

# Seroprevalence and clinical profile of scrub typhus in patients presenting with undifferentiated acute febrile illness in a tertiary care centre



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## ABSTRACT

**Background:** Scrub typhus (ST) is a common Rickettsial infection which has been increasingly reported from the various states of southern part of India. **Aims and Objective:** With very few reports from Central Kerala, we aimed to study the seroprevalence of scrub typhus and its clinical profile. **Materials and Methods:** Patients presenting with acute undifferentiated febrile illness and other symptoms and signs of Rickettsial infections during a period of two years were included in this prospective study. Serodiagnosis of ST was based on a positive Scrub typhus IgM Enzyme Linked Immunosorbent Assay (ELISA) and/or agglutination for OXK in Weil Felix test. The cut-off for ELISA was calculated. The clinical and laboratory details of the positive patients were obtained and bivariate analysis was performed. **Results:** The cut-off for ST IgM ELISA was calculated and found to be 0.38. Of the 636 samples screened, 34 (5.3%) were positive for ST IgM ELISA. Out of the 34 positive samples, only 5 (0.8%) were positive by Weil Felix test. Most of the ST patients presented during the months of May to January. Rashes were observed in 8.8% of the ST patients and none had signs of eschar. The commonest clinical features included fever, myalgia (52.9%), nausea & vomiting (32.4%), headache (29.4%) and hepatosplenomegaly (29.4%). The commonest abnormal laboratory finding and complication observed were elevated serum transaminases (52.9%) and acute respiratory distress syndrome (17.6%) respectively. **Conclusion:** The seroprevalence of ST in Central Kerala was 5.3% using Weil Felix test and ST IgM ELISA.

**Key words:** Cut-off; Enzyme Linked Immunosorbent Assay; Fever; Rash; Weil Felix Test

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## INTRODUCTION

Scrub typhus (ST) is one of the emerging and re-emerging zoonotic Rickettsial infections with a trend in increasing incidence of the disease worldwide including India. It is an important etiology of acute undifferentiated febrile illness (AFI). The disease is caused by *Orientia tsutsugamushi* and transmitted to humans by the bite of the larvae of trombiculid mite or chigger. The name scrub typhus is derived from the prevalence of the disease and mites in

areas with heavy scrub vegetation. The disease is endemic in the geographical region known as “tsutsugamushi triangle” which includes the Indian subcontinent as well.<sup>1</sup>

The disease remains underdiagnosed in our country due to the non-specific clinical presentations and lack of diagnostic tests in most places. The clinical symptoms of ST 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

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fever. The severity of the illness varies from mild to severe with multi-organ system involvement and can cause death if not treated accurately in the early stage of the illness.<sup>2</sup>

The most common screening test used for the diagnosis of Rickettsial infections in India is the Weil Felix test. Although economical, easily available and technically simple, the test lacks specificity and sensitivity. Enzyme Linked Immuno Sorbent Assay (ELISA) is currently the preferred method. Scrub typhus IgM ELISA is considered a cost-effective test with good sensitivity and specificity when compared to other tests. The gold standard for serology, Indirect Immunofluorescence Antibody (IFA), is expensive and requires considerable training for both performing the test and interpretation of the result.<sup>3</sup>

In the clinical setting, a diagnosis of ST is considered when a patient with AFI has an eschar and a positive IgM ELISA for scrub typhus where other causes of fever have been excluded. In the absence of an eschar, a positive IgM ELISA in the appropriate clinical setting with defervescence within 48-h of initiation of doxycycline or scrub IgM ELISA seroconversion on convalescent sera with other etiologies of AFI ruled out also suggests scrub typhus infection.<sup>4</sup> Weil Felix test can be used to find out the possibility of other Rickettsial infections also. Combining the two methods of serological tests increases the positive predictive value.

The present study has been aimed to know the prevalence and clinical profile of ST in central Kerala that would enable clinicians for its early diagnosis and prompt management in AFI.

## MATERIALS AND METHODS

### Study design

This was a prospective study which was carried out from January 2018 to January 2020, in a tertiary care centre hospital in Central Kerala.

### Ethical approval

The study was conducted after obtaining ethical clearance from Institutional Ethical Committee (IEC/2018/02/26).

