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# COEVAL MENACE OF SCRUB TYPHUS – AN IMMUNOLOGICAL APPROACH TO DIAGNOSIS IN A TERTIARY CARE HOSPITAL

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## Abstract

**Background:** One of the vector-borne zoonotic infectious diseases known as Scrub typhus is caused by bacteria *Orientia tsutsugamushi.* Diagnosis of scrub typhus may be difficult as the symptoms and clinical signs are similar to other causes of febrile illnesses leading to untoward fatality. **Aim:** Present study was conducted to determine the seropositivity rate of Scrub typhus in patients with acute febrile illness at Salem and compare the efficacy of Weil-Felix test and Scrub typhus IgM Enzyme linked immunosorbent assay. **Material and Methods:** The cross- sectional study was conducted from July 2023 to October 2023 for a period of 4 months in the Department of Microbiology, Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamilnadu. Enzyme linked immunosorbent assay(IgM) and Weil Felix test were performed in 200 samples of patients with acute febrile illness using commercially available IgM ELISA kit and Weil Felix Antigen OXK tube agglutination test kit from King institute. **Result:** Out of 200 serum samples analyzed 21 were tested positive by IgM ELISA and 14 were positive by Weil Felix test. The overall seropositivity rate was 10.5% by ELISA. Among 21 ELISA positives, 11 samples were tested positive by Weil Felix test. Data collected was entered into a Microsoft excel sheet, analysed using SPSS software version 21.0.with appropriate statistical tests like Chi-square test as per objectives of the study. Confidence interval was calculated. The p-value ≤0.005 was considered statistically significant. **Conclusion:** Present study showed 10.5% seropositivity rate of Scrub typhus in patient with acute febrile illness in Salem. Easy availability of Weil Felix test makes it a common preliminary screening test for diagnosing Scrub typhus in the community level health setup. This study confirms that Scrub typhus IgM ELISA has good sensitivity & specificity compared to Weil-Felix test and is the best tool to diagnose early.

## INTRODUCTION

Scrub typhus is a vector borne re-emerging rickettsial disease with steadily increasing in incidence throughout India. The seroprevalence varies from 8-50% in Tamilnadu.[1] It is caused by the slow growing obligate intracellular bacterium, *Orientia tsutsugamushi*. This zoonotic disease attributed to 35-50% of acute febrile illness requiring hospital admission with case fatality rate ranging from 1.3%-33.5% which is related to organ involved.[2] In India, it is transmitted by bite of trombiculid mite larvae of genus *Leptotrombidiumdeliensis*.[3,4] This red minute insect, a parasite of rodents is usually found in moist places with suitable climate, and scrub vegetation. The larval stage of the mite is called Chiggers which require a blood meal from vertebrates for their development. Scrub typhus is also called as chiggerosis. These larvae seek moist areas of the body like the groin and axilla. Their bite causes black eschar, a pathognomonic finding in Scrub typhus seen in intertriginous region like groin and axilla.[5] Humans are the accidental and terminal hosts, infected when they enter mite infested areas.


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The clinical triad consists of eschar, maculopapular rashes and regional lymphadenopathy. Bacteria invade endothelial cells; produce vasculitis and perivascular inflammation leading to end organ damage.[6] The incubation period is 1-3weeks.[6] The most common complications include hepatitis, thrombocytopenia, acute respiratory syndrome, acute kidney injury, meningitis, myocarditis and shock[2]. Antimicrobial agent of choice for chiggerosis is Doxycycline.[7] Early initiation of antimicrobial therapy is essential for prevention of life-threatening complications.

In India, Weil Felix is the most common screening test used widely whereas Scrub typus IgM Enzyme linked immunosorbent assay can be used to test bulk samples at a time. This study was conducted to assess the seropositivity rate of scrub typhus in patients admitted with acute febrile illness at Salem and compare the diagnostic performance of Weil Felix and Scrub Typhus IgM ELISA in the diagnosis of Scrub typhus.

