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### A COMPARATIVE STUDY ON DIAGNOSIS OF SCRUB TYPHUS BY IMMUNO CHROMATOGRAPHY AND IgM ELISA METHODS IN CLINICALLY SUSPECTED CASES

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**Abstract:** India being a tropical country, fevers are caused by different etiological agents. Fever of unknown origin (FUO) is said when the body temperature increases to 38.3°C (101°F) or more several times a day lasting longer than 3 weeks or failure to reach a diagnosis despite a week of inpatient evaluation. Scrub typhus (ST), a rickettsial disease caused by *Orientia tsutsugamushi*, is a very less known cause of FUO. In India, the presence of scrub typhus has been known for several years. A Total of 247 blood samples were collected from patients attending S. V. R. R. Government General Hospital, Tirupati, with clinical features suggestive of scrub typhus. The serum samples were tested for IgM antibodies by IgM ELISA (In Bios International; Inc., Netherlands) and Rapid method was done using SD Bio line Tsutsugamushi, one-step scrub typhus antibody test. ELISA is more sensitive to detect IgM antibody for scrub typhus as per the present study.

**Keywords:** Fever of unknown origin, scrub typhus, *Orientia tsutsugamushi*



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## INTRODUCTION

India being a tropical country, fevers are caused by different etiological agents. When a patient presents with history of fever with rash/ thrombocytopenia, rickettsial infections is one of the differential diagnosis along with dengue, measles, rubella, meningococcal infection, malaria, leptospirosis and other viral exanthems.<sup>1</sup> Fever of unknown origin (FUO) is said when the body temperature increases to 38.3°C (101°F) or more several times a day lasting longer than 3 weeks or failure to reach a diagnosis despite a week of inpatient evaluation<sup>2</sup>. Scrub typhus (ST), a rickettsial disease caused by *Orientia tsutsugamushi*, is a very less known cause of FUO.<sup>3</sup>

Also known as tsutsugamushi disease<sup>4</sup>. Caused by *Orientia tsutsugamushi* (*O.tsutsugamushi*) an obligatory intra-cellular gram negative bacterium. It is transmitted to humans by the bite of larval mites (chiggers) of *Leptotrombidium deliense*<sup>5</sup>, which are almost microscopic, often brilliantly coloured (red)<sup>5</sup>. Infected chiggers are found particularly in areas of heavy scrub vegetation during the wet season, (therefore this disease has also been called river/flood fever when mites lay eggs)<sup>5</sup>. Four factors are essential for the establishment of a micro focus of infection, namely, coexistence and intimate relationship among *O. tsutsugamushi*, Chiggers, rats and secondary or transitional forms of vegetation and are known as zoonotic tetrad<sup>6</sup>. In India, the presence of scrub typhus has been known for several years. The disease is widely spread all over the country, and was reported in several states Haryana, Jammu, and Kashmir, Himachal Pradesh, Uttaranchal, West Bengal, Assam, Maharashtra, Kerala and Tamilnadu<sup>7</sup>.

In India, the burden of rickettsiosis is underestimated as there is lack of both community based studies and of specific laboratory tests<sup>8</sup>. The observation of the eschar is often missed and other signs and symptoms of the disease are not characteristic leading to delayed diagnosis by the clinician. In view of low index of suspicion, non-specific signs and symptoms, and absence of widely available sensitive and specific diagnostic tests, these infections are difficult to diagnose<sup>9</sup>.

Failure of timely diagnosis leads to significant morbidity and mortality. As antimicrobials effective for rickettsial diseases are usually not included in empirical therapy of nonspecific febrile illnesses, treatment of rickettsial diseases is not provided unless they are suspected<sup>1</sup>, with timely diagnosis there is dramatic response to antimicrobials. The clinical manifestations of this disease range from sub-clinical disease to organ failure<sup>10</sup>. Fever is the most common feature of scrub typhus and due to lack of awareness among clinicians the condition is labelled as "fever of unknown origin". Human beings usually get infected when they accidentally encroach upon an area of infected chiggers mainly in rural and sub-urban areas. Delayed diagnosis, may lead to serious complications like renal failure, myocarditis, septic shock,

meningoencephalitis and rarely, acute respiratory distress syndrome. The mortality rate is 7 to 30%<sup>11</sup>.

