Section: Microbiology

Original Research Article

SEROPREVALENCE OF SCRUB TYPHUS AMONG CASES OF ACUTE UNDIFFERENTIATED FEBRILE ILLNESS IN A TERTIARY CARE HOSPITAL IN CENTRAL KERALA

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ABSTRACT

Background: Scrub typhus is a major cause of Acute Febrile illness (AFI) in tropical countries, which is caused by Rickettsial bacteria name *Orientia tsutsugamushi*. It is often underdiagnosed due to overlapping clinical features with other acute febrile illnesses and a low index of suspicion among clinicians. **Objective:** To determine the Seroprevalence of Scrub typhus among cases of acute undifferentiated febrile illness in a tertiary care hospital in Central Kerala. **Materials and Methods:** This cross-sectional hospital-based study was conducted in a tertiary care hospital from June 2025 to January 2026 at central Kerala. A total of 318 samples from Acute febrile cases which were negative for Dengue fever, Leptospirosis and Enteric fever were included. Detection of scrub typhus IgM antibody was performed using the ELISA method. **Results:** Out of 318 acute febrile illness (AFI) cases, 17 tested positive for scrub typhus IgM antibodies, resulting a seroprevalence of 5.3%. The majority of cases were in the 16–30 years age group, with a higher proportion among females. The highest positivity was observed in patients presenting between 5 -10 days of illness duration (70.6 %), and most cases occurred during the post-monsoon season (58.8%). Common clinical features included fever, vomiting, myalgia, abdominal pain, headache, chills, and thrombocytopenia. Coronary artery disease was the co-morbidity noted in the study. Laboratory findings showed predominant hepatic involvement, with elevated direct bilirubin, SGOT, and SGPT. **Conclusion:** Scrub typhus is a significant cause of acute febrile illness in Central Kerala, particularly during the post-monsoon period, and IgM antibodies against Scrub typhus are best detected between 5 – 10 days of illness in this study. Hepatic involvement is common. Routine screening in AFI cases for Scrub typhus is recommended for early diagnosis and management.

INTRODUCTION

It is found that many of the acute febrile illness (AFI) cases are remaining as undifferentiated fever (no etiological diagnosis) even at the time of discharge of the patient. Some deaths are also occurring among these undifferentiated fever.

The existing literature highlights the growing burden of scrub typhus in various regions of India, but there is a lack of specific data for Central Kerala, leaving a significant gap in understanding the regional epidemiology of scrub typhus in this area. Addressing this gap is vital for optimizing diagnostic protocols, enhancing clinical awareness, and implementing public health measures to manage scrub typhus effectively in Central Kerala.

The overlap of scrub typhus symptoms with other AFI causes like dengue, enteric fever and leptospirosis further complicates diagnosis in endemic regions. [1,2] If we could find out their etiology as Scrub Typhus which is a treatable and curable condition, it would be helpful to the patient. In addition, if the seroprevalence of Scrub Typhus is considerably high, all acute febrile illness cases may have to be investigated routinely for Scrub Typhus

Acute febrile illness (AFI) is common in tropical countries but the etiology may vary from country to country. Similarity in clinical presentation, lack of focal signs and symptoms, and scarce diagnostic tools make diagnosis a challenge.[3] In many areas of

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developing countries, where diagnostic facilities are limited, etiologies of acute febrile illness (AFI) remain largely unknown. Physicians often diagnose patients presumptively based on clinical features and assumptions regarding circulating pathogens. These AFI that includes scrub typhus, dengue fever, malaria, enteric fever, and leptospirosis among others cause significant mortality and morbidity.[4] Among the causes of AFI, Scrub typhus has re-emerged as a major cause of acute undifferentiated febrile illnesses (AUFI) with the case fatality rate (CFR) ranges from 1.3% to 33.5% depending on the organ involvement and complications present.[5]

Scrub typhus is a zoonotic infection that is caused by a rickettsial bacteria called Orientia tsutsugamushi and transmitted to humans through chigger mites. Human beings get infected accidentally following exposure to mite-infested rural and suburban areas. It is often acquired during recreational, occupational or agricultural exposure because crop fields are an important reservoir for transmission.[6] The clinical symptoms of Scrub typhus are fever, headache, myalgia, malaise, rash and lymphadenopathy which are also commonly seen in other acute febrile illnesses like malaria, enteric fever, leptospirosis and dengue fever.[7] In some patients, an eschar may develop at the site of chigger feeding, the presence of which is diagnostic. Unfortunately, it is reported to be less frequently observed especially in South Asian patients than in East Asian.[8]