## MATERIALS AND METHODS

The study was a cross sectional study conducted in the Department of Microbiology, Government Mohan Kumaramangalam Medical College Hospital, Salem, Tamilnadu for a period of 4 months from July 2023 to October 2023 and approved by Institutional Ethics Committee Ref.No. GMKMC&H/114/IEC/2023 dated 14.06,2023. Sample size calculated using formula, n=Z2X(p X q)/e2 = 1.962 X 0.17 X 0.83/0.052 = 216.7. Sample size =200[8].

**Inclusion criteria**

The study population included undiagnosed patients of febrile illness aged more than 18 years with clinical suspicion of Scrub typhus admitted in this hospital. The patients with history of fever for 5-7 days duration (Temperature > 37.2° C) with or without rashes, eschar, lymphadenopathy and had symptoms of malaise, headache, myalgia, nausea, abdominal pain were included.

**Exclusion criteria**

Laboratory confirmed cases of Typhoid fever, Leptospirosis and Dengue, Urinary tract infection caused by *Proteus Spp* were excluded.

**Sample collection:** After getting informed consent, under aseptic precautions 5ml of venous blood was drawn in plain tube from 200 patients with acute febrile illness on 7th day of fever. Sera was separated and subjected to Weil Felix test for detection of OXK Antigen and Scrub typhus IgM ELISA for the identification of IgM antibodies to *Orientia tsutsugamushi* bacteria in the serum.

**Weil Felix tube agglutination Test:** Weil Felix test is a heterophile tube agglutination test based on antigenic cross reactivity. Group specific alkali stable lipopolysaccharide antigen is shared between *Orientia tsutsugamushi* and non motile strains of *Proteus mirabilis* OXK.[9]

Testing was performed in single acute phase sera using OXK antigen received from King Institute of Preventive Medicine, Guindy which detects OXK antibodies. Using normal saline as a diluent, serum samples were diluted ranging from 1/20 to 1/1280. OXK antigen suspension was added into each tube and incubated overnight at 37°C. Complete clearing of supernatant and formation of white flocculent masses at the bottom of the tube was considered as positive. Highest dilution of the serum showing agglutination was interpreted as serum titre. Formation of button at the bottom of the tube was considered as negative. Agglutination titre of ≥80 was considered as positive for *Orientia tsutsugamushi*.[10]

**Scrub Typhus IgM ELISA:** The test was performed as per manufacturer's protocol using Scrub Typhus DetectTMIgM ELISA system, In Bios International, inc. kit. It is a qualitative kit containing ELISA plates with 96 wells coated with recombinant antigen mix of *Orientia tsutsugamushi*. During testing, samples were diluted to 1/100. OD (Optical Density) readings were taken at 450nm using a microtitre plate ELISA reader.[11]

**Calculation of cut off:**100 serum samples from 3 categories- diseased (Laboratory confirmed Scrub typhus), confirmed unrelated febrile illness like Typhoid, Dengue, Leptospirosis and normal healthy adults were tested for determination of cut-off using Receiver operating characteristic curve analysis.[11] The cut off OD values ≥0.48 were considered positive.

**Statistical analysis:** Data collected was entered into a MS excel sheet, analysed using Statistical package for social studies software version 21.0 with appropriate statistical tests like Chi-square test as per objectives of the study. Confidence interval was calculated. The p-value ≤0.005 was considered statistically significant.

