

Seroprevalence of Human Leptospirosis in a Tertiary Care Hospital

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Abstract

Background: Leptospirosis is an important zoonotic cause of acute febrile illness in tropical regions, yet its local burden is frequently underestimated because clinical manifestations overlap with other infections and laboratory testing is not uniformly applied.

Objectives: To estimate *Leptospira* seroprevalence among tested patients at Government Medical College, Ongole, and describe its distribution by age, sex, patient category, residence, and month of sample collection.

Methods: A prospective, hospital-based cross-sectional study was conducted at Government Medical College, Ongole, a tertiary-care teaching institution located in Prakasam district, Andhra Pradesh, India, from 1 May 2025 to 30 April 2026. The institution serves patients from Ongole and the surrounding urban, peri-urban, and rural communities, including referrals from neighbouring districts. A total of 110 eligible patients undergoing *Leptospira* testing were consecutively included. Seroprevalence was calculated with an exact 95% confidence interval, and categorical associations were assessed using two-sided Fisher's exact tests.

Results: Among 110 individuals tested, 30 were seropositive, yielding a seroprevalence of 27.3% (95% CI: 19.2%-36.6%). The mean age was 10.9 ± 12.7 years, and 98 participants (89.1%) were younger than 16 years. Seropositivity was significantly higher among females than males (39.1% vs 18.8%; $p=0.029$). All seropositive cases were inpatients and residents of Prakasam district; positivity was 30.6% in Prakasam district versus 0% in other districts ($p=0.034$). Month-wise positivity varied from 0% to 50.0%, with the highest proportions observed in January and July.

Conclusion: Human leptospirosis represented a substantial proportion of tested patients in this tertiary-care setting. The one-year findings support continued diagnostic suspicion, timely laboratory evaluation of compatible febrile illness, and strengthened surveillance across the hospital catchment area.

Recommendations: Strengthened laboratory surveillance, complete recording of clinical and exposure variables, and year-round testing of clinically suspected cases are recommended. Larger multicentre prospective studies should characterize risk factors, diagnostic performance, disease severity, and outcomes.

Keywords: Leptospirosis; seroprevalence; acute febrile illness; zoonosis; Andhra Pradesh; tertiary care; epidemiology

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1. Introduction

Leptospirosis is a globally distributed zoonotic infection caused by pathogenic spirochetes of the genus *Leptospira*. Humans acquire infection predominantly through direct or indirect contact with urine from infected mammals, particularly when contaminated water or soil contacts abraded skin or mucous membranes. Rodents, livestock, dogs, and several wild mammals can act as maintenance hosts. The ecological persistence of leptospires, together with occupational, agricultural, recreational, and flood-related exposures, makes transmission especially relevant in warm and humid environments. Clinical expression ranges from a mild, self-limited febrile illness to jaundice, acute kidney injury, pulmonary haemorrhage, meningitis, shock, and multiorgan dysfunction. Because the early syndrome commonly resembles dengue, malaria, scrub typhus, enteric fever, and other tropical infections, leptospirosis is easily overlooked when laboratory confirmation is not pursued (1,2).

The public-health burden is substantial despite persistent under-recognition. A systematic analysis estimated approximately 1.03 million cases and 58,900 deaths from

leptospirosis worldwide each year, with particularly high morbidity and mortality in South and Southeast Asia and other resource-constrained tropical regions (3). Burden estimates expressed as disability-adjusted life years further demonstrate the importance of severe disease, premature mortality, and socioeconomic vulnerability (4). These estimates also reflect limitations in routine surveillance, since clinically mild infections, diagnostic uncertainty, delayed seroconversion, and restricted access to confirmatory testing can all reduce case ascertainment.

