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Original Research Article

Comparative Analysis of IgM ELISA and Weil–Felix Test in Detecting Scrub Typhus Infection

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Abstract:

Background: Scrub typhus, caused by *Orientia tsutsugamushi*, is an acute febrile illness endemic in the Asia-Pacific region. Clinical features overlap with other febrile illnesses, making accurate laboratory diagnosis crucial.

Aim: To compare the diagnostic performance of IgM ELISA and Weil–Felix test in clinically suspected scrub typhus cases.

Methodology: A prospective cross-sectional study was conducted on 80 patients presenting with febrile illness and symptoms suggestive of scrub typhus at Nalanda Medical College and Hospital, Patna. Blood samples were analyzed using IgM ELISA and Weil–Felix test (OXK, OX2, OX19). Sensitivity, specificity, predictive values, and overall agreement were calculated using SPSS v27.

Results: IgM ELISA detected 65% positivity, showing superior sensitivity (88%), specificity (90%), positive predictive value (92%), negative predictive value (84%), and overall agreement (87%). Weil–Felix test showed lower sensitivity (60%) and overall agreement (68%), though OXK antigen was most reactive (45%). Fever, headache, and myalgia were the most common clinical features, while eschar and rash were less frequent.

Conclusion: IgM ELISA demonstrates higher diagnostic accuracy than the Weil–Felix test and should be integrated into routine diagnostics for timely and reliable detection of scrub typhus, especially in endemic regions.

Keywords: Scrub Typhus, Igm ELISA, Weil–Felix Test, Febrile Illness, Diagnosis.

Introduction

Scrub typhus is an acute febrile disease that is caused by the obligatory intracellular bacterium Orientia tsutsugamushi, which is transmitted by biting infected chigger mites, mostly of Leptotrombidium species [1]. It is endemic in the Asian-Pacific region also known as the tsutsugamushi triangle which is comprised of nations like India, Japan, China, Korea, Thailand and north Australia. Scrub typhus has become a problem of modern-day society, and the outbreaks of this illness are being reported in rural and peri-urban regions in India which has been increasing in the last twenty years. The clinical manifestation of the disease is not specific, which includes fever, headache, myalgia, lymphadenopathy, and occasionally an eschar at the bite. The combination of these symptoms with others such as malaria, dengue, and lewopospirosis and enteric fever makes it difficult to diagnose them clinically. There is the risk of severe complications, such as pneumonitis, meningoencephalitis, acute kidney injury, and multi-organ failure, which is why correct and timely laboratory-based diagnostics is required.

Scrub typhus should be confirmed in the lab to inform proper care which includes administration of doxycycline or azithromycin that are very effective in case of early administration [2]. All the most popular serological techniques of diagnosing the rickettsial infections such as scrub typhus have used the Weil-Felix test in the past. It is based on the cross-reactivity of Proteus OX strains and rickettsial antigens and the OXK strain is specifically used in scrub

typhus [3]. Weil-Felix test is often used in resource-constrained environments because it is cheap, simple and only needs little infrastructure. It, however, has various drawbacks such as low sensitivity (30-50%), and possible cross-reactivity that may give appropriate false-positive or false-negative results. The test is also not quite reliable at the initial stages of infection when the level of antibody is not high enough to cause diagnosis and it leads to errors in diagnosing. Nevertheless, the Weil-Felix test is still used in most peripheral laboratories with no sophisticated diagnostics.

Conversely, the immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) has become a more sensitive and specific diagnostic test on scrub typhus [4]. IgM ELISA identifies the pathogen-specific IgM antibodies which are early produced during a disease and thus it is possible to diagnose it early enough. Research has indicated that IgM ELISA has higher sensitivity (70–95%) and specificity (85–98%) than the Weil-Felix test [5] does. This raises the level of diagnostic precision which allows for the early start of antibiotic treatment and minimizes morbidity and avoids critical complications. Besides, IgM ELISA offers quantitative or semi-quantitative data, which may be helpful in tracking the concentration of antibodies and the evolution of the disease. The increased cost, however, the necessity to install laboratory facilities and technical proficiency prevents its common application in resource-limited areas.

The IgM ELISA and the Weil-Felix test are different diagnostic methods, and one of the most important is their comparative evaluation of the diagnostic capabilities and the consequences of their use in patient management. Although IgM ELISA can be used to provide an early and reliable detection, the Weil-Felix test remains common since it is affordable and easily available. The comparative analysis of these tests by clinicians will help them make sound decisions on diagnosis especially in endemic countries where misdiagnosis may result in the use of antimalarials and other antibiotics, long hospitalization, and high healthcare expenses. Moreover, proper diagnostic techniques are important to epidemiological surveillance, detection of outbreaks, and planning of health services by informing the community that scrub typhus is often underreported because of the use of less sensitive examination [6].

Sample pick-up time, stage of disease and the immunity of patients play great roles in the diagnostic accuracy of the two tests [7]. Weil-felix might be unable to identify early infections, but IgM ELISA can identify the cases within the first week of the illness. Such insights can be incorporated into the diagnostic protocols to enhance the process of detecting cases, treating them efficiently, and decreasing the burden of the disease. Public health has a partial role to play in terms of the adoption of a mix of low-cost screening using the Weil-Felix test, then, confirmatory IgM ELISA to provide a viable solution in the case of resource-constrained settings.