The clinical symptoms vary depending on the duration of illness, strain of *O.tsutsugamushi*, immune status and the other host factors of the patient. It is difficult to distinguish scrub typhus clinically from many other febrile diseases especially in the absence of characteristic eschar.<sup>12</sup> Bacteraemia is detectable 1 to 3 days before the onset of fever<sup>13</sup>. Respiratory symptoms are frequent. Interstitial pneumonia, pulmonary oedema, pleural effusion, cardiomegaly and focal atelectasis are observed by chest radiography in patients<sup>14</sup>. Several tests are available with their own advantages and limitations<sup>15</sup>. Among all the serological tests available Weil-Felix test is the cheapest and easily available, but this is notoriously unreliable. Indirect immunofluorescence test, the gold standard is beyond affordability especially in poor countries and needs expertise for interpretation as the choice of cut-off values for positive diagnosis is influenced by several factors such as antibody kinetics, geography, negative seroconversion and seasonality<sup>16,17</sup>. IgM ELISA has been evaluated and found to be quite satisfactory in comparison to the gold standard, but samples need to be pooled for ELISA which can lead to delayed diagnosis thus influencing the overall outcome. Rapid tests which are economic, rapid and single tests can be carried out.

This study was carried out to know the seroprevalence of scrub typhus in clinically suspected cases and to compare a rapid test with IgM ELISA for the diagnosis of scrub typhus.

#### **MATERIALS AND METHODS:**

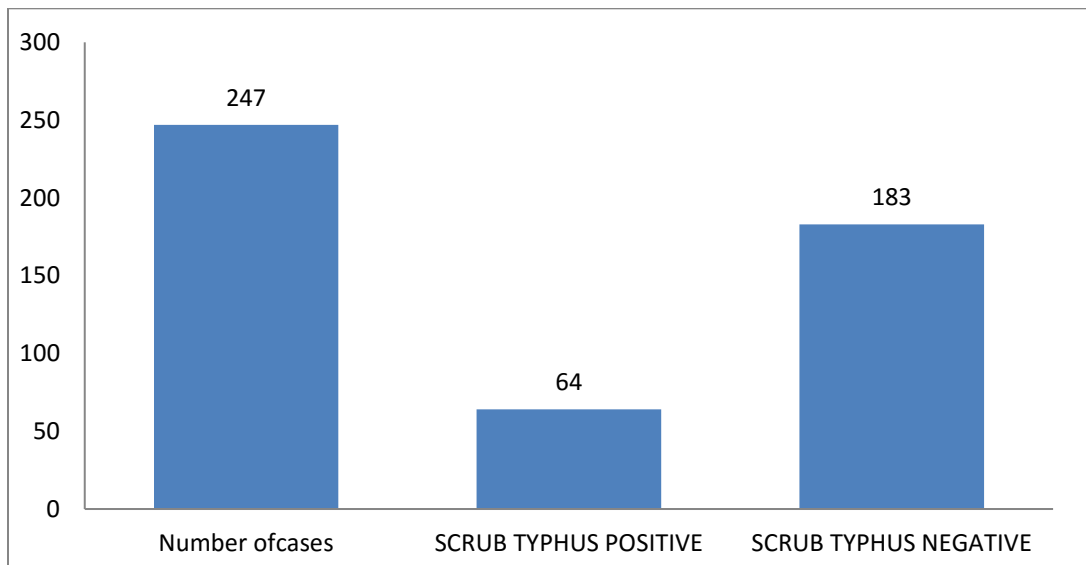
The present study was conducted in the Department of Microbiology, S.V. Medical College, Tirupati from November 2014 to October 2015. A Total of 247 blood samples were collected from patients attending S.V.R.R. Government General Hospital, Tirupati, with clinical features suggestive of scrub typhus. The blood samples were collected from the patients by venepuncture following strict aseptic precautions and allowed to clot at room temperature and then centrifuged. The serum was separated. Serum samples were refrigerated (2-8 °C) or stored frozen in a deep freezer (-20 °C), if not tested within two days. The serum samples were tested for IgM antibodies by IgM ELISA (In Bios International; Inc., Netherlands) and Rapid method was done using SD Bio line Tsutsugamushi, one-step scrub typhus antibody test. The serum samples received from clinically suspected patients were also tested for the presence of scrub typhus by SD Bio line Tsutsugamushi, One step scrub typhus antibody test.

**RESULTS:** All the serum samples were processed for the detection of IgM antibodies for the diagnosis of scrub typhus by ELISA (In Bios International TM IgM ELISA.) and Rapid Immunochromatographic test (SD Bio line Tsutsugamushi, one-step scrub typhus antibody test).