Diagnosis depends on the phase of illness and the laboratory methods available. Culture is definitive but slow and technically demanding, while molecular methods are most useful early in infection. Serological methods remain central to routine diagnosis, particularly after the development of detectable antibodies; however, interpretation varies according to timing, assay platform, background exposure, and use of confirmatory microscopic agglutination testing (5). In India, hospital-based studies have demonstrated considerable geographic and temporal heterogeneity. A retrospective study from Hyderabad highlighted leptospirosis as an underreported infection in Andhra

Pradesh and documented the practical role of serological testing (6). Outbreak investigations from Chittoor district have also established transmission within Andhra Pradesh, emphasizing the need for local surveillance rather than extrapolation from distant regions (7).

Ongole and the surrounding Prakasam district include urban, peri-urban, agricultural, and rural communities where patients with undifferentiated fever are frequently referred to tertiary-care services. Institution-specific data can therefore provide an important signal of disease occurrence and guide diagnostic awareness, even when they do not represent community prevalence. The objective of the present study was to estimate the seroprevalence of human leptospirosis among patients whose samples were tested at Government Medical College, Ongole, during the prospective one-year study period from May 2025 to April 2026. Secondary objectives were to describe seropositivity according to age, sex, inpatient or outpatient status, district of residence, and month of sample collection, and to explore selected categorical differences using exact statistical tests.

2. Methodology

2.1 Study design and setting

This prospective, hospital-based cross-sectional study was conducted from 1 May 2025 to 30 April 2026 at Government Medical College and its affiliated Government General Hospital, Ongole, Prakasam district, Andhra Pradesh, India. The institution is a government tertiary-care teaching hospital providing outpatient, inpatient, emergency, and specialized healthcare services, including general medicine, paediatrics, general surgery, obstetrics and gynaecology, and diagnostic laboratory services. The hospital is equipped with intensive care facilities and serves as a referral centre for patients from Ongole, surrounding rural and peri-urban communities, and neighbouring districts. Leptospira testing was performed as part of routine diagnostic evaluation, and eligible patients with documented laboratory results were included in the study.

2.2 Study population and eligibility

Patients were eligible when they underwent Leptospira testing during the study period and had a documented positive or negative result together with sufficient demographic information for analysis. Patient-level seroprevalence, rather than test-episode positivity, was the primary measure. Repeat test entries were checked using concordant demographic and contact information so that each individual contributed only once to the patient-level denominator. The final analytical population comprised 110 unique individuals.

2.3 Sample size and sampling

A total of 110 patients were included. Consecutive inclusion was applied to all eligible, unique patients undergoing

Leptospira testing during the predefined one-year prospective study period. The final sample comprised 110 participants; no additional sampling fraction was applied.

2.4 Variables and data collection

Study data included age, sex, inpatient/outpatient category, village, subdistrict, district, sample-collection date, testing date, and laboratory result. These variables were recorded for eligible patients during the study period. Age was standardized to completed years, and sex entries were categorized as male or female. District names were standardized for minor spelling variations. Age was summarized continuously and grouped as ≤ 5 , 6–10, 11–15, and ≥ 16 years for descriptive presentation. For exploratory comparison, age was also dichotomized as < 16 versus ≥ 16 years. Month-wise analysis was based on sample-collection date.

2.5 Laboratory classification

Leptospira results were classified as positive or negative exactly as recorded in the routine laboratory dataset. Consequently, no assay-specific sensitivity or specificity adjustment was applied. This conservative approach is important because Indian studies have shown that serological and molecular methods can yield different detection proportions across clinical settings (8–10).

2.6 Measures to Minimize Bias

: To minimize selection bias, all eligible patients undergoing Leptospira testing during the predefined study period were consecutively included without additional sampling. Potential duplication bias was addressed by reviewing demographic and contact information to ensure that each patient contributed only once to the analysis. Data consistency was supported by standardizing age categories, sex classification, and district names before analysis. Laboratory results were classified according to the recorded positive or negative findings without retrospective reclassification. To reduce analytical bias associated with small subgroup sizes, Fisher's exact test was used for categorical comparisons, while monthly seropositivity was reported descriptively. Nevertheless, potential referral and selection biases arising from clinician-directed testing at a single tertiary-care hospital could not be eliminated, and the findings may not be representative of the general population

2.7 Statistical analysis

Categorical variables were summarized as frequencies and percentages, while age was described using mean, standard deviation, median, and range. Overall seroprevalence was calculated as the proportion of unique individuals with a positive laboratory result, with a two-sided exact binomial 95% confidence interval. Two-sided Fisher's exact tests were used for exploratory comparisons by sex, age category

(<16 versus ≥ 16 years), inpatient/outpatient status, and residence in Prakasam versus other districts because several cells contained small expected counts. A p value <0.05 was considered statistically significant. Month-wise findings were summarized descriptively because of small and uneven monthly denominators.

