

Original Research Article

SEROPREVALENCE AND CLINICAL-LABORATORY CORRELATES OF SCRUB TYPHUS AMONG FEBRILE INPATIENTS IN A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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Received : 19/06/2026
Received in revised form : 06/08/2026
Accepted : 21/08/2026

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DOI: 10.70034/ijmedph.2026.3.643

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (3); 4123-4129

ABSTRACT

Background: Scrub typhus is an important cause of acute febrile illness in India and may present with nonspecific clinical manifestations. This study assessed the seroprevalence of Scrub Typhus and its clinical and laboratory correlates among febrile inpatients.

Materials and Methods: A prospective study was conducted among 100 febrile inpatients at Coimbatore Medical College and Hospital from July 2021 to June 2022. Serum samples were tested for *Orientia tsutsugamushi* IgM antibodies using Scrub Typhus Detect™ IgM ELISA. Clinical and laboratory parameters were correlated with serological findings.

Results: Seventeen patients (17%) were IgM positive. Among positive patients, 70.6% were male. Eschar and lymphadenopathy were significantly associated with positivity. Thrombocytopenia, elevated urea and creatinine, and abnormal liver-function tests were significantly more frequent among positive patients.

Conclusion: Scrub Typhus contributed substantially to febrile illness. Early recognition and IgM ELISA testing can facilitate timely diagnosis and treatment.

Keywords: Scrub Typhus, IgM ELISA, Seroprevalence, Renal dysfunction, Hepatosplenomegaly, Tamil Nadu.

INTRODUCTION

Acute febrile illness is a major clinical and public-health problem in tropical countries, particularly in India, where patients may present with overlapping manifestations caused by dengue, malaria, enteric fever, leptospirosis, viral infections and rickettsial diseases.^[1,2] Scrub Typhus is an important but frequently under-recognized cause of such illness. It is a zoonotic, vector-borne infection caused by the obligate intracellular bacterium *Orientia tsutsugamushi* and transmitted primarily by infected larval trombiculid mites.^[3] The disease is traditionally associated with the geographical region known as the Tsutsugamushi triangle, although its recognized distribution has expanded considerably.^[4] The dissertation source describes Scrub Typhus as a re-emerging infection with

reports from several Indian states, including Tamil Nadu.^[2] The clinical spectrum of Scrub Typhus ranges from an uncomplicated febrile illness to severe systemic disease.^[5] Fever, headache, myalgia, rash, gastrointestinal symptoms and lymphadenopathy are common manifestations.^[6] Eschar at the site of mite feeding is considered a characteristic clinical finding; however, its frequency varies considerably according to geographical region and may be absent in many patients.^[7] Severe disease can involve multiple organ systems, producing complications such as hepatitis, acute kidney injury, pneumonitis, acute respiratory distress syndrome, myocarditis, meningoencephalitis, disseminated intravascular coagulation and shock.^[8] Diagnosis can be challenging because the initial clinical manifestations are nonspecific. Conventional

bacterial culture is not routinely useful, and isolation of *O. tsutsugamushi* requires specialized cell-culture facilities and high-containment laboratory infrastructure.^[9] Molecular assays such as polymerase chain reaction can detect infection, particularly during the early phase, but their availability is limited in many healthcare facilities.^[10] Serological methods therefore remain important in routine diagnostic practice. Several serological methods have been described, including the Weil–Felix test, indirect immunofluorescence assay, immunochromatographic tests and ELISA.^[11,12] The Weil–Felix test has limited sensitivity and specificity, whereas immunofluorescence requires specialized equipment and trained personnel.^[13,14] IgM ELISA is particularly useful for detecting recent infection and is more practical for routine hospital laboratories.^[15] The study used Scrub Typhus Detect™ IgM ELISA based on recombinant *O. tsutsugamushi* antigens. Previous studies from India have reported considerable geographical variation in Scrub Typhus seroprevalence.^[16] The dissertation cites reported prevalence values from different Indian regions and notes increasing recognition of Scrub Typhus with improved use of ELISA and molecular diagnostic methods.^[17] Despite its clinical importance, Scrub Typhus can remain unrecognized because many patients lack an eschar and present with nonspecific fever. Identifying clinical and laboratory parameters associated with seropositivity can therefore help clinicians recognize the disease earlier.^[18] The present study was undertaken to determine the seroprevalence of Scrub Typhus among febrile inpatients in a tertiary-care hospital in Coimbatore, Tamil Nadu, using IgM ELISA, and to evaluate the association between Scrub Typhus positivity and demographic, clinical and laboratory characteristics.

