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Original Research Article

PROPORTION OF LEPTOSPIROSIS USING MOLECULAR METHODS AMONG ADMITTED PATIENTS WITH ACUTE FEBRILE ILLNESS IN TERTIARY CARE CENTER – CENTRAL KERALA

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ABSTRACT

Background: Leptospirosis is an endemic infection in tropical countries and often underdiagnosed and underreported due to lack of diagnostics for early detection. Polymerase chain reaction (PCR) and appear to be more sensitive and can detect leptospirosis in the early phase of illness. This study was aimed to assess the proportion of leptospirosis using PCR among admitted patients with acute febrile illness. **Materials and Methods:** Study was conducted at Government Medical College, Ernakulam, a tertiary care centre in central Kerala included 221 patients admitted with febrile illness less than five days duration from July 2025 to January 2026.Clinical history collected using predefined Performa. DNA extracted from EDTA Blood samples were subjected to PCR for the detection of leptospirosis. **Result:** Out of 221 patients, target genes for leptospira were found to be amplified in 23 cases, with positivity rate of 10.4%. Lab parameter analysis exhibit complexity of the disease in positive cases. **Conclusion:** This study found significant role of PCR in early diagnosis of leptospirosis. Also designated that outrageous positivity rate in study area. it warrants inclusion of molecular methods for detection of leptospirosis in screening panels to differentiate acute febrile illness.

INTRODUCTION

Acute febrile illness (AFI) ranges from self-limiting illness to life-threatening infection.[1] Leptospirosis is a common cause of febrile illness globally, caused by pathogenic spirochetes of the genus Leptospira. Leptospirosis is one of the most important zoonotic bacterial infections. It is an endemic infection in tropical countries and in some temperate regions and is prevalent globally. [2,3] The epidemiology of infection is complex and dynamic, comprising of humans, animals, and contaminated soil and water bodies. It is a widespread and potentially fatal zoonosis that can cause large epidemics after heavy rainfall and flooding.[4-7]

The existing literature highlights the growing burden of leptospirosis across various regions of India,[8-11] but there is a lack of specific data for molecular diagnosis for leptospirosis in Central Kerala, leaving a significant gap in understanding the regional and seasonal epidemiology of leptospirosis in this region. Leptospirosis is often underdiagnosed and underreported because its clinical symptoms in humans are non-specific.[12] The lack of reliable diagnostic tools for early detection greatly affects disease recognition, especially in patients with acute febrile illness and vague symptoms. Molecular diagnostic methods like Polymerase Chain Reaction (PCR) provide quick and sensitive detection of leptospiral infection in the early stages of the disease.[13,14] Since leptospirosis is a treatable condition, early diagnosis and prompt initiation of the right antibiotic therapy are crucial to improve patient outcomes and lower the risk of death associated with the disease.[15]

If proportion of Leptospirosis is high in our study it warrants inclusion of molecular methods for detection of leptospirosis in screening panels to differentiate acute febrile illness.

A comprehensive approach is vital for effectively managing acute febrile illness, ensuring timely diagnosis, appropriate treatment, and preventive measures, especially crucial in resource-limited regions where healthcare challenges are most pronounced.

Objectives

Estimate the proportion of leptospirosis using PCR among admitted patients with acute febrile illness in Government Medical College, Ernakulam.

Describe the clinical and demographic profile of the sample population.

MATERIALS AND METHODS

A hospital based cross sectional study was conducted from July 2025 to January 2026 at VRDL, microbiology department, Government medical college Ernakulam.

Case Definition of Acute febrile fever: Sudden onset of fever of 35ºC or 95 º F with a duration of less than 5 days.

A total of 221 participants who met the case definition criteria and provided informed consent were included in the study. The sample size was calculated using the formula $n = Z^2 \times p \times q/d^2$, based on a reported leptospirosis prevalence of 10.29% from a study conducted in North India.[16]

Sample collection and storage: All EDTA blood samples included in the study were subjected to PCR for Leptospirosis.

EDTA blood, was centrifuged at 3000 g for 20 min at room temperature for separation of plasma. PCR was done as a two -step procedure, DNA extraction and PCR amplification.

Assay kits and Instrument used: Extraction kit: QIAamp® DNA Mini Kit (QIAGEN; Germany).

PCR kit: DIAGSure Leptospirosis Real time PCR kit

Instrument: Biorad CFX 96

Batch number, lot number and expiry were noted.

DNA Extraction: All samples were brought to room temperature. Spin column-based extraction method was used. DNA was eluted in 50 µl elution buffer from 200 µl of sample.