2.8 Ethical considerations

The study was approved by the Institutional Ethics Committee, Government Medical College, Ongole (approval no. IEC/GMC-UGL/255/2025). Participant confidentiality was maintained throughout data collection and analysis, and only de-identified findings were reported.

3. Results

3.1 Participant Flow and Baseline Characteristics

During the study period from 1 May 2025 to 30 April 2026, patients undergoing *Leptospira* testing at Government

Medical College, Ongole, were consecutively screened for eligibility. Patients with documented laboratory results and sufficient demographic information were considered eligible. Laboratory records were reviewed to identify repeat testing and ensure that each individual was included only once. A total of 110 unique patients fulfilled the eligibility criteria and were included in the final analysis. All included participants had available laboratory results for the primary outcome assessment.

Of the 110 participants, 30 were seropositive and 80 were seronegative, yielding an overall *Leptospira* seroprevalence of 27.3% (30/110; exact 95% CI: 19.2%–36.6%). The mean age of the study population was 10.9 ± 12.7 years, with a median age of 8 years and an age range of 1–74 years. Most participants were younger than 16 years (98/110; 89.1%), and 64 (58.2%) were male. The demographic and clinical characteristics of participants according to *Leptospira* serostatus are presented in Table 1.

Table 1

Demographic and clinical distribution of participants according to Leptospira serostatus (N = 110)

Characteristic	Total, n (%)	Positive, n	Seropositivity, %
Overall	110 (100.0)	30	27.3
Age group, years			
≤ 5	34 (30.9)	11	32.4
6-10	41 (37.3)	9	22.0
11-15	23 (20.9)	5	21.7
≥ 16	12 (10.9)	5	41.7
Sex			
Male	64 (58.2)	12	18.8
Female	46 (41.8)	18	39.1
Patient category			
Inpatient	105 (95.5)	30	28.6
Outpatient	5 (4.5)	0	0.0
District			
Prakasam	98 (89.1)	30	30.6
Bapatla	7 (6.4)	0	0.0
Guntur	3 (2.7)	0	0.0
Nellore	2 (1.8)	0	0.0

Note. Percentages are calculated using 110 unique individuals. Seropositivity percentage is within each category.

Seropositivity varied across demographic subgroups (Table 2). Twelve of 64 males (18.8%) and 18 of 46 females (39.1%) were seropositive; the difference was statistically significant (Fisher's exact $p=0.029$). Among participants aged <16 years, 25 of 98 (25.5%) were positive compared with 5 of 12 (41.7%) aged ≥ 16 years, although this difference was not statistically significant ($p=0.303$). All 30

positive cases occurred among inpatients, giving an inpatient positivity of 28.6%; none of the five outpatients tested positive ($p=0.320$). All positive participants were residents of Prakasam district, where the observed positivity was 30.6%, compared with 0% among 12 participants from other districts; this difference reached statistical significance ($p=0.034$).

Exploratory associations between selected characteristics and Leptospira seropositivity

Characteristic	Positive, n	Negative, n	Seropositivity, %	p value
Sex: male	12	52	18.8	0.029
Sex: female	18	28	39.1	
Age <16 years	25	73	25.5	0.303
Age ≥16 years	5	7	41.7	
Inpatient	30	75	28.6	0.320
Outpatient	0	5	0.0	
Prakasam district	30	68	30.6	0.034
Other districts	0	12	0.0	

Note. Two-sided Fisher's exact tests were used. *p* values refer to the paired comparison shown in consecutive rows. Statistical significance was set at $p < 0.05$.