### Determination of cut-off value for Scrub typhus IgM ELISA

**Samples:** Thirty serum samples from healthy blood donors residing in Central Kerala, thirty serum samples positive for Dengue NS1 Antigen and thirty serum samples positive for *Leptospira* IgM by ELISA. Past infection of ST was ruled out from the information collected through detailed questionnaire in case of healthy blood donors. In the case of Dengue and Leptospirosis proven cases, thorough

clinical history and investigations were performed to rule out other causes of acute febrile illnesses.

**Calculation of cut-off:** All the ninety serum samples were tested for the detection of scrub typhus IgM antibodies by ELISA using the commercially available kit (InBios International Inc.) according to the manufacturer's instructions. The cut-off value was determined by the geometric mean (GM) of optical density (OD) of the ninety samples. The upper limit of the GM (99.99% confidence interval) was taken as the cut-off value. The cut-off value was calculated as 0.38 for the geographical area in study. Accordingly, samples with an OD  $\geq$  0.38 were considered as positive for ST.

### Seroprevalence of scrub typhus and clinical profile

**Inclusion criteria:** Patients presenting with fever for more than five days duration irrespective of their age were included in the study. A suspected case of AFI with symptoms and signs suggestive of Rickettsial infections like high grade fever with or without chills & rigor, fever with rash/eschar/hepatosplenomegaly/jaundice/lymphadenopathy/thrombocytopenia, fever with constitutional symptoms like malaise, myalgia, nausea, vomiting; fever with bleeding diathesis (petechia, purpura); fever with shock were included in the study.

**Exclusion criteria:** Known cases of immunocompromised patients like AIDS/lymphomas; malignancy secondaries; tuberculosis and seropositive cases of dengue fever, leptospirosis and enteric fever were excluded from the study.

**Specimen collection and processing:** A total of 636 blood samples were collected from patients with AFI suspecting Rickettsial infections after getting informed consent. The serum samples were stored at 2-8 °C and subjected to Weil Felix test and ELISA for IgM antibodies to ST within 48 hours.

**Weil Felix test:** The test was performed with the commercial kit (FAR Diagnostics) as per the manufacturer's instructions. An Agglutination titre of  $\geq$  160 for OX K antigen was considered as significant for ST.

**IgM ELISA for *O. tsutsugamushi* (scrub typhus):** Qualitative detection of IgM antibodies was carried out using the same commercially available kit (InBios International Inc.) as used for the determination of cut-off value for the test.

**Diagnosis of ST** was made when the samples were tested positive for OXK agglutination in Weil Felix and/or IgM ELISA tests. Clinical and demographical details of ST positive patients were collected.

### Statistical analysis

Data were entered into Microsoft Excel and analysed using SPSS version 21. Cut-off was calculated using upper limit of 99% confidence interval for geometric mean. Apart from descriptive measures, we performed bivariate analysis using Pearson Chi-square test for finding associations.

## RESULTS

The cut-off for scrub typhus IgM ELISA was calculated as 0.38 using geographically relevant ninety serum samples. After screening 636 patients with acute febrile illness for ST, 34 samples were found positive for ST by IgM ELISA test (5.3%). Out of the 34 ELISA positive samples, 5 (0.8%) were reactive by Weil Felix test (agglutination for OX K). Out of the 636 samples tested, 14 samples (2.2%) were positive by Weil Felix test but negative by ELISA and these cases were not included in the study. Negative results were observed by both the tests in 588 (92.5%) samples.

Of the 636 patients tested, 389 (61.2%) were males and 247 (38.8%) were females and out of the 34 ST positive patients, 19 (55.9%) were males and 15 (44.1%) were females. The ST positive patients included 27 adults (20 to 99 age group) and 7 children (0-19 years). The mean age of ST patients was found to be 43.8 years. The youngest patient was 1 year old and the oldest was 81-year-old. Highest seropositivity was observed in the age group 40 to 69 (n=20, 58.8%). The age and gender distribution of ST patients are illustrated in Figure 1.