**Table 1: positive percentage among study group**

Number of cases	SCRUB TYPHUS POSITIVE	SCRUB TYPHUS NEGATIVE
247 (100%)	64 (25.91%)	183 (74.09%)

**Chart 1: positive percentage among study group**

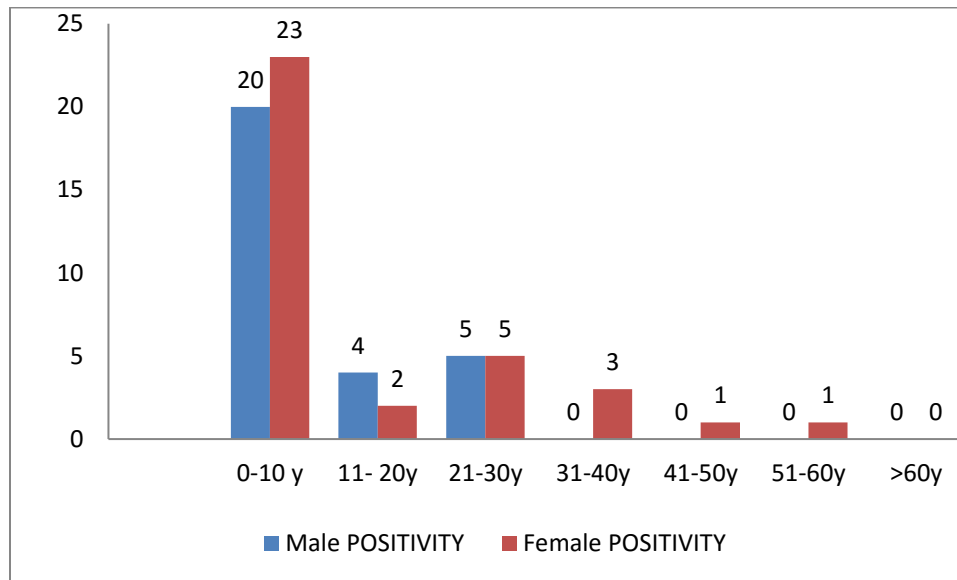


**Table 2: IgM positivity: Age- wise and Sex –wise distribution**

Age (in years)	Male		Female		Total
	Total no. of cases	IgM positivity	Total no. of cases	IgM positivity	
0 – 10	31	20(31.25%)	134	23(35.93%)	165
11-20	17	4(6.25%)	17	2(3.12%)	34
21-30	14	5(7.81%)	12	5(7.81%)	26
31-40	5	0	6	3(4.68%)	11
41-50	5	0	1	1(1.56%)	6
51-60	1	0	2	1(1.56%)	3

>60	0	0	2	0	2
<b>Total</b>	<b>73</b>	<b>29(45.31%)</b>	<b>174</b>	<b>35(54.69%)</b>	<b>247</b>

**Chart 2: IgM positivity: Age- wise and Sex –wise distribution**

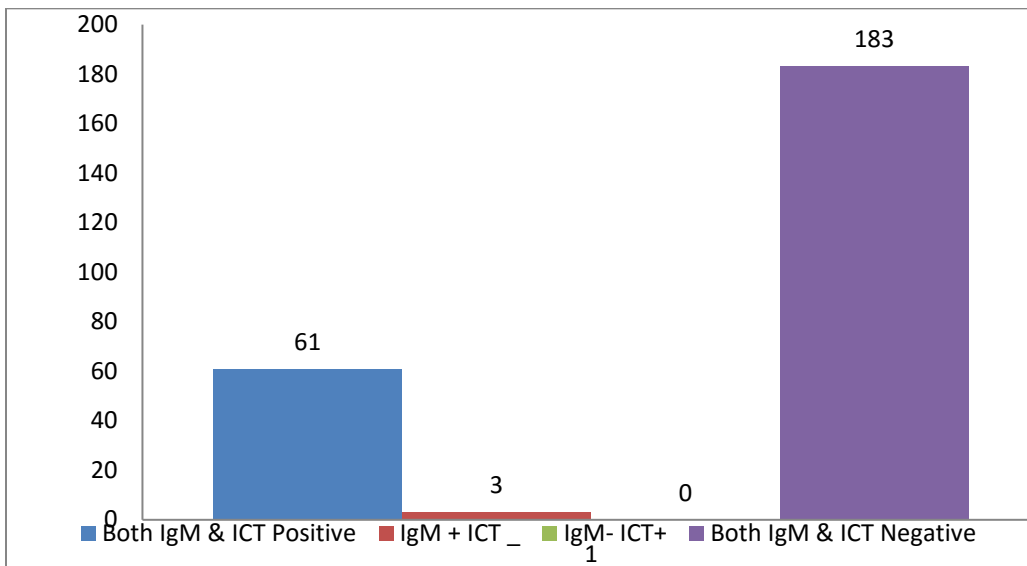


The prevalence of Scrub typhus was significantly higher ( $p < 0.05$ ) in 0-10 y age group followed by 21-30 y age group compared to rest of age groups. No significant difference exists between remaining groups. All the 247 cases were tested for IgM antibodies to scrub typhus by ELISA and Rapid Immuno chromatographic test. Of the 247 serum samples tested, 64 samples were positive for IgM antibodies to scrub typhus. More number of positive cases were observed among the females. Of the total 73 male cases, in the age group 0-10y twenty cases were IgM positive for scrub typhus. Of the total 174 Female cases, in the age group 0- 10y twenty three cases were IgM positive for scrub typhus.