MATERIALS AND METHODS

Study design and setting

This was a prospective hospital-based observational study conducted in the Department of Microbiology, Coimbatore Medical College and Hospital, Coimbatore, Tamil Nadu. The study was performed over a period of one year, from July 2021 to June 2022. Patients admitted to the medicine and paediatric wards with febrile illness were evaluated for possible Scrub Typhus.

Ethical considerations

Institutional Ethical Committee approval was obtained before initiation of the study. Written informed consent was obtained from the participants or reliable informants before sample collection.

Study population

A total of 100 febrile patients were included in the study.

Inclusion Criteria

Patients fulfilling one or more of the following criteria were included:

1. Fever for more than five days.
2. Fever associated with eschar or rash.
3. Fever associated with hepatosplenomegaly or lymphadenopathy.
4. History of travel to hilly or known endemic areas.

Exclusion Criteria

Patients with established alternative causes of fever were excluded, including:

- Malaria
- Dengue
- Leptospirosis
- Enteric fever
- Meningitis.

Clinical evaluation

Demographic and clinical information was recorded using a structured data collection format. Particular attention was given to duration of fever, presence of eschar, lymphadenopathy and hepatosplenomegaly. Relevant laboratory parameters including complete blood count, platelet count, renal-function parameters and liver-function tests were recorded.

Sample collection and processing

Approximately 3 mL of venous blood was collected under aseptic precautions into yellow-capped BD Vacutainer tubes following informed consent. The samples were centrifuged at 6000 rpm for 15 minutes. Approximately 1 mL of separated serum was transferred to appropriately labelled vials. Samples not tested immediately were stored at –20°C until testing.

Serological testing

Serum samples were tested using the Scrub Typhus Detect™ IgM ELISA system manufactured by InBios International Inc., USA, according to the manufacturer's instructions. The assay is a qualitative ELISA designed to detect IgM antibodies against *O. tsutsugamushi*. The assay utilizes recombinant antigen and an enzyme-labelled anti-human IgM conjugate. Following incubation and washing steps, tetramethylbenzidine substrate was added and the reaction was stopped before optical density was measured at 450 nm. The assay cut-off was calculated according to the manufacturer's procedure using the mean optical density plus three standard deviations. Samples with absorbance values above the calculated cut-off were considered reactive, while samples below the cut-off were considered non-reactive. Reactive samples were repeated for confirmation.

Laboratory parameters

The following laboratory variables were evaluated:

- Total white blood cell count
- Platelet count
- Blood urea
- Serum creatinine
- Liver-function tests.

The relationship between these parameters and Scrub Typhus IgM positivity was assessed.

Statistical Analysis

The study compared demographic, clinical and laboratory characteristics between IgM-positive and IgM-negative participants. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized using means. Statistical significance was considered at $p < 0.05$, as reported in the source dissertation.

RESULTS

Demographic characteristics

A total of 100 febrile patients were enrolled. The mean age of the study population was 29.8 ± 17.6

years, with ages ranging from 6 months to 78 years. The largest proportion of participants belonged to the 21–30-year age group (29%), followed by the 11–20-year age group (19%). Paediatric patients below 12 years accounted for 18%, while adults constituted 82% of the study population (Table 1). Females constituted 60% of the study population and males 40%. However, among Scrub Typhus IgM-positive patients, males predominated, accounting for 71%, while females constituted 29% (Figure 1). The dissertation reports a statistically significant association between sex and Scrub Typhus positivity ($p < 0.05$).