The trend of seasonal pattern of scrub typhus over the two years is shown in Figure 2. Maximum number of ST patients presented in the monsoon, post-monsoon and winter period (May to January). The ST patients were from four districts around the hospital, which is illustrated in

Figure 3. Repeat testing with IgM ELISA was performed in 3 samples within a period of 1 to 2 weeks and seropositivity was seen in all. Co-infection with Leptospirosis IgM ELISA and Dengue IgM ELISA was seen in 5 and 1 ST positive sera samples respectively.

Fever was the commonest symptom which was seen in all patients (100%) and the mean duration of fever prior to presentation to hospital was 6.4 days. Although rash was observed in 8.8% of the ST patients, eschar was not seen in any of the cases. The different clinical presentations, abnormal laboratory findings and complications of the seropositive patients of children and adult age group categories are mentioned in Table 1. Significant associations were observed with age group, gender, fever duration and absorbance value in ELISA in comparison with the clinical and laboratory parameters (Table 2).

## DISCUSSION

The cut-off value for scrub IgM ELISA was standardised initially and was found to be 0.38 in our study. There is a significant lack of literature regarding the methodologies and cut-offs for ELISA used for the diagnosis of ST infections. The optimal ELISA cut-off values can be assessed by two methodologies: 1) Using the manufacturer’s cut-off formula of mean OD plus 3SDs from healthy controls and non-scrub typhus cases; 2) By comparing ELISA OD values against IFA IgM titres using Receiver Operator Characteristic (ROC) analysis.<sup>5,6</sup> In the present study, calculation of cut-off was performed by the first method. By using the same method, the cut-off ranged from 0.38 to 0.6 in few studies in India.<sup>7-11</sup> Gupta N et al observed a cut-off of 0.89 in New Delhi by using the second method.<sup>12</sup>