## RESULTS

Total of 200 inpatients having undiagnosed acute febrile illness were analysed by Weil Felix test and Scrub typhus IgM ELISA. Among these, 21(10.5%) samples were tested positive by Scrub typhus IgM ELISA [Table1] and 14(7%) samples were tested positive by Weil Felix test [Table 2]. Black eschar was present in 8/21 (38.09%) cases [Figure1]

Out of 21 Scrub typhus ELISA positives, 8(38.1%) were males and 13(61.9%) were females [Table3]. Age-wise analysis in the present study showed high seropositivity in the age group 20-40 years (85.7%)[Table3]. 11(5.8%) samples were tested positive both by ELISA and Scrub typhus [Table 4]. Among 21 ELISA positives, 11(52.4%) showed positive by Weil Felix test and 10(47.6%) were negative[Table5]. Three Weil Felix test positive samples were tested negative by Scrub typhus ELISA.


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The validity of Weil Felix test results was compared with IgM ELISA which is taken as the gold standard method. In this study, Weil Felix test showed sensitivity of 52.4% and specificity of 98.3% [Table6]. Weil Felix test had a positive predictive value of 78.6% and a negative predictive value of 94.6% [Table6].

**Table 1: Sero- positivity of scrub typhus among acute febrile illness patients by IGM ELISA**

| No of Patients with Acute Febrile illness | No of Scrub typhus IgM Positive | | No of Scrub typhus IgM ELISA* Negative | | 95%CI† |
|---|---|---|---|---|---|
| | No | % | No | % | |
| 200 | 21 | 10.5% | 179 | 89.5% | 87.3-91.7% |
| *Enzyme Linked Immunosorbent Assay †CI- confidence interval | | | | | |

**Table 2: Sero-positivity of scrub typhus among acute febrile illness patients by Weil Felix test**

| No of Patients with Acute Febrile illness | No of Weil Felix Test Positive | | No of Weil Felix Test Negative | | 95%CI* |
|---|---|---|---|---|---|
| | No | % | No | % | |
| 200 | 14 | 7% | 186 | 93% | 89.4-96.6% |
| *CI- confidence interval | | | | | |

**Table 3: Age and genderwise distribution of scrub typhus sero-positivity by IgM ELISA**

| Age group (in years) | Males | | Female | | Total | |
|---|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | Positive | Negative |
| 21-30 | 0 | 13 | 9 | 31 | 9 | 44 |
| 31-40 | 6 | 39 | 3 | 17 | 9 | 56 |
| 41-50 | 2 | 32 | 0 | 20 | 2 | 52 |
| 51-60 | 0 | 17 | 1 | 10 | 1 | 27 |
| Total | 8 | 101 | 13 | 78 | 21 | 179 |

**Table 4: Results of Weil felix test and scrub typhus IgM ELISA in the diagnosis of scrub typhus**

| Result | Weil Felix test (n=200) | | ScrubtyphusIgM ELISA* (n=200) | | Weil Felix test and IgM ELISA*(n=200) | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| Positive | 14 | 7% | 21 | 10.5% | 11 | 5.8% |
| Negative | 186 | 93% | 179 | 89.5% | 189 | 94.5% |
| *Enzyme Linked Immunosorbent Assay | | | | | | |

**Table 5: Comparison of results of Weil Felix test and scrub typhus IgM ELISA***

| Result | ScrubtyphusIgM ELISA *Positive | Scrub typhus IgM ELISA* Negative | Total | Chi square value &p value |
|---|---|---|---|---|
| Weil Felix Positive | 11(a) | 3(b) | 14 | †Chi square statistics 74.2271 p value <0.00001 |
| Weil Felix Negative | 10(c) | 176(d) | 186 | |
| Total | 21 | 179 | 200 | |
| *Enzyme Linked Immunosorbent Assay †Chi square statistics :74.2271, p-value <0.00001, the result is significant at p<0.005 | | | | |

**Table 6: validity of Weil Felix test with respect to ELISA**

| S.NO | Parameters | Results | 95% Confidence Interval |
|---|---|---|---|
| 1. | Sensitivity | 52.4% | 32.4-71.7% |
| 2. | Specificity | 98.3% | 95.2-99.4% |
| 3. | Positive predictive value | 78.6% | 52.4-92.4 |
| 4. | Negative predictive value | 94..62% | 90.4-97.1% |