Table 1: Demographic characteristics of the study population

Demographic characteristic	Number (n)	Percentage (%)
Total participants	100	100
Age group		
<12 years	18	18.0
12–20 years	19	19.0
21–30 years	29	29.0
31–40 years	—	—
41–50 years	—	—
51–60 years	—	—
>60 years	—	—
Age		
Mean age	29.8 ± 17.6 years	—
Age range	0.5–78 years	—
Gender		
Male	40	40.0
Female	60	60.0

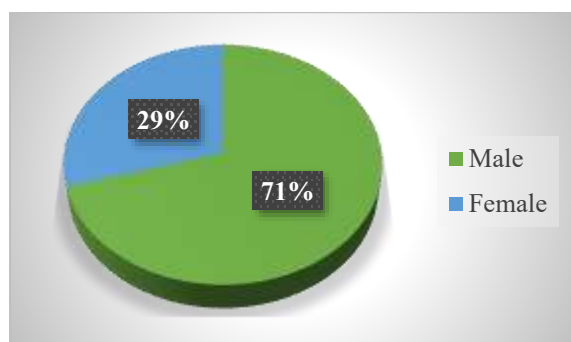


Figure 1: Gender-wise distribution of Scrub Typhus IgM-positive patients

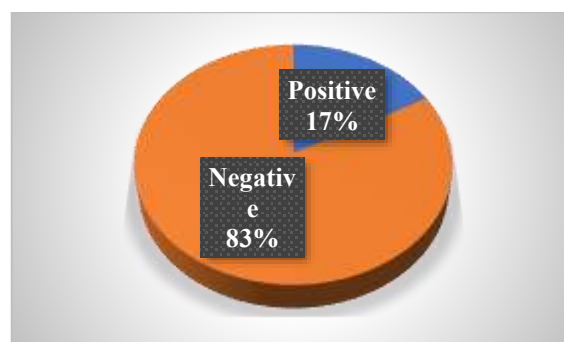


Figure 2: Detection of Scrub Typhus IgM antibodies

Seroprevalence of Scrub Typhus

Of the 100 febrile patients tested, 17 were positive for Scrub Typhus IgM antibodies and 83 were negative. Thus, the overall seroprevalence/detection rate of Scrub Typhus IgM was 17.0% (Figure 2). The finding demonstrates that approximately one in every six febrile inpatients tested had evidence of Scrub Typhus IgM positivity.

Clinical characteristics

The overall mean duration of fever was 5.9 days, with a range of 3–14 days. Among IgM-positive patients, the mean duration of fever was 6.7 days compared with 5.7 days among IgM-negative patients, and this difference was statistically significant ($p = 0.028$). Eschar was identified in five patients. All five patients with eschar were positive for Scrub Typhus IgM, while none of the IgM-negative patients had an eschar. The association was statistically significant ($p < 0.001$). Similarly, lymphadenopathy was observed in five patients, all of whom were Scrub Typhus IgM positive. No lymphadenopathy was documented among IgM-negative patients, giving a statistically significant association ($p < 0.001$). Hepatosplenomegaly was observed in 12 participants overall. Five of the 17 IgM-positive patients had hepatosplenomegaly

compared with seven of the 83 IgM-negative patients. This association was statistically

significant ($p=0.015$). [Table 2]

Table 2: Association between clinical findings and Scrub Typhus IgM positivity

Clinical parameter	IgM positive (n=17)	IgM negative (n=83)	p value
Mean fever duration (days)	6.7	5.7	0.028
Eschar present	5	0	<0.001
Lymphadenopathy present	5	0	<0.001
Hepatosplenomegaly present	5	7	0.015