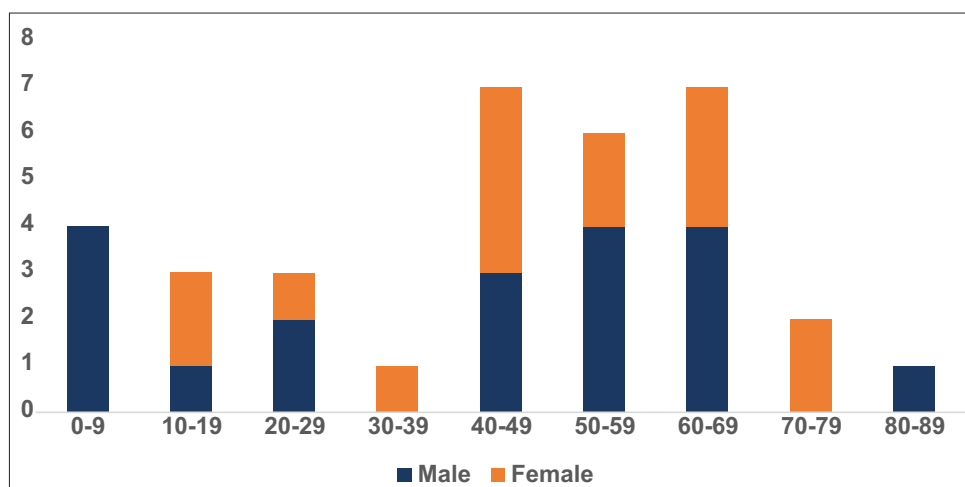
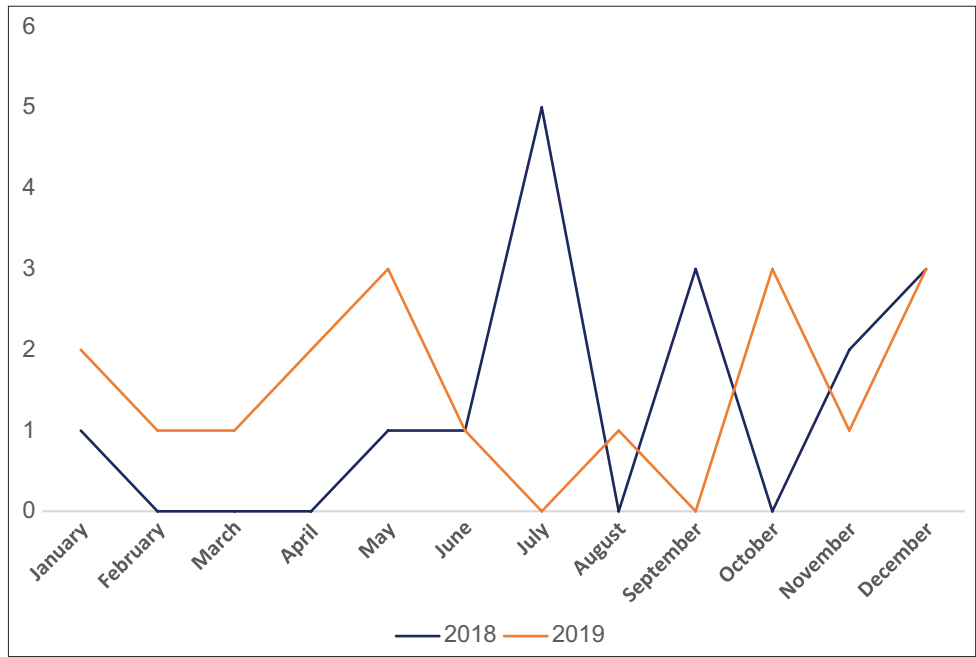
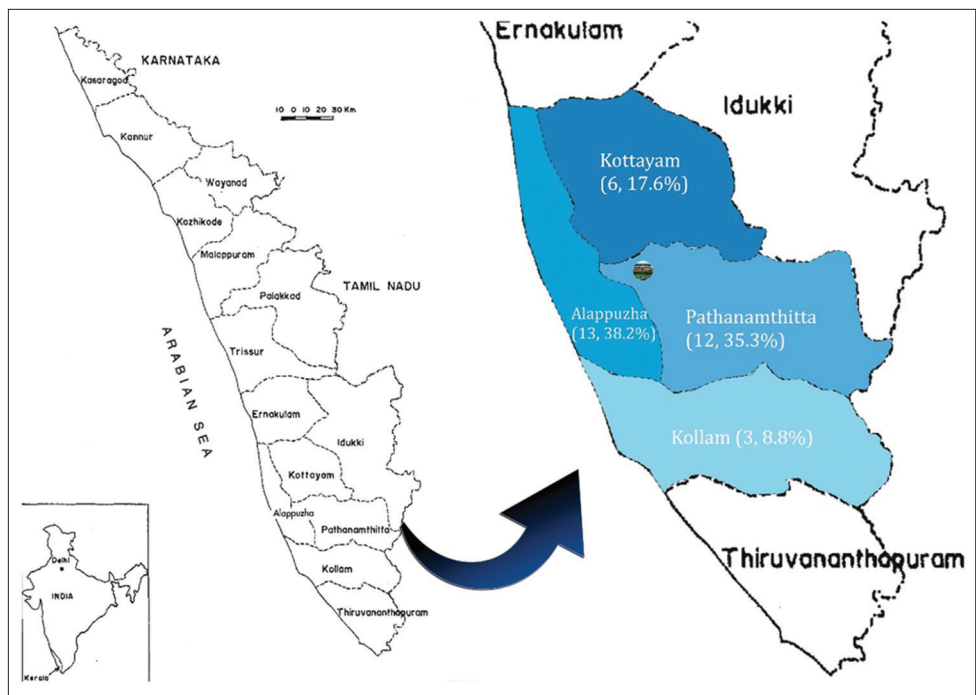


Figure 1: Age – gender distribution of scrub typhus positive patients



**Figure 2:** Seasonal trend of scrub typhus over the years 2018 and 2019



**Figure 3:** Distribution of scrub typhus positive patients identified across the study sites in Central Kerala

In the present study, 5.3% (34 out of 636) of patients who presented with AFI had scrub typhus. The prevalence of ST reported in various parts of Central Kerala ranged from 11 to 19% in different studies.<sup>7,13,14</sup> Other studies from south India have reported a seroprevalence of 39% (Andhra Pradesh), 24% (Pondicherry) and 9% (Tamil Nadu).<sup>8,15,16</sup> Studies by Oberoi A et al in Punjab and Singh R et al in Uttarakhand have reported the seroprevalence of ST as 12% and 6% respectively.<sup>17,18</sup>