Real time PCR: DNA elutes used for PCR, using DIAGSure Leptospirosis Real time PCR kit. Kit contains endogenous internal control which is used as an extraction and PCR inhibition control, probe labelled with a fluorophore Cy5, TaqMan Mastermix and Primer probe mix specific to detect the LipL32 and 16SrRNA genes of Leptospira spp. Probe for LipL32 labelled with the fluorophore channel FAM and 16SrRNA probe labelled with fluorophore channel HEX.

The limit of detection is 30 DNA copies/reaction under in-vitro condition. CT cut-off for fluorescent channel FAM (Lip32 gene) and HEX (16SrRNA) is ≤ 40. CT cut-off of fluorescent channel Cy5 for internal control is ≤ 35.

Steps were performed as per manufactures guidelines -in a specific order in distinct areas designated for each to minimize the risk of sample contamination and ensure accuracy of the results.

Data collection and entry: Informed consent was obtained from the patient for including them in the study. Clinical details were collected and entered into the proforma. The same were numerically coded and entered in Microsoft Excel spreadsheet.

Data Analysis: Qualitative data were analysed using proportion and percentages. 95% confidence interval were also estimated.

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RESULTS

a) Positivity Rate (N = 221)

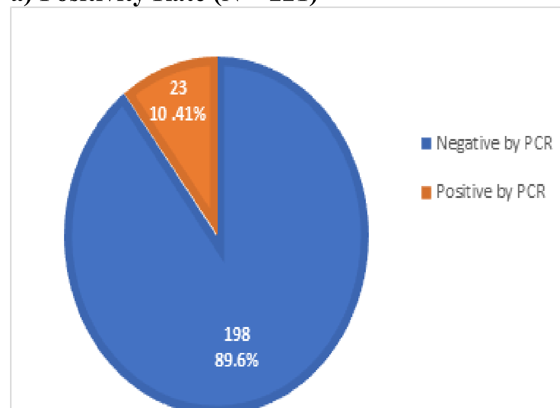

Figure 1: Leptospira PCR Positivity rate

Leptospira PCR positivity was 10.41%
95% confidence interval: 6.4%–14.5%.

b) Age distribution

Figure 2: Age distribution of the study population and positive cases

Highest percentage of study population and positive cases were among the age group 46-60 years,14.8%.
95% confidence interval: 5.9%–23.7%

c) Gender Distribution

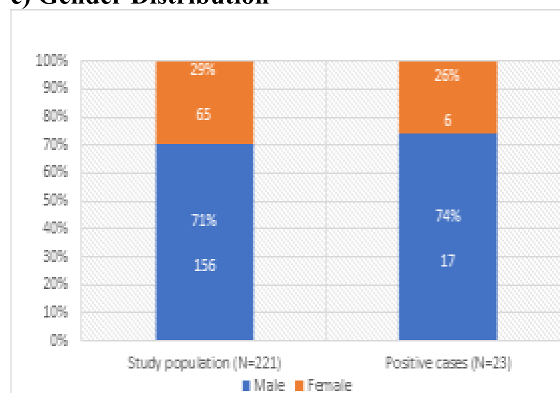

Figure 3: Gender distribution among study population and positive cases

Males dominated in both the study population and in positive cases.
10.9% Positivity in male.

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95% confidence interval :6.0%–15.8%

d) Month wise distribution

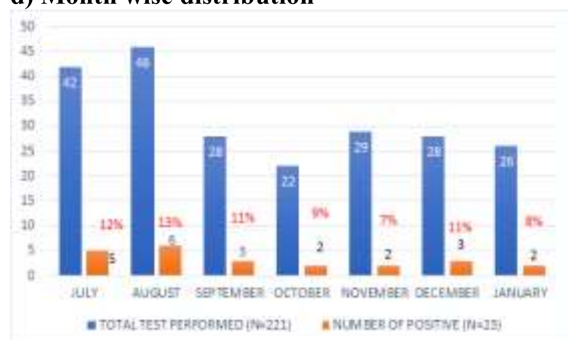

Figure:4 Month wise distribution of study population and leptospirosis positive cases

Both acute febrile illness and leptospirosis cases were predominant in the months July and August.

The positivity rate during the monsoon season (July, August and September) was high 12.1% (95% CI: 6.2%–18.0%) compared to 8.6% (95% CI: 3.2%–13.9%) during the post-monsoon season (October, November, December and January).

e) Clinical features

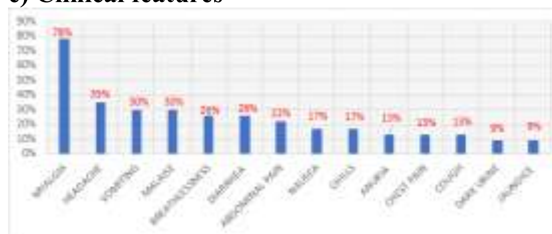

Figure 5: Clinical features in positive cases

Myalgia, headache, vomiting, malaise, diarrhea, breathlessness and abdominal pain were the major symptoms of the study population, apart from fever. Among the positive cases also, myalgia (78%) was the predominant symptom apart from fever. Diarrhea and abdominal pain were found in around 24% of positive cases. The frequent combinations of clinical features in positive cases other than fever were myalgia, headache, vomiting, malaise, breathlessness, diarrhea and abdominal pain.