The month-wise distribution based on sample-collection date is shown in Table 3. Positivity varied across the year, from 0% in February to 50.0% in January. Relatively higher positivity was observed in January (50.0%), July (41.7%), May (36.4%), and September (33.3%). Positivity during the

remaining months ranged from 14.3% to 30.0%. Because the number tested differed by month and some monthly denominators remained small, these findings were interpreted descriptively rather than as evidence of a definitive seasonal pattern.

Table 3

Month-wise distribution of Leptospira seropositivity by sample-collection date

Month (May 2025–April 2026)	Tested, n	Positive, n	Seropositivity, %
May 2025	11	4	36.4
June 2025	10	2	20.0
July 2025	12	5	41.7
August 2025	10	3	30.0
September 2025	9	3	33.3
October 2025	9	2	22.2
November 2025	8	2	25.0
December 2025	7	1	14.3
January 2026	8	4	50.0
February 2026	7	0	0.0
March 2026	7	1	14.3
April 2026	12	3	25.0
Total	110	30	27.3

Note. Month-wise percentages are descriptive because denominators were small and unequal

4. Discussion

The principal finding of this one-year study was a patient-level *Leptospira* seroprevalence of 27.3% among 110 individuals tested at a tertiary-care institution in Ongole. This proportion is close to the 26.9% seropositivity reported in a 10-year tertiary-care study from North India, although direct comparison requires caution because referral patterns, case definitions, laboratory platforms, and study periods differ (8). The larger analytical sample provides a more stable estimate than a small case series and indicates that leptospirosis represented a meaningful fraction of patients selected for testing in this setting rather than an occasional laboratory diagnosis.

Indian studies have reported variable detection rates. Sethi and colleagues documented increasing leptospirosis positivity over successive years in northern India, showing that hospital-based burden can change with time and clinical recognition (9). Bhatia et al. demonstrated differing diagnostic yields across dark-field microscopy, culture, and commercial serological tests, emphasizing that seroprevalence depends partly on the assay used (10). In a multicentre investigation of acute undifferentiated fever across India, leptospirosis occurred within a diagnostic landscape that also included malaria, dengue, scrub typhus, chikungunya, and bacteraemia; limitations of routine diagnostic methods were highlighted (11). These observations support cautious interpretation of a laboratory-

based positive/negative endpoint when assay details are unavailable.

The age distribution in the Ongole dataset was predominantly paediatric, with 89.1% of participants younger than 16 years. This likely reflects testing and referral practices within the hospital rather than a population-level age pattern. A Chennai paediatric study reported antileptospiral antibody prevalence of 30.8% and demonstrated year-round occurrence among children (12), broadly supporting the relevance of leptospirosis in paediatric febrile illness. In the present analysis, females had significantly higher seropositivity than males (39.1% versus 18.8%; $p=0.029$). This association should be interpreted cautiously because these observational data cannot determine whether the difference reflects exposure patterns, healthcare-seeking behaviour, referral practices, or a true epidemiological difference.

All positive cases occurred among inpatients and residents of Prakasam district. The concentration among inpatients is consistent with clinician-directed tertiary-care testing of more symptomatic or diagnostically uncertain patients; however, the inpatient-outpatient difference was not statistically significant. Residence in Prakasam district was significantly associated with positivity in the exploratory analysis (30.6% versus 0% in other districts; $p=0.034$), but this finding likely reflects the hospital's principal catchment area and the small number of patients referred from neighbouring districts. A South Indian acute febrile illness study reported *Leptospira* IgM seropositivity of 14.7% (13), whereas a prospective study from central and eastern Uttar Pradesh found leptospirosis in 26.6% of clinically suspected patients by at least one diagnostic test (14), a figure close to the present estimate. Month-wise positivity was heterogeneous across the complete year, with relatively higher proportions in January and July, but unequal monthly denominators preclude a firm conclusion regarding seasonality.