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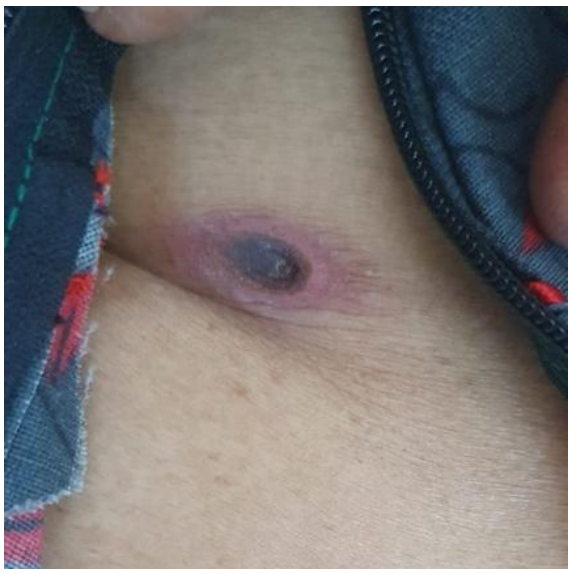

**Figure 1: Eschar**

## DISCUSSION

Scrub typhus is a re-emerging zoonotic disease often presents as a life-threatening febrile illness if left untreated. Epidemics have been reported from Puducherry, Andhra Pradesh and other southern states. Urbanization, deforestation and climatic change have impacted the epidemiology. The causative agent is a minute intracellular bacteria named *Orientia tsutsugamushi* transmitted by an arthropod mite called *Leptotrombidium*.

This zoonotic disease often under diagnosed due to its vague clinical presentation and paucity of definitive tool for diagnosis. Culture of the *Orientia tsutsugamushi* is difficult, technically demanding, time consuming and impractical in many of the laboratories. It is also dangerous which requires Biosafety Level III facility.[12] Various serological tests like Weil Felix test, Scrub IgM ELISA, Rapid immunochromatographic test, Indirect Immunoperoxidase test, Indirect Immunofluorescence antibody test and molecular tests like Polymerised chain reaction are available.[3]

This study showed seropositivity of 10.5% by IgM ELISA in Acute febrile illness patients which is lower than studies from Krishnagiri(31%), Vellore (47.5%) and Pondicherry(70%).[13-15] Paul raj et al study reported seropositivity of 6.07% by IgM ELISA among febrile illness cases which is lower than present study.[1]A systematic review done by Emily et al showed that the overall burden of Scrub typhus cases were 15.3%, Hospital based studies 15.1%, Acute undifferentiated febrile illness studies 25.3% and seroprevalence studies 34.2% in India.[2] According to Emily et al, higher proportion of scrub typhus cases reported in South India (55.5%) compared to North India (31.5%).[2] Tamil Nadu (37.6%) has contributed highest proportion of cases among Southern states.[2]

Age-wise analysis in the present study showed higher seropositivity (85.7%) in the age group 20-40 years which is comparable to Paulraj et al with 21-65 years and Singh et al reported 30-60years.[1,16] Vivekanandan et al with high seropositivity in 40-60years and Yaqoob et al where their study showed 31-60years having highest seropostivity.[15,17] This could be explained that people in their active years of life involved in agriculture related works are exposed to scrub vegetations.

In this study, there is high prevalence in female gender (61.9%) which is concurrent with Vivekanandan et al, Yaqoob et al, Seema Rani et al (70%) and PK Sharma et al(54.8%).[15,17-19] This association is not clear but may be due to different types of roles of women and men assumed in their society, with rural women spending more time in places of scrub vegetation.[20]

Clinical diagnosis of scrub typhus may pose problem without laboratory investigation as eschar was observed only in 8/21 (38.09%) cases, which is higher than Vivekananthan et al (21.43%).[15]According to Munilakshmi et al, it is observed in 10-90% of the cases and often missed if covered parts are not examined.[4]

Weil Felix test is a widely used test in resource poor settings in India despite its low sensitivity and specificity. It detects agglutinating antibodies against heterophile antigen which appears only in the second week of infection.[2] As the Weil Felix test shows false negative results in the early days of illness, delay in initiation of antimicrobials can lead to complication and death. The IgM ELISA with its good sensitivity and specificity is suitable for testing large number of samples in tertiary care centers.