f) Lab parameters

Table 1: Lab parameters in positive cases (N=23)

Lab parameters	Number of frequencies	Percentage	95% Confidence Interval
Elevation in Urea	23	100%	85.2%–100%
Hyperbilirubinemia	21	91.30%	79.8%–100%
Elevation in creatine	19	82.60%	67.1%–98.1%
Thrombocytopenia	18	78.26%	61.4%–95.1%
High SGOT	14	60.86%	40.9%–80.8%
Low Hb level	12	52.17%	31.8%–72.5%
High SGPT	11	47.82%	27.5%–68.2%

In majority of positive cases, there was derangement of lab parameters, frequent combination being elevated urea, bilirubin, creatine and thrombocytopenia. Urea was elevated in all positive cases, closed followed by hyperbilirubinemia in (91%).

DISCUSSION

Using a standardized study design and PCR diagnosis, we showed that leptospirosis was a common cause of febrile illness and evaluated the 10.40% of positivity. Similar positivity rate was reported by Chaudhry et al,[16] (2013) study conducted in a tertiary care hospital in New Delhi, India, this study states, PCR from blood was positive in 14 of 136 (10.29 %) cases. Positivity rate of current study shedding light on the burden of leptospirosis and its endemic nature in central Kerala.

Age distribution among positive cases in this study strongly support that adults age group,46-60 engaging in outdoor activities have a higher risk of exposure to infections (39%). This trend was also observed in a cross-sectional study conducted in a tertiary care centre, Government Medical College, Trivandrum, done by Mohammed Muhsin et al. (2017).[17] In contrast study done by Sultana et al. (2024),[18] notice different trend in age distribution among leptospirosis positive cases, positivity was significantly lower in age group 46-60(17%), as per this study leptospirosis was more commonly found in the age groups16–30 years (38.6%).

Male predominance was observed in study population and in positive cases. Similar observations were documented by various studies, Delight et al. (2024),[19] Arumugam et al. (2011),[20] Thalva et al (2017).[21] This may be due to the fact that male have more outdoor activity and more occupational exposure. This observation denied in the study by Dung et al (2022),[22] which stated more females were observed to be infected than males.

Study was reported a sharp increase in leptospirosis cases in July and August month. Similarly Das et al. (2025),[23] reported higher rates of leptospirosis during July, August and September, coinciding with monsoon season. The risk raised significantly during Kerala's monsoon months due to flooding and waterlogging, reported by James et al (2018).[24] This infection transmitted from animals to humans, particularly through contact with water contaminated by rodent urine. Kerala's southwest monsoon typically brings intense rainfall, increasing the risk of waterborne diseases. Stagnant water and improper

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waste disposal further aggravate the spread, Nair et al (2025).[25]

Apart from fever, majority of the patients present with myalgia similar observations were reported in studies by Lane et al.(2016)[26] and Haake et al.(2015).[27] According to the study carried out by Becirovic et al (2020),[28] the most common symptoms in leptospirosis patients were fever (95,6%), headache (93,8%), malaise (87,5%) and myalgia (85,6%),this observation was agreed with our findings .Other than these symptoms, frequency of gastro intestinal symptoms like vomiting, nausea, abdominal pain, diarrhoea were also comparable with our study.

In the present study find out that elevated urea and creatine, hyperbilirubinemia, thrombocytopenia, decrease of haemoglobin level also Liver function tests showed elevated SGOT and SGPT were reported, this observation is consistent with a prospective study conducted among leptospirosis suspected cases by Ganvir D and Turbadkar D. (2025),[29] who reported Leptospirosis manifests as a variety of test abnormalities and clinical manifestations.

CONCLUSION

This study which focused on understanding proportion of leptospirosis using PCR among admitted patients with acute febrile illness (AFI)in territory care hospital, central Kerala during July 2025 to January 2026. Proportion was found to be 10.4%. Study provided sociodemographic, clinical and epidemiological characters consistent with reports published. It was observed that males of the age group of 46-65 were the most affected by the disease during the study period. With respect to clinical characteristics, disease manifestations were commonly observed irrespective of age and gender. Findings of this study was strong enough for inclusion of molecular methods for detection of leptospirosis in screening panels of AFI. So that early detection and effective treatment can be given so as to reduce mortality.

Limitation of the study

As the study was conducted in a single tertiary care centre, the generalizability of the findings may be limited.

The relatively short study duration of seven months may limit the robustness and generalizability of the findings.

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