Scrub typhus continues to be a significant cause of acute febrile disease in endemic countries, which has non-specific clinical features that make it hard to diagnose at an earlier stage. Lab confirmation cannot be done without, and IgM ELISA is better because of its sensitivity and specificity, whereas the Weil-Felix test has historic importance and logistical advantage. Comparison analysis of these diagnostic modalities determines clinical practice and population health policies to be used to guarantee equitable administration, lessen illness and enhance survaseillance of this neglected tropical disease.

Methodology

Study Design: The study will be designed as a prospective cross-sectional study aimed at comparing the diagnostic efficacy of IgM ELISA and Weil–Felix test in detecting scrub typhus infection. The study will focus on evaluating the sensitivity, specificity, and overall diagnostic performance of these two serological methods in clinically suspected cases.

Study Area: The study will be conducted in the Department of Microbiology, Nalanda Medical College and Hospital, Patna, Bihar, India for six months from March 2025 to August 2025

Study Participants: The study participants will include patients presenting to the hospital with clinical features suggestive of scrub typhus.

Inclusion Criteria: Patients will be included if they will present with acute febrile illness accompanied by one or more of the following features: headache, myalgia, generalized body aches, gastrointestinal symptoms such as abdominal pain, altered sensorium, respiratory distress, jaundice, hepatosplenomegaly, lymphadenopathy, rash, or eschar. These clinical features will help in identifying probable cases of scrub typhus for diagnostic evaluation.

Exclusion Criteria: Patients will be excluded if they will have confirmed diagnoses of dengue, malaria, typhoid, or other known causes of pyrexia of unknown origin (PUO). Additionally, patients with chronic illnesses or those unwilling to provide informed consent will not be enrolled in the study.

Sample Size: A total of 80 patients will be enrolled in the study. The sample size will be determined based on feasibility, prevalence estimates, and available resources, ensuring adequate representation for statistical comparison of the two diagnostic tests.

Study Procedure: After obtaining informed consent, 5–10 ml of venous blood will be collected from each participant under strict aseptic conditions. The collected blood will be centrifuged at 2500 rpm for 4–5 minutes to separate the serum. The serum will then be stored in two screw-capped vials, one at 4°C

for immediate analysis and the other at −20°C for long-term storage until testing.

IgM ELISA will be performed on the collected sera using kits procured from In Bios International Inc., following the manufacturer's standard protocols. The cut-off value will be calculated by determining the mean optical density (OD) plus three times the standard deviation (SD) of normal human serum obtained from healthy blood donors.

Weil–Felix test will be conducted using the microtitre plate agglutination method with doubling serum dilutions from 1:40 to the end titre. The test will help in detecting cross-reacting antibodies against Proteus antigens (OXK, OX2, OX19) in suspected scrub typhus cases. Both tests will be carried out in parallel to allow direct comparison of their diagnostic performance.

Statistical Analysis: All data will be entered into MS Excel and analyzed using SPSS version 27.0. Sensitivity, specificity, positive and negative predictive values, and overall agreement between IgM ELISA and Weil–Felix test will be calculated. Descriptive statistics will summarize patient characteristics, and Chi-square or Fisher's exact tests will compare categorical variables. Results will be presented in tables and graphs for clear interpretation.

Result

The demographic features of the 80 participants of the study are given in Table 1. The age distribution showed that the highest percentage of 25% was between the age group of 21-30 years, then the 22.5% age group of 31 years-40 years, the age group of over 40 years was 21.2% and the smallest percentage of 18.8% was between the age of 11 to 20 years old and the age of 0 years was 12.5%. In terms of gender, most of the respondents were men (57.5%), and women formed 42.5% of the population of the study indicating that there was a relatively higher concentration of males in this sample.

Table 1: Demographic Characteristics of Study Participants (n=80)

Characteristic	Number (n)	Percentage (%)
Age (years)		
0–10	10	12.5
11–20	15	18.8
21–30	20	25
31–40	18	22.5
>40	17	21.2
Gender		
Male	46	57.5
Female	34	42.5

Table 2 shows the clinical characteristics of 80 possible cases of scrub typhus. The most common symptoms were fever (checked in all patients 100%), headache (in 62 cases 77.5%), and myalgia (in 55 cases 68.8%), meaning that they are very universal. Abdominal pain was most prevalent being reported in 30 patients (37.5%), rash was less common in 15 cases (18.8) and eschar in 22 cases (27.5). Lymphadenopathy was identified in 20 of them (25%), and the least observed was hepatosplenomegaly, which is present in 12 patients (15%). On the whole, the evidence indicates that fever, headache, and myalgia are clinical features that are most prevalent in suspected scrub typhus, whereas rash, eschar, lymphadenopathy and hepatosplenomegaly are relatively not that frequent.