If left untreated, Scrub typhus can lead to severe complications including multiple organ dysfunction syndrome(MODS), acute respiratory distress syndrome (ARDS), hepatitis, myocarditis, and meningoencephalitis.[9] In Kerala, the first case of scrub typhus reported from Thiruvananthapuram district in the year 2000. Since then, scattered cases have been reported every year from Kerala.[11] Scrub typhus is seen predominantly in the monsoon and post monsoon months in Kerala.[12]

Scrub typhus is under- diagnosed in India due to lack of awareness, a low index of suspicion among clinicians, paucity of confirmatory diagnostic facilities and clinical symptoms mimicking other more prevalent diseases like malaria, enteric fever, dengue or leptospirosis.[6]

The methods available for diagnosis are Weil Felix test, indirect immunofluorescence assay, enzyme‑linked immunosorbent assay (ELISA), rapid test like immunochromatographic tests, culture, polymerase chain reaction (PCR) and LAMP. Weil Felix test is the oldest test, which is cheap, easy to perform, but it lacks sensitivity. Indirect Immunofluorescence assay is the gold standard test, but the cost and need of technical expertise limit the use. Culture requires Biosafety Level‑3 and is, therefore difficult. PCR, although sensitive and specific, is expensive.[10] Hence ELISA is the most popular method currently used.

The mortality rate of untreated cases ranges between 7- 13%. However, early diagnosis and treatment with antibiotic like doxycycline or azithromycin reduce morbidity and mortality.[7] Given the diagnostic challenges and the treatable nature of scrub typhus, estimating its seroprevalence among AFI cases in tertiary care settings is crucial.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted from June 2025 to January 2026 at the Viral Research and Diagnostic Laboratory (VRDL), Department of Microbiology, Government Medical College Ernakulam, among patients admitted with acute febrile illness (AFI).

Serum samples received in the Microbiology laboratory during the study period from AFI cases, defined as sudden onset of fever ≥38°C (100.4°F), which tested negative for dengue fever, leptospirosis, and enteric fever, were included in the study. Hemolysed, lipemic, contaminated, and insufficient quantity samples were excluded from the study.

A total of 318 study samples (Sample size calculated using the formula: $n = z^2 \times pq \div d^2$, based on a study conducted in South Kerala,, where the prevalence of scrub typhus was 15.7%)[10] were included in this study. Study was conducted after obtaining ethical clearance from the institute (IEC No: 22/25 dated 23.06.2025).

Serum samples tested negative for Dengue NS1 and IgM ELISA, Leptospira IgM ELISA and Widal test for enteric fever were stored at -20^{O}C and subjected to Scrub typhus IgM ELISA test once in a week.

ELISA was performed using Scrub Typhus Detect™ In-Bios International Inc (WA, USA) ELISA kit. Lisa wash 4000 microplate washer (Plate washer) and Bio-Rad PR 4100 Absorbance ELISA plate analyser (Plate Reader) were the instruments used to perform the ELISA. This was a qualitative ELISA test for the detection of IgM antibodies specific to *Orientia tsutsugamushi* in human serum. The ELISA procedure was carried out following the manufacturer's guidelines.

ELISA plate with 96 wells was used in which the first 5 wells were dedicated to negative controls, positive controls and blank. All the samples needed to be diluted with sample diluent provided with the kit in 1:100 ratio. 100µl diluted samples were added to the respective wells which were precoated with the recombinant antigens of *O. tsutsugamushi*. After 30 minutes incubation at 37°C, washing the wells 6 times was carried out with wash buffer using ELISA plate washer. Further 100µl of diluted polyclonal Goat anti-human IgM antibodies labelled with the enzyme horseradish peroxidase (HRP) were added to each well and incubated again for 30 minutes at 37°C. After 6 times wash by wash buffer, the wells were incubated with 150µl of EnWash reagent at room temperature (20–25°C) for 10 minutes. Once again wash step was performed for 6 times with wash buffer. Further 100µl of tetramethylbenzidine (TMB) substrate was added to each well and incubated at room temperature in dark place for 10 minutes. After

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the incubation, an acidic stopping solution was then added to cease the reaction and the absorbance was read at 450nm wavelength using ELISA plate reader. The Optical density (OD) values of samples obtained were compared with the cutoff value to determine if the sample was positive or negative for Scrub Typhus IgM antibodies.

The cutoff value was calculated using the formula: Cutoff value = Optical density (OD) value of the negative control (NC) + 0.4 which represented the geometric mean of Scrub typhus IgM in our geographical region [11, 12]. Samples with OD values ≥ cutoff were considered positive for Scrub typhus.

Data collection method: Clinical details of the study samples were collected from the case files and entered into the proforma. The same were numerically coded and entered into a Microsoft Excel spreadsheet. Permission from the Medical Superintendent was obtained for the same.