Total 200 serum samples were analysed by both Weil Felix test and IgM ELISA and results were compared. 14(7%) samples were tested positive by Weil Felix test and 21(10.5%) by ELISA in contrast to K.S.Roopa et al with positivity 25%, 30.8% respectively[5]. Out of 21 ELISA positive 11(52.4%) showed positivity by Weil Felix test at 1:80 titre which is similar to Jacob et al(54%) and higher than Issac et al (30%).[21,22]

In the present study, only 52.4% ELISA positive samples showed reactivity in Weil Felix test. Sensitivity, specificity, positive predictive value and negative predictive value of Weil Felix test at cut off titre 1:80 with respect to IgM ELISA was 52.4%, 98.3%, 78.6% and 94.6% respectively. This finding correlates well with study done by Prakash et al showing sensitivity 43%, specificity 98%.[23] In contrast, Issac et al demonstrated sensitivity and specificity 30% & 100% respectively.[22] Differences in sensitivity of Weil Felix test may be attributed to differences in circulating genotypes of organism and antigen used.

Among 179 ELISA negative samples 3 were tested positive for Weil Felix positive. False positives in Weil Felix test could be due to Urinary tract Infection caused by *Proteus* and other febrile illness. The specificity of this heterophile agglutination test was reasonably good even at cut off titre 1:80 in the present study which is similar to Issac et al and Prakash et al.[22,23] In spite of its major drawbacks


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like poor sensitivity & cross reactivity, cost effectiveness and easy availability makes Weil Felix test a useful preliminary screening tool for diagnosis of Scrub typhus in resource poor settings.

Scrub typhus IgM ELISA is a qualitative test that uses recombinant antigen for detection of antibodies to *Orientia tsutsugamushi* in serum. 21/200(10.5%) tested positive by ELISA. Present study showed lower ELISA positivity than Seena Rani et al (29.46%), K Usha et al (58.21%).[18,24]. 10/21 ELISA positive samples were tested negative by Weil Felix test. According to Pooja et al, sensitivity and specificity of ELISA was 100% & 94.67% which correlates with Issac et al.[22,25]

Therefore, IgM ELISA with its statistically significant (*p* value <0.005) sensitivity and specificity have become a test of choice in tertiary care hospitals. False positive results can occur in dengue, leptospirosis and malaria which can be overcome by hiking the cut off. False negative result can occur in secondary infection with *Orientia tsutsugamushi*.

Molecular methods like Polymerized chain reaction (PCR) with higher sensitivity, specificity and short turnaround time can be used in the initial stages of illness. False negative results due to genetic diversity and unavailability of technique in remote places limits its application. Development of Loop mediated isothermal amplification method, biosensor, DNA sensor and immuno-sensor based diagnostic systems are the future of early diagnosis.[26]

## CONCLUSION

Present study with seropositivity rate of 10.5% conclude that Scrub typhus is emerging in this locality and should be considered in the differential diagnosis of acute febrile illness especially in monsoon and post monsoon season. An early initiation of antimicrobial therapy can prevent complications and reduce mortality. This study confirms Scrub typhus IgM ELISA has good sensitivity & specificity compared to Weil Felix test and is the best tool to diagnose early and test large number of samples simultaneously.

**Limitation**

Present study could not assess the validity of Rapid immunochromatographic test, indirect immunofluorescence assay and Real time PCR in the diagnosis of scrub typhus due to financial constraints

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