Taken together, the findings reinforce the need to retain leptospirosis in the differential diagnosis of compatible febrile illness in and around Ongole. The full-year observation period provides a broader temporal view than a partial-year assessment, although small, unequal monthly samples limit assessment of seasonal determinants. Better linkage of laboratory results with exposure history, rainfall or flooding, occupational contact, clinical manifestations, organ dysfunction, treatment, and outcomes would transform routine surveillance into a more informative epidemiological system. Future surveillance studies using a documented testing algorithm and, where appropriate, confirmatory methods would permit stronger estimates of disease burden and clearer identification of locally relevant risk groups.

4.1 Generalizability

These findings are most directly applicable to patients selected for *Leptospira* testing at GMC, Ongole, and similar tertiary-care referral settings in coastal or south-eastern Andhra Pradesh. They should not be interpreted as community seroprevalence because testing was clinically directed and the sample remained predominantly paediatric and inpatient. Nevertheless, the one-year dataset of 110 tested individuals provides a useful local signal for diagnostic vigilance and supports broader population-based or multicentre surveillance in Prakasam district and neighbouring areas using systematic recruitment and standardized laboratory methods.

5. Conclusion

Human leptospirosis accounted for a substantial proportion of tested patients at GMC, Ongole, with a one-year patient-level seroprevalence of 27.3%. Most tested individuals were children, and all seropositive cases were identified among inpatients and residents of Prakasam district. Female participants showed significantly higher seropositivity than males, while the age-group and inpatient-outpatient comparisons were not statistically significant. Month-wise positivity varied across the year, with relatively higher proportions in January and July, although unequal monthly denominators limit seasonal inference. These findings support maintaining leptospirosis in the differential diagnosis of compatible acute febrile illness, strengthening routine laboratory surveillance, and improving linkage of laboratory data with clinical, exposure, treatment, and outcome information across the hospital catchment area.

6. Limitations

This study has limitations. It was conducted prospectively at a single tertiary-care institution, but clinician-directed testing and hospital referral patterns may have introduced selection bias; the observed proportion therefore cannot be interpreted as community prevalence. Several subgroup denominators remained small, limiting statistical precision. Detailed information on assay platform, kit characteristics, exposure history, clinical manifestations, treatment, and outcomes was unavailable in the analytical dataset. Although the observation period covered an entire year, unequal monthly testing volumes limit definitive assessment of seasonality.

7. Recommendations

GMC, Ongole should continue strengthening year-round leptospirosis surveillance using a standardized testing algorithm and complete recording of the assay performed, date of illness, exposure history, occupation, animal contact, rainfall or flood exposure, clinical severity, treatment, and outcome. Clinicians should consider leptospirosis in patients with compatible acute febrile illness, particularly when

epidemiological exposure is present. Laboratory-positive cases should be linked with district surveillance systems to identify clustering and changing transmission patterns. Future research should use larger prospective cohorts, systematic enrolment, and appropriate confirmatory testing to estimate seasonal burden, identify independent risk factors, and evaluate complications, treatment response, and short-term outcomes in Prakasam district.

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List of abbreviations

CI – confidence interval
 IEC – Institutional Ethics Committee
 IP – inpatient
 OP – outpatient
 SD – standard deviation
 ELISA – enzyme-linked immunosorbent assay
 IgM – immunoglobulin M
 MAT – microscopic agglutination test
 PCR – polymerase chain reaction

Conflict of interest

The authors declare no conflict of interest.

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Data availability

De-identified data supporting the findings of this study are available from the corresponding author on reasonable request, subject to institutional and ethics requirements.

Author contributions

Dr. Sulakshana Sony Cheemala: Conceptualization, study design, methodology, data collection, literature review, interpretation of findings, manuscript drafting, and critical revision of the manuscript.

Dr. Vijaya Swetha Vuppapapati: Methodology, data acquisition, data analysis and interpretation, literature review, manuscript preparation, critical review, correspondence with the journal, and final approval of the manuscript.

Dr. N. Padma Priya: Supervision, study methodology, academic guidance, interpretation of results, critical intellectual review of the manuscript, and final approval of the version to be published.

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