**Table 4: Correlation between ELISA and ICT in IgM detection.**

Assay	IgM ELISA		Total
	Positive	Negative	
Immuno Chromagography			
Postive	61	0	61
Negative	3	183	186
<b>TOTAL</b>	<b>64</b>	<b>183</b>	<b>247</b>

**Chart 4: Correlation between ELISA and ICT in IgM detection.**



This Table shows correlation between ELISA and Immunochromatography in IgM detection in which positivity by IgM ELISA was 64 and Immunochromatography was 61 out of 247 samples tested. In the present study using IgM ELISA and rapid test, there was 95.31% correlation between the two tests. Chi- square test revealed no significant ( $P=0.821$ ) difference between the detection rates of both tests. In the present study the sensitivity and specificity of rapid Immunochromatography in relation to ELISA were 95.31% and 100%. The positive predictive value of Immunochromatography is 100% and the negative predictive value is 98.38%.

**Table 5: Clinical features of scrub typhus cases at the time of presentation.**

Clinical features	positives	percentages
Fever with chills	247	100%
Rigors	54	84.37%
cough	35	54.68%
Head ache	10	15.62%
Hepato splenomegaly	52	81.25%
lymphadenopathy	58	90.62%
Maculo papular rash	52	81.25%

crackles/ wheeze	8	12.50%
Altered sensorium	12	18.75%
conjunctival congestion	31	48.43%
joint pains	3	4.68%
Breathlessness	39	60.93%
Myalgia	26	40.62%
Diarrhoea and vomiting	19	29.68%
Oedema	9	14.06%
seizures	13	20.31%
Eschar	6	9.37%

The commonest presenting features in the patients were fever with chills in all cases (100%), rigors (84.37%), Breathlessness (60.93%), Maculo papular rash (81.25%), Hepato splenomegaly (81.25%), lymphadenopathy (90.62%). The other common features are cough, head ache, myalgia.

#### DISCUSSION:

In southern India outbreak of scrub typhus occur during cooler months as reported by Mathai et al. The present study also shows outbreak during November to February. This correlated with the months with a higher number of field rats infected with *O. tsutsugamushi* and the months with more mites attached to rodents. Scrub typhus is prevalent but an undiagnosed disease in India. It should be considered in the differential diagnosis of patients suffering from acute febrile illness, This is particularly important after the rainy season and in cooler months.

Munilakshmi et al (2014) showed higher incidence during rainy and early cool months. Positive cases were more in October to January in the study done by Jyothi et al., 2015. In a study conducted by Garuda Rama (2014) there was high incidence cases during the months of October and November. There were no deaths arising from scrub typhus in the present study which correlates with Jitendra et al. There was also no mortality in the study by Garuda Rama (2014).

Gurung et al also reported more scrub typhus cases were positive by ELISA and ICT tests than Weil-Felix test. Although Indirect IgM ELISA may give false positive results due to rheumatoid factor<sup>62</sup> and false negative results due to rise of IgG levels occur at the time of secondary infection. Primary infection produces a rapid rise in IgM antibodies within 8 days, whereas secondary or re-infection is characterized by a sharp rise in IgG levels, with a variable IgM response. More scrub typhus cases were reported by ELISA and ICT tests than Weil-Felix test in a study done by Gopal et al (2014).

Kammili et al 2013, from Secunderabad, India tested for the prevalence of scrub typhus among patients who were provisionally diagnosed as dengue fever. Among the 100 patients studied, 19 were found to be positive for antibodies for scrub typhus by Rapid Immunochromatography and Weil-Felix test. The present study shows superiority of IgM ELISA in the Detection of IgM antibodies with respect to Rapid Immunochromatography. In the Resource poor settings where ELISA facilities are not available rapid tests are useful for early detection and administration of Doxycycline but if the rapid test is negative and still the patient persists with symptoms further evaluation of clinical specimens is necessary with ELISA along with the consideration of clinical manifestations.

In the present study, 59 (92.18%) Patients recovered fully and responded to doxycycline<sup>101</sup> alone with improvement starting within 48 hours. This correlated with study by H.c. Lee, et al<sup>103</sup> in which 12 out of 15 cases showed defervescence in  $\leq 3$  days. This also correlates with the study done by Garuda Rama (2014) in which all the patients have shown dramatic response with early administration of Doxycycline.

#### CONCLUSION:

This study emphasizes the need for the general awareness of Rickettsial infections in Andhra Pradesh. Diagnostic tests such as ELISA using 56 KDA antigens specific for *Orientia Tsutsugamushi* can provide a cost effective alternative diagnostic tool. More research is essential regarding the epidemiology, pathogenesis and lab diagnosis of diseases in Indian context particularly in Andhra Pradesh.

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