Statistical analysis: Qualitative data were analysed using proportions and percentages. The Chi-square test and Fisher's exact test were used to determine the association between the variables. The analysis was performed using Jamovi software – version 2.7.24. The level of statistical significance was considered as a p value < 0.05 in this study.

RESULTS

This hospital based cross sectional study was conducted with the objectives of estimating the seroprevalence of Scrub typhus among cases of acute undifferentiated febrile illness at Government medical college, Ernakulam and to describe the clinical and demographic profile of the study population.

Out of 318 study samples, 17 samples (5.3%) were positive for Scrub Typhus IgM ELISA test with 95% Confidence interval.

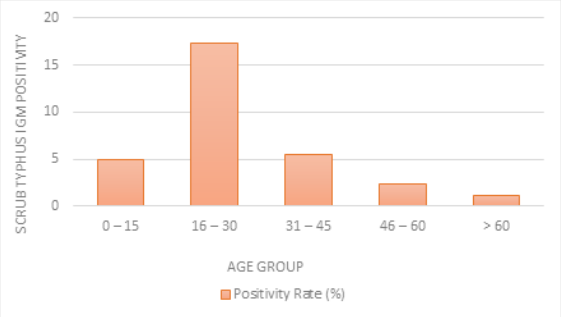

Figure 1: Age distribution among IgM positive patients for Scrub typhus (n=17)

IgM positivity for Scrub typhus was more among the 16 – 30 years age group, though participants mainly belonged to > 60 years age group. P value was 0.002, showing statistically significant association.

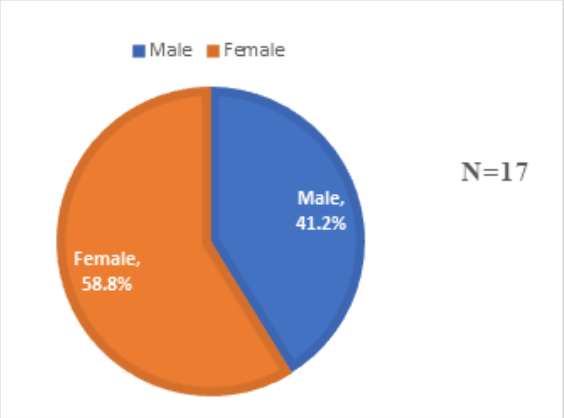

Figure 2: Gender wise distribution among the Scrub typhus IgM positive cases

Scrub typhus positivity was observed predominantly among females (58.8%) despite male participants comprising majority of the study population. P value was 0.014, showing statistically significant association.

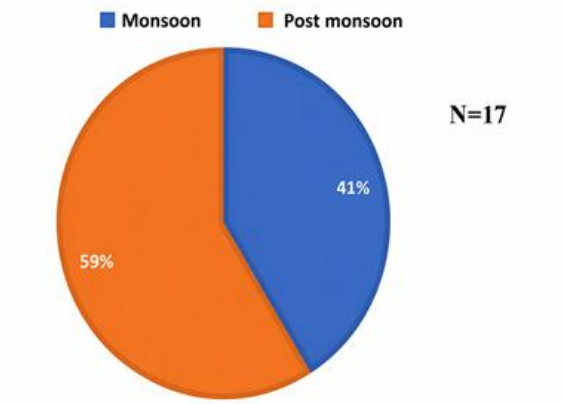

Figure 3: Distribution of Scrub Typhus IgM Positive cases in Monsoon and Post monsoon Period

Seasonal distribution showed a statistically significant association between scrub typhus positivity and post-monsoon period (p = 0.013), with the majority of positive cases occurring during the post-monsoon season.

Table 1: Distribution of Scrub typhus positivity according to Duration of illness(n=17)

Duration of illness	Scrub typhus positive cases (%)
1–4 days	3(17.7)
5–10 days	12(70.6)
11–15 days	2 (11,8)
>15 days	0 (0)

The highest scrub typhus positivity rate was observed in patients presenting between 5–10 days from the onset of illness (70.6%). P value was 0.039, showing statistically significant association.

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Table 2: Clinical presentation of Scrub typhus positive cases apart from fever

Clinical presentations	Scrub positive cases (n =17)	Percentage (%)
Vomiting	11	64.7
Myalgia	8	47.1
Thrombocytopenia	7	41.2
Abdominal Pain	5	29.4
Headache	4	23.5
Chills	4	23.5
Giddiness	3	17.7
Cough	2	11.8
Rash	2	11.8
ARDS	2	11.8
Chest Pain	2	11.8
Loose Stool	2	11.8
Breathlessness	2	11.8
Splenomegaly	2	11.8
Malaise	1	5.9
Hepatomegaly	1	5.9
Altered Sensorium	1	5.9
Tachycardia	1	5.9

All cases included had fever. Most of the positive patients had more than one clinical features and the most frequent combination were Myalgia, Vomiting, Abdominal Pain and Chills.

