (65.44%) males and 216 (34.56%) females.

Since the study excluded patients with positive blood or urine culture, they were tested for all possible infectious causes of fever with an unknown origin. The tests included ELISA for the detection of *Leptospira* IgM, Dengue NS1 antigen, Dengue IgM, and Scrub

Variables	Total population (N=625)	Patients tested positive for leptospirosis (n=63)
<b>Gender, n (%)</b>		
Male	409 (65.44)	40 (63.49)
Female	216 (34.56)	23 (36.50)
<b>Age group (in years), n (%)</b>		
0-20	127 (20.32)	8 (12.6)
21-40	213 (34.08)	29 (46.03)
41-60	185 (29.6)	17 (26.98)
61-80	95 (15.2)	9 (14.28)
81-100	5 (0.8)	0
<b>Residence, n (%)</b>		
Rural	118 (18.88)	4 (6.34)
Urban	507 (81.12)	59 (93.65)

[Table/Fig-1]: Demographic details of study population (n=625).

typhus IgM, as well as, a rapid diagnostic test for the detection of *Plasmodium falciparum* HRP2 and the widal test for typhoid.

It was found that 63 (10.08%) patients tested positive for leptospirosis IgM, 30 (4.8%) patients tested positive for Dengue NS1 antigen, and 32 (5.12%) patients tested positive for Dengue IgM. Scrub typhus IgM was detected in 19 (3.04%) patients, malaria was detected in 16 (2.56%) patients, and typhoid was detected in 25 (4%) patients. These tests were negative in all the other patients under study, who were classified under unknown causes (n=440). The unknown causes may include any other infective cause such as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection, Human Immunodeficiency Virus (HIV) infection, or other viral infections, or non infective causes such as inflammatory causes or neoplastic causes. None of the patients who tested positive for any of the diseases showed co-infection, except for 11 (17.4%) patients who tested positive for both leptospirosis IgM and Scrub typhus IgM.

Of the study population, 63 patients were found to be positive for leptospirosis. The seroprevalence of leptospirosis was found to be 10.08%. The majority of the patients positive for leptospirosis belonged to the age group of 21-40 years, with 29 patients (46.03%) [Table/Fig-1]. The clinical symptoms, laboratory findings and outcomes of the total study population and the population, tested positive for leptospirosis are given in [Table/Fig-2].

Variables	Total population (N=625)	Patients tested positive for leptospirosis (n=63)	Odds Ratio (OR)	95% CI	p-value
<b>Symptoms, n (%)</b>					
Rash	102 (16.32)	15 (23.80)	1.70	0.91 to 3.18	0.09
Vomiting	143 (22.88)	15 (23.80)	1.05	0.57 to 1.95	0.85
Myalgia	423 (67.68)	58 (92.06)	6.26	2.47 to 15.86	<b>0.0001</b>
Weakness	498 (79.68)	60 (95.23)	5.66	1.74 to 18.36	<b>0.003</b>
Shortness of breath	321 (51.36)	15 (23.80)	10.02	4.71 to 21.30	<b>&lt;0.0001</b>
Jaundice	243 (38.88)	54 (85.71)	11.84	5.72 to 24.49	<b>&lt;0.0001</b>
Any CNS related complaints	98 (15.68)	12 (19.04)	1.30	0.66 to 2.54	0.43
Epigastric pain	587 (93.92)	60 (95.23)	1.32	0.39 to 4.44	0.64
Headache	87 (13.92)	15 (23.80)	2.12	1.13 to 3.99	<b>0.01</b>
Decreased urine output	123 (19.68)	9 (14.28)	0.65	0.31 to 1.36	0.25

Laboratory investigations (normal values), n (%)					
Elevated bilirubin levels (0.2-1.2 mg/dL)	203 (32.48)	60 (95.23)	58.6	18.09 to 189.75	<b>&lt;0.0001</b>
Abnormal PT/INR (11-13.5 seconds)	260 (41.6)	63 (100)	235.03	14.46 to 3819.37	<b>0.0001</b>
Abnormal APTT (30-40 seconds)	263 (42.08)	63 (100)	229.61	14.13 to 3731.21	<b>0.0001</b>
Multiple Organ Dysfunction Syndrome (MODS) with sepsis	30 (4.8)	5 (7.93)	1.85	0.68 to 5.02	0.22
Outcome, n (%)					
Recovered	594 (95.04)	58 (92.06)	1.77	0.65 to 4.80	0.25
Death	31 (4.96)	5 (7.93)			