Laboratory findings

The mean WBC count for the entire study population was 7079.7/ μ L. The WBC count did not differ significantly between IgM-positive and IgM-negative groups ($p=0.755$). The mean WBC count was 6869.4/ μ L in the positive group and 7122.7/ μ L in the negative group. In contrast, platelet counts were significantly lower among IgM-positive patients. The mean platelet count was 77,882/ μ L among positive patients compared with 103,349/ μ L among negative patients ($p=0.030$). Mean serum

urea was significantly higher in the IgM-positive group, at 56.3 mg/dL compared with 37.8 mg/dL among negative patients ($p=0.001$). Similarly, mean creatinine was 1.3 mg/dL among positive patients and 0.9 mg/dL among negative patients, with a highly significant difference ($p<0.001$). Liver-function abnormalities were found in 12 of the 17 IgM-positive patients (70.6%), compared with 22 of the 83 IgM-negative patients. This difference was statistically significant ($p<0.001$).

Table 3: Laboratory parameters according to Scrub Typhus IgM status

Laboratory parameter	IgM positive (n=17)	IgM negative (n=83)	p value
WBC/ μ L	6,869.4	7,122.7	0.755
Platelets/ μ L	77,882.4	103,349.4	0.030
Urea (mg/dL)	56.3	37.8	0.001
Creatinine (mg/dL)	1.3	0.9	<0.001
Elevated liver-function tests	12 (70.6%)	22	<0.001

Treatment and outcome

Among the 17 Scrub Typhus IgM-positive patients, 15 adults were treated with doxycycline and two children were treated with azithromycin. No deaths were reported among the Scrub Typhus -positive patients. Seven patients who developed respiratory distress and required intensive-care management recovered following antimicrobial and supportive treatment.

DISCUSSION

Scrub Typhus is an important cause of acute undifferentiated febrile illness in India and is increasingly recognized in South India. In the present prospective study conducted among 100 febrile inpatients at a tertiary-care hospital in Coimbatore, 17 patients (17%) were positive for *Orientia tsutsugamushi* IgM antibodies by ELISA. This finding indicates that Scrub Typhus contributed substantially to the burden of febrile illness in the study population. However, since the study was hospital based and included clinically selected patients, the observed 17% IgM positivity should be interpreted as the detection rate among the study population rather than the prevalence of Scrub Typhus in the general population. Previous studies from South India have also demonstrated a considerable burden of Scrub Typhus among hospitalized patients with acute febrile illness. Varghese et al. reported Scrub Typhus among hospitalized patients with febrile illness in South India and identified it as an important and treatable

cause of fever.^[19] Similarly, Abhilash et al. reported Scrub Typhus as one of the commonest identified causes of acute undifferentiated febrile illness in a tertiary-care hospital in South India.^[20] Differences in the proportion of positive cases between studies may be related to variations in geographical location, season, patient selection, duration of fever, inclusion and exclusion criteria, disease severity and diagnostic methods. The mean age of the participants in the present study was 29.8 ± 17.6 years, with the largest proportion belonging to the 21–30-year age group. Both children and adults were affected, indicating that Scrub Typhus is not restricted to a particular age group. Similar observations have been reported from Indian studies involving both adult and paediatric populations. Kalal et al. reported a substantial burden of Scrub Typhus among hospitalized children in South India, demonstrating that the infection should also be considered in paediatric patients presenting with prolonged fever.^[21] In the present study, males constituted 70.6% of the IgM-positive patients despite females forming 60% of the total study population. The association between male sex and Scrub Typhus positivity was statistically significant. A possible explanation may be greater environmental or occupational exposure among males. However, this interpretation should be made cautiously because detailed occupational and environmental exposure histories were not systematically assessed in the present study. Previous epidemiological studies have demonstrated associations between Scrub Typhus and exposure to

vegetation, agricultural activities and rural environments.^[22]

The mean duration of fever was significantly longer among IgM-positive patients than among IgM-negative patients (6.7 versus 5.7 days; $p=0.028$). This finding supports the importance of considering Scrub Typhus in patients with persistent fever, particularly after common causes such as dengue, malaria, enteric fever and leptospirosis have been excluded. Scrub Typhus frequently presents initially as an undifferentiated febrile illness, and the clinical features may become more suggestive only after several days of illness. Varghese et al. similarly demonstrated the importance of prolonged fever in hospitalized patients with Scrub Typhus.^[19] Therefore, persistent fever in an endemic region should prompt appropriate serological or molecular testing, even when characteristic clinical findings are absent.