Table 3: Co-morbidities among scrub typhus positive cases(n=17)

Co-morbidities	Scrub typhus positive cases n, (%)
Hypertension	1 (5.9)
Diabetes	1 (5.9)
Coronary artery disease (CAD)	2 (11.8)
Chronic Obstructive Pulmonary Disease (COPD)	1 (5.9)

In 2 patients had more than one co-morbidities such as coronary artery disease (CAD) and Hypertension in one and Chronic Obstructive Pulmonary Disease (COPD) with CAD in the other.

Table 4: Abnormal Laboratory Parameters in Scrub Typhus Positive Cases(n=17)

Lab Parameters	Scrub typhus positive cases n, (%)
Elevated Direct Bilirubin	14 (82.4)
Elevated SGOT	10(58.8)
Elevated SGPT	8(47.1)
Elevated Urea	6(35.3)
Elevated Serum Creatinine	1(5.9)

In most of the patients, lab parameters indicating hepatic involvement such as increased Bilirubin, SGOT and SGPT were deranged. Renal derangement as evidenced by increased blood urea and Serum Creatinine was comparatively less.

DISCUSSION

This study evaluated the seroprevalence of scrub typhus among acute undifferentiated febrile illness (AUFI) cases in a tertiary care hospital in Central Kerala and demonstrated a seroprevalence of 5.3%. The findings emphasize that scrub typhus continues to be an important cause of AFI in Kerala, despite being frequently underdiagnosed because of its nonspecific clinical presentation and overlap with other febrile illnesses.

The seroprevalence observed in this study was lower compared to several studies conducted in India. Previous Indian studies conducted between 2010 and 2020 have reported scrub typhus seroprevalence rates among AFI cases ranging from 5% to 56%,[5,13 -23] with studies from Kerala reporting prevalence rates of 11%–19%.[10,13-15] In contrast, the prevalence observed in the present study was comparable to the findings of one of the studies from Thiruvalla (2021),[12] whose seroprevalence was 5.3%. The lower prevalence in the current study may be attributed to regional epidemiological differences, climatic variations and seasonal distribution during the study period, differences in study population and lesser duration of illness.

Age distribution analysis in the present study demonstrated significantly higher positivity among the 16–30 years age group ($p = 0.002$). Similar findings were reported by other studies,[14,15,23] from India where young adults constituted the major affected population. The predominance among younger adults in the present study may be related to increased outdoor and occupational exposure to mite-infested vegetation, particularly in agricultural activities.

The present study demonstrated a statistically significant association between gender and scrub typhus positivity ($p = 0.014$), with females accounting for 58.8% of positive cases, which is comparable to the findings reported by a study from Chandigarh.[20] However, most other Indian studies

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have reported male predominance among scrub typhus cases.[11,13,15-18,21] Variations in gender distribution may be influenced by local occupational patterns and environmental exposure.

Seasonal distribution in the present study showed a statistically significant association with scrub typhus positivity (p = 0.013), with predominance during the post-monsoon period. Similar seasonal clustering during monsoon and post-monsoon months has been reported by many studies,[11,13,,15,16,18,20] from Kerala. The findings of the present study further support the established seasonal trend of scrub typhus occurrence in South India.

The present study demonstrated a statistically significant association between duration of illness and scrub typhus positivity (p = 0.039), with the highest positivity observed among patients presenting between 5–10 days of illness duration.[24] This finding corresponds with the natural course of IgM antibody production, which becomes detectable after the first week of illness.

Scrub typhus is a disease, where the clinical features is protean and hence varied presentations were observed in different studies conducted. The commonest clinical features observed was fever in all the patients and the other symptoms were vomiting, myalgia, thrombocytopenia, abdominal pain, head ache and chills which were in agreement with other studies conducted in Kerala and other parts of India.[11,13- 15,17-21] Major co-morbidities found in our study were CAD, Hypertension, Diabetes and COPD. We could not find any observation regarding co-morbidities in any of studies cites and hence could not compared. Majority of the Scrub typhus positive patients showed deranged liver function which was in comparison with most of the studies.[11,17,18,20]

CONCLUSION

Seroprevalence of Scrub typhus in our study was 5.3%, but it varies different parts of the country. Statistical significance association could be shown for age and gender distribution, seasonality and duration of illness in our study. Majority of IgM positive patients showed hepatic derangement which was in agreement with other studies cited.

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