In our study, we noted a male preponderance (55.9% males and 44.1% females), which was similar to studies by Ramyasree A et al and Roopa KS et al.<sup>8,15</sup> Most of the ST patients were in the age group 40 to 60 (58.8%) and this was in par with the studies by Ramyasree A et al and Jyothy R et al.<sup>14,15</sup>

Scrub typhus is seen predominantly in the monsoon and post-monsoon months in Kerala which is similar to our findings.<sup>7,13,14</sup> Similar seasonal trend was observed in

**Table 1: Classification of scrub typhus patients (children and adults) according to the clinical features, abnormal laboratory findings and complications**

Scrub typhus patients (n=34)	Children (n=7)	Adults (n=27)
<b>Clinical features</b>		
Mean fever duration (prior to hospital admission)	9.1 days	5.7 days
Myalgia (n=18, 52.9%)	1 (14.3%)	17 (63%)
Nausea, vomiting (n=11, 32.4%)	1 (14.3%)	10 (37%)
Diarrhoea (n=3, 8.8%)	0	3 (11.1%)
Dyspnoea, cough (n=8, 23.5%)	3 (42.9%)	5 (18.5%)
Headache (n=10, 29.4%)	1 (14.3%)	9 (33.3%)
Seizures (n=2, 5.9%)	0	2 (7.4%)
Altered sensorium (n=6, 17.6%)	0	6 (22.2%)
Lymphadenopathy (n=3, 8.8%)	2 (28.6%)	1 (3.7%)
Hepatosplenomegaly (n=10, 29.4%)	2 (28.6%)	8 (29.6%)
Rash (n=3, 8.8%)	1 (14.3%)	2 (7.4%)
<b>Abnormal laboratory findings</b>		
Leukocytosis (n=9, 26.5%)	2 (28.6%)	7 (26%)
Elevated serum transaminases (n=18, 52.9%)	3 (42.9%)	15 (55.6%)
Thrombocytopenia (n=11, 32.4%)	1 (14.3%)	10 (37%)
Elevated serum creatinine (n=8, 23.5%)	0	8 (29.6%)
<b>Complications</b>		
Acute respiratory distress syndrome (ARDS) (n=6, 17.6%)	1 (14.3%)	5 (18.5%)
Invasive ventilation (n=1, 2.9%)	0	1 (3.7%)
Renal failure (n=4, 11.8%)	0	4 (14.8%)
Meningoencephalitis (n=1, 2.9%)	0	1 (3.7%)
Shock requiring inotropic agent (n=2, 5.9%)	0	2 (7.4%)
Multiorgan dysfunction syndrome (MODS) (n=2, 5.9%)	0	2 (7.4%)

**Table 2: Bivariate analysis of the demographic variables against clinical or laboratory findings**

Demographic variables	Presence of Clinical/Laboratory findings (n, %)	Chi-square value	p-value
Age group	Myalgia	5.287	0.035
Less than 20	1 (5.6%)		
20 and above	17 (94.4%)		
Gender	Thrombocytopenia	4.437	0.035
Males	9 (81.8%)		
Females	2 (18.2%)		
Gender	Dyspnoea/cough	4.047	0.044
Males	2 (25%)		
Females	6 (75%)		
* ELISA Absorbance value	Elevated serum transaminases	4.250	0.042
More than 1.00	12 (66.7%)		
Less than 1.00	6 (33.3%)		
* ELISA Absorbance value	Hepatosplenomegaly	5.100	0.029
More than 1.00	8 (80%)		
Less than 1.00	2 (20%)		

\* Scrub typhus IgM ELISA Absorbance value or Optical density (1.00)

another study in Kerala and outbreaks during the cooler months are reported from Puducherry.<sup>9,19</sup> There are also reports of high incidence of ST during September to December from other parts of India.<sup>14,16,17,20</sup>