**[Table/Fig-2]:** Clinical, laboratory investigations, and outcome details of the total study population and patients tested positive for leptospirosis.  
MODS: Multiple organ dysfunction syndrome; PT/INR: Prothrombin time/International normalised ratio; APTT: Activated partial thromboplastin time; CNS: Central nervous system; Statistical significance was estimated using OR, 95% CI and p-value. The p-value in bold font indicates statistically significant values

The month-wise distribution of the total population tested and samples tested positive for leptospirosis is shown in [Table/Fig-3]. It is evident that the maximum number of positive cases for leptospirosis were reported in the months of July to October (60.31%), with the highest number of positive cases in September (28.57%).

Months	Total samples tested (n=625)	Total samples tested positive for leptospirosis (n=63)
January	24 (3.84)	4 (6.34)
February	15 (2.4)	0
March	41 (6.56)	4 (6.34)
April	34 (5.44)	1 (1.58)
May	47 (7.52)	6 (9.52)
June	51 (8.16)	4 (6.34)
July	46 (7.36)	4 (6.34)
August	49 (7.84)	7 (11.11)
September	98 (15.68)	18 (28.57)
October	88 (14.08)	9 (14.28)
November	86 (13.76)	3 (4.76)
December	46 (7.36)	3 (4.76)

**[Table/Fig-3]:** Month-wise distribution of the total samples tested (n=625) and samples tested positive (n=63) for leptospirosis.  
Values are presented as n (%)

The geographical distribution of the patients who tested positive for Leptospira IgM is shown in [Table/Fig-4]. It can be seen that the majority of the cases of leptospirosis were from the Lucknow district (23.8%), followed by Prayagraj (11.11%) and Raebareli (7.93%).

Districts of Uttar Pradesh from where samples were received	Number of cases positive for leptospirosis (n=63)
Ambedkar nagar	1 (1.58)
Amethi	3 (4.76)
Azamgarh	4 (6.34)
Barabanki	1 (1.58)
Basti	1 (1.58)
Deoria	2 (3.17)
Farukkabad	1 (1.58)
Ghazipur	2 (3.17)

Gonda	2 (3.17)
Gorakhpur	2 (3.17)
Hardoi	1 (1.58)
Kanpur	4 (6.34)
Lakhimpur kheri	2 (3.17)
Lucknow	15 (23.8)
Pratapgarh	3 (4.76)
Prayagraj	7 (11.11)
Raebareli	5 (7.93)
Shahjahanpur	1 (1.58)
Siddharth nagar	3 (4.76)
Siwan	1 (1.58)
Unnao	1 (1.58)
Varanasi	1 (1.58)

**[Table/Fig-4]:** District wise distribution of the patients positive for leptospirosis in Uttar Pradesh, India.  
Values are presented as n (%)

The common symptoms of acute febrile illness in patients included weakness, epigastric pain, myalgia, and jaundice. Other symptoms such as shortness of breath, altered sensorium, vomiting, and headache were also reported in cases of leptospirosis. Prolonged PT/INR ratio and APTT values were important findings in all patients with leptospirosis. Elevated bilirubin levels indicated hepatic dysfunction in these patients.

Using modified Faine's criteria, a presumptive diagnosis could be made in 46% of cases and a possible diagnosis in 23% of cases [Table/Fig-5]. MODS was observed in five patients with leptospirosis, and all five patients died [Table/Fig-2].

Category	Score
Part A: Clinical data	
Headache	2
Fever	2
If fever, temperature 39° or more	2
Conjunctival suffusion (bilateral)	4
Meningism	4
Muscle pain	4
Conjunctival suffusion+Meningism+Muscle pain	10
Jaundice	1
Albuminuria or nitrogen retention	2
Part B: Epidemiological factors	
Rainfall	5
Contact with contaminated environment	4
Animal contact	1
Part C: Bacteriological and laboratory findings	
Isolation of leptospira on culture	Diagnosis certain
Positive serology	
ELISA IgM positive, SAT positive, MAT single high titre (Any one of the three)	15
MAT rising titre	25
A presumptive diagnosis of leptospirosis may be made if: (i) Score of Part A+Part B=26 or more (Part C laboratory report is usually not available before fifth day of illness; thus it is mainly a clinical and epidemiological diagnosis during early part of disease) or Part A+Part B+Part C ≥25. (ii) A score between 20 and 25: Suggests a possible but unconfirmed diagnosis of leptospirosis.	
[Table/Fig-5]: Modified Faine's criteria used for assessing severity of leptospirosis. SAT: Standard agglutination test; MAT: Microscopic agglutination test	