Eschar was observed in five patients in the present study, and all five were IgM positive, while none of the IgM-negative patients had an eschar. The association was highly significant ($p<0.001$). This finding emphasizes the diagnostic importance of an eschar when present. However, only 5 of the 17 positive patients had an eschar, meaning that 70.6% of seropositive patients did not have a documented eschar. This is consistent with previous reports showing considerable geographical variation in the frequency of eschar. Varghese et al. reported that eschar was present only in a proportion of patients with Scrub Typhus in South India,^[19] while Chrispal et al. also demonstrated variable clinical presentation among hospitalized adults.^[22] The absence of an eschar should therefore not be used to exclude Scrub Typhus. Small lesions may occur in concealed anatomical sites and may be missed during routine examination. Careful examination of the axilla, groin, inframammary region and other intertriginous areas is therefore important. Lymphadenopathy was present in five patients, all of whom were IgM positive, giving a statistically significant association ($p<0.001$). Hepatosplenomegaly was observed in 5 of the 17 positive patients compared with 7 of the 83 negative patients, and this association was also significant ($p=0.015$). These findings are consistent with previous Indian studies describing lymphadenopathy, hepatomegaly and splenomegaly among important manifestations of Scrub Typhus, particularly in children.^[21] The presence of lymphadenopathy and hepatosplenomegaly may reflect systemic involvement of the reticuloendothelial system and should increase clinical suspicion when associated with prolonged fever. Thrombocytopenia was significantly more pronounced among IgM-positive patients, with a mean platelet count of $77,882/\mu\text{L}$ compared with $103,349/\mu\text{L}$ in IgM-negative patients ($p=0.030$). This finding agrees with previous studies in which thrombocytopenia was frequently observed in Scrub Typhus.^[19,22] However, thrombocytopenia is not

specific to Scrub Typhus and is also common in dengue, malaria and other tropical infections. Therefore, thrombocytopenia should be interpreted together with other clinical and biochemical findings. Interestingly, the mean WBC count did not differ significantly between the two groups ($p=0.755$). This differs from some earlier studies in which leukocytosis was reported as a useful predictor of Scrub Typhus.^[19] The absence of a significant difference in WBC count in the present study may be related to the small sample size and variation in disease severity. It also emphasizes that a normal WBC count does not exclude Scrub Typhus. Renal dysfunction was significantly associated with scrub typhus positivity. Mean serum urea was 56.3 mg/dL in positive patients compared with 37.8 mg/dL in negative patients ($p=0.001$), while mean creatinine was 1.3 mg/dL and 0.9 mg/dL , respectively ($p<0.001$). Similar findings have been reported in previous South Indian studies, where renal impairment was recognized as an important manifestation and prognostic marker of severe Scrub Typhus.^[19,22] Renal involvement may result from systemic inflammation, endothelial injury, altered renal perfusion and multiorgan involvement. The presence of elevated urea and creatinine in a patient with prolonged fever and thrombocytopenia should therefore increase suspicion for Scrub Typhus.

Liver-function abnormalities were present in 70.6% of IgM-positive patients compared with 26.5% of IgM-negative patients, with a statistically significant association ($p<0.001$). Hepatic involvement is well recognized in Scrub Typhus and has been reported in several Indian studies. Varghese et al. demonstrated that elevated transaminases, particularly when associated with thrombocytopenia and other laboratory abnormalities, could assist in identifying patients with Scrub Typhus.^[19] Hepatic dysfunction may range from mild transaminase elevation to severe hepatitis and multiorgan dysfunction. Thus, the combination of fever, thrombocytopenia, abnormal liver-function tests and renal dysfunction should be considered a useful clinical pattern suggestive of Scrub Typhus in endemic regions. The use of IgM ELISA provided a practical method for laboratory confirmation in the present study. ELISA is more suitable for routine hospital laboratories than methods requiring specialized facilities such as culture or immunofluorescence. Studies evaluating the InBios Scrub Typhus Detect assay have demonstrated useful diagnostic performance compared with reference serological methods.^[23,24] However, IgM detection has limitations, including persistence of antibodies after recent infection and possible cross-reactivity with other febrile illnesses. The absence of paired acute and convalescent serum samples and the lack of PCR confirmation in the present study limit the ability to establish acute infection with absolute certainty. Therefore, the results should be interpreted in conjunction with clinical findings and