Fever was found to be the commonest symptom present in all the patients who were tested positive for ST. The mean duration of fever prior to hospital admission was 6.4 days. Sharma N et al observed mean duration of  $10 \pm 5.3$  days of fever in patients from Chandigarh.<sup>21</sup> Myalgia (52.9%), nausea/vomiting (32.4%), diarrhoea (8.8%), headache (29.4%) and dyspnoea/cough (23.5%) were the other common symptoms present. Myalgia was

noticed predominantly in adults and dyspnoea/cough in females. Scrub typhus is accompanied by varied clinical manifestations as seen in other studies.<sup>13,22,23</sup> Fever and myalgia were the commonest presentations in ST as noted by Mathai E et al.<sup>14</sup> The commonest neurological presentations were altered sensorium (17.6%) and seizures (5.9%). Similar observations were reported by Vivekanandan M et al in Pondicherry.<sup>22</sup> None of the children with ST presented with diarrhoea or neurological manifestations in our study. In a study among paediatric group from South India, neurological manifestations were observed in a few patients, while none of them presented with diarrhoea.<sup>19</sup> Although rashes were observed in 8.8% of

the patients, eschar was not seen in any of them. Eschar is considered as a classical sign in ST, but absence of eschar has been reported in many studies.<sup>8,10,24</sup> Lymphadenopathy was observed in 8.8% of the ST positive patients and it was resolved after the course of antibiotic treatment with Doxycycline. The percentage of lymphadenopathy reported in various studies ranged from 18-53%.<sup>22,24,25</sup>

Among the laboratory parameters, the most consistent abnormality observed in the ST positive patients was elevated transaminases which was in concordance with other studies.<sup>6,22,23</sup> Other laboratory findings included thrombocytopenia, leucocytosis and elevated serum creatinine. Thrombocytopenia was significantly noticed among males. In patients with an absorbance value more than 1.00 in ST IgM ELISA, hepatosplenomegaly and thrombocytopenia were observed. Although ST ELISA is a qualitative test, absorbance value can also be considered in a case of scrub typhus.<sup>19</sup> Acute respiratory distress syndrome (ARDS), renal failure, shock, multi-organ dysfunction syndrome, requirement of invasive ventilation and meningoencephalitis were the complications noted in ST patients in the present study. Recovery in children was with less complications when compared to adults. Similar findings were observed by Stephen S et al in Puducherry.<sup>13</sup> All the patients with ST in our study recovered after treatment with Doxycycline and/or Ceftriaxone along with other supportive measures. Similarly, in a study conducted by Ramyasree A et al in Andhra Pradesh, mortality was not observed.<sup>8</sup> Co-infections with Leptospirosis and Dengue infections were seen in our study, which were also reported from others.<sup>13,19</sup>

The available diagnostic tests for ST include Scrub typhus IgM ELISA, IFA, PCR and Weil Felix test. PCR is the gold standard test for the diagnosis of ST whenever inconclusive results are obtained in IFA tests.<sup>7</sup> The non-availability and higher cost of these tests are the major obstacles for their use in India. The evaluation of baseline titres and cut-off among the population of the testing facility are essential elements for the serodiagnosis of ST.

### Limitations

As the present study is hospital-based, the seroprevalence can't be generalised to the population or community. Comparison with the gold standard tests were not performed to assess the exact seroprevalence of scrub typhus. The value calculated as cut-off for ST IgM ELISA falls in the lower range of those determined in similar studies and this can increase the chance of false positive test result.

### CONCLUSION

Scrub typhus is a re-emerging zoonotic infection which is prevalent in Central Kerala. Our study has shown a

seroprevalence of 5.3% for scrub typhus among AFI on the basis of cost-effective tests like IgM ELISA and Weil Felix. The cut-off value for ELISA was calculated as 0.38 for our area. Assessment of cut-off values for serological tests in a locality would help in the accurate estimation of the seroprevalence of the disease in that region. The diverse presentations of scrub typhus upraise a high index of suspicion and its early diagnosis can help in prompt management of this condition.

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**Author's Contribution:**

**RAJ-** Interpreted the results, Concept and design of the study; Data analysis and prepared first draft of manuscript; **HJ-** Interpreted the results; reviewed the literature and manuscript; **AAJ-** Interpreted the results, revision of manuscript; **PT-** Statistically analysed and interpreted, preparation of manuscript and revision of the manuscript; **RM-** Concept, coordination of overall study and revision of manuscript; **MT-** Concept, revision of manuscript.

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