DISCUSSION

Leptospirosis is considered one of the most neglected tropical diseases [4]. Among patients with fever of unknown origin, infectious causes account for 17-35% of cases, inflammatory



causes account for approximately 24-36% of cases, neoplastic causes account for 10-20% of cases, and miscellaneous causes account for 3-15% of cases [14]. In the current study, it was found that 29.6% cases had an infectious aetiology, while the others were classified as unknown causes. It is important to note that, in the present study only included testing for leptospirosis, dengue, scrub typhus, malaria, and typhoid, so it does not represent the overall percentage of infectious diseases responsible for fever of unknown origin.

As documented in the present study, a male predominance among patients positive for leptospirosis has been observed in other Studies, as well [2,15]. It was also observed that the majority of the study population with acute febrile illness and patients with leptospirosis belonged to the age group of 21-40 years. A similar observation was documented in a study conducted in Punjab, India [3].

The seroprevalence of leptospirosis in the present study was found to be 10.08%, which is consistent with many studies in the literature. A study conducted in Vietnam estimated the seroprevalence of leptospirosis to be 9.5% [1]. Similar seroprevalence of leptospirosis has also been documented in other studies, including those conducted in Morocco (10.4%), New Delhi, India (6.47%) and Tamil Nadu, India (10%) [7,9,16].

The highest seroprevalence was found during the months of July to October in the present study. A study by Kaur P et al., in Punjab also found the highest seroprevalence during the monsoon season (June to September), followed by the autumn season (October to November) [3]. This might help to establish the epidemiological link between seasonal occurrence and leptospirosis. The higher incidence of infection during these months suggests that rain and dampness promote the spread of infection and favour the survival of *Leptospira* bacteria in the soil.

In the current study, fever was reported in all cases of leptospirosis, and other common symptoms included weakness, epigastric pain, myalgia, and jaundice. Similar clinical presentations were reported in other studies as well [3,17]. An important finding in this study was the prolonged Prothrombin Time/International Normalised Ratio (PT/INR) ratio and Activated Partial Thromboplastin Time (APTT) values observed in all patients with leptospirosis, indicating an association between coagulopathy and leptospirosis. Wagenaar JF et al., reported in their study that the coagulation system becomes activated in patients with leptospirosis, leading to severe bleeding and poor outcomes [18]. The presence of hepatic dysfunction and multiple organ dysfunction in the patients in the current study is supported by findings from other studies [3,16,19].

Another interesting finding in the current study was that out of the 63 patients detected positive for leptospirosis, 11 (17.4%) patients were also positive for scrub typhus. This incidence of overlap in the study may be due to co-infections rather than cross-reactivity, considering the similar exposure risk and susceptible population. Similarly, a multicentric study conducted in six states of India found that among patients with acute undifferentiated fever, leptospirosis was detected in 7% of the patients, and co-infection of leptospirosis and scrub typhus was observed in 24% [11]. There is evidence of co-infections in the previous literature, as well [20,21]. Several case reports from India have also documented evidence of co-infection between leptospirosis and scrub typhus [22].

The finding that death occurred in five patients with leptospirosis who developed MODS with sepsis indicates that mortality occurs when multiple organs are involved. This highlights the importance of early diagnosis in patients with acute febrile illness.

### Limitation(s)

A large proportion of leptospirosis infections are subclinical, exhibiting mild symptoms. These cases are often not reported in

healthcare facilities, resulting in an underestimation of the community prevalence of leptospirosis. In the present study, IgM ELISA was utilised a genus-specific test that cannot detect the specific serovars of *Leptospira* bacilli. Due to limited resources, it was not possible to perform serovar-specific tests such as the Microscopic Agglutination Test (MAT). However, serological tests like ELISA are effective and useful for estimating seroprevalence, particularly in resource-limited settings.

### CONCLUSION(S)

The seroprevalence of leptospirosis was found to be 10.08%. To reduce morbidity and mortality associated with leptospirosis, it is necessary to enhance physicians' diagnostic skills and knowledge for screening the disease in patients with acute febrile illnesses, given the involvement of multiple systems and the overlap in common presenting symptoms.

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