epidemiological context. All 17 IgM-positive patients received appropriate antimicrobial treatment, with doxycycline administered to adults and azithromycin to children. No deaths were reported among the positive patients, although seven patients developed respiratory distress requiring intensive-care management and subsequently recovered. Previous studies have reported substantial morbidity and mortality in severe Scrub Typhus, particularly among patients with acute respiratory distress syndrome, renal dysfunction, shock and multiorgan involvement.^[22] The favorable outcome in the present study may be related to early recognition, timely antimicrobial therapy and availability of intensive-care support. These observations reinforce the importance of early diagnosis, because untreated or delayed treatment can result in severe respiratory, renal, hepatic, cardiovascular and neurological complications. Overall, the findings of the present study are comparable with previous Indian studies demonstrating that Scrub Typhus is an important and treatable cause of acute febrile illness. The significant association of Scrub Typhus IgM positivity with prolonged fever, eschar, lymphadenopathy, hepatosplenomegaly, thrombocytopenia, renal dysfunction and liver-function abnormalities provides useful clinical and laboratory clues. Importantly, absence of an eschar should not exclude the diagnosis because most seropositive patients in the present study did not have a documented eschar. In endemic areas such as Tamil Nadu, Scrub Typhus should therefore remain an important differential diagnosis in patients with persistent fever, particularly when thrombocytopenia is accompanied by hepatic or renal dysfunction. Further multicentre studies involving larger populations, paired serological samples and molecular confirmation would help establish the true burden of disease and improve understanding of clinical predictors and outcomes.

Limitations

This study has several limitations. First, the sample size was relatively small, with only 100 participants, and the study was conducted at a single tertiary-care hospital; therefore, the findings may not be generalizable to the wider population. Second, the study was hospital-based and included clinically selected febrile patients, so the observed 17% IgM positivity should not be considered community prevalence. Third, diagnosis was based on a single serum sample tested by IgM ELISA. IgM antibodies may persist after recent infection and may show cross-reactivity with other febrile illnesses, potentially resulting in false-positive results. Paired acute and convalescent serum samples were not obtained to demonstrate seroconversion or a significant rise in antibody levels. Fourth, molecular confirmation by PCR was not performed. Finally, detailed information regarding occupational, environmental and vector exposure was limited, preventing assessment of specific risk factors. These

limitations should be considered when interpreting the study findings.

CONCLUSION

Scrub Typhus was an important cause of febrile illness among hospitalized patients in this tertiary-care hospital in Coimbatore, with 17% of patients showing IgM positivity. Scrub Typhus positivity was significantly associated with prolonged fever, male sex, eschar, lymphadenopathy and hepatosplenomegaly. Laboratory abnormalities including thrombocytopenia, elevated blood urea and serum creatinine, and abnormal liver-function tests were also significantly associated with IgM positivity. Eschar, although strongly associated with Scrub Typhus, was absent in most seropositive patients; therefore, its absence should not be used to exclude the diagnosis. These findings highlight the importance of maintaining a high index of suspicion for Scrub Typhus in patients presenting with persistent fever, particularly in endemic regions of South India. Early laboratory confirmation and prompt appropriate antimicrobial therapy may reduce progression to severe disease and multiorgan complications. Further multicentre studies using larger sample sizes, paired serological testing and molecular confirmation are recommended to better define the epidemiology and diagnostic characteristics of Scrub Typhus in this region.

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