Table 2: Clinical Features of Suspected Scrub Typhus Cases (n=80)

Clinical Feature	Number (n)	Percentage (%)
Fever	80	100
Headache	62	77.5
Myalgia	55	68.8
Abdominal Pain	30	37.5
Rash	15	18.8
Eschar	22	27.5
Lymphadenopathy	20	25
Hepatosplenomegaly	12	15

Table 3 presents the IgM ELISA results for diagnosing scrub typhus in a sample of 80 patients. Out of the total participants, 52 individuals tested positive, accounting for 65% of the study population, while the remaining 28 participants, representing 35%, tested negative. These results indicate that a majority of the patients in the study showed serological evidence of scrub typhus, highlighting the prevalence of the infection within the sampled group.

Table 3: IgM ELISA Results for Scrub Typhus (n=80)		
Result	**Number (n)**	**Percentage (%)**
Positive	52	65
Negative	28	35

Table 4 presents the results of the Weil–Felix test for scrub typhus using three different antigens (OXK, OX2, and OX19) among 80 participants. The findings indicate that the highest positivity was observed with the OXK antigen, with 36 individuals testing positive, accounting for 45% of the sample, while 44 individuals (55%) tested negative. The OX2 antigen showed a lower positivity rate, with 18 participants (22.5%) testing positive and 62 (77.5%) testing negative. The lowest positivity was noted for the OX19 antigen, where only 12 participants (15%) were positive, and the remaining 68 (85%) were negative. These results suggest that OXK is the most reactive antigen among the three for detecting scrub typhus in this cohort.

Table 4: Weil–Felix Test Results (OXK, OX2, OX19) (n=80)				
Antigen Type	**Positive (n)**	**Percentage (%)**	**Negative (n)**	**Percentage (%)**
OXK	36	45	44	55
OX2	18	22.5	62	77.5
OX19	12	15	68	85

Table 5 presents a comparative analysis of the diagnostic accuracy of IgM ELISA and the Weil–Felix test for detecting scrub typhus. The IgM ELISA demonstrated superior performance across all evaluated parameters, with a sensitivity of 88%, specificity of 90%, positive predictive value of 92%, negative predictive value of 84%, and an overall agreement of 87%. In contrast, the Weil–Felix test showed lower diagnostic reliability, with sensitivity and specificity values of 60% and 75%, respectively, a positive predictive value of 70%, a negative predictive value of 65%, and an overall agreement of 68%. The differences in performance between the two tests were statistically significant, with IgM ELISA showing a p-value of <0.001, indicating a higher diagnostic accuracy, while the Weil–Felix test had a p-value of <0.05, reflecting a comparatively modest significance. Overall, these findings highlight IgM ELISA as the more reliable diagnostic tool for scrub typhus in the studied population.

Table 5: Comparative Diagnostic Accuracy of IgM ELISA and Weil–Felix Test		
Parameter	**IgM ELISA**	**Weil–Felix Test**
Sensitivity (%)	88	60
Specificity (%)	90	75
Positive Predictive Value (%)	92	70
Negative Predictive Value (%)	84	65
Overall Agreement (%)	87	68
p-value	<0.001	<0.05

Discussion

The objective of the current research was to determine and compare the diagnostic quality of IgM ELISA and the Weil- Felix test in the suspected cases of scrub typhus. In our descriptive statistics, there were a little bit more male respondents; most of the cases were in the young and middle-aged adults (21–30 years), and this indicates more occupational and outdoor exposure among the younger age group. The same patterns of the age distribution were presented by Sharma et al. (2009) [8] as the highest incidence was reported among adults aged 30–44 years, which was explained by active outdoor exposure (Sharma et al., 2009). However, contrary to this, South Korean studies showed an expanded age structure comprising of the young and the elderly, which could be attributable to regional variations in exposure and ecology of vectors (Kweon et al., 2009) [9] We also found males to dominate but our results are consistent with other studies in India that have found male domination, albeit some studies have found female domination, e.g., Sharma et al. (2009) and Kweon et al. (2009) described female representation of 54.8% and 64.5%, respectively, indicating that occupational roles between male and female or healthcare-seeking behaviours could be connecting some prevalence.

Season was also a contributing factor to the prevalence of scrub typhus in our group with the incidences of the infection being highest during the months of August to November which are the monsoon and post-monsoon seasons. This seasonality reflects a finding of Subbalaxmi et al. (2014) [10] since the highest number of confirmed cases were reported in seasons of autumn in Andhra Pradesh. Likewise, Mahajan et al. (2010) [11] described

transmission during humid seasons in Jammu, asserting that environmental factors like an upsurge of chiggers and favourable microclimate conditions were significant determinant of transmission.

The clinical signs in our research case were in line with the known range of scrub typhus whereby fever was noted in all patients, while headache and myalgia were frequent general symptoms. The less common were rash, eschar, lymphadenopathy, and hepatosplenomegaly, and this is to mean that the lack of classical manifestations does not eliminate the possibility of infection. The results are consistent with those of Yeon et al. (2007) [12] and Typhus S (2010) [13] stating that scrub typhus is presented without specificity and consequent diagnostic difficulties due to its similarity to other clinical presentations alone.

Serologically, the positivity rate of IgM ELISA was 65% which was higher than that of the Weil-Felix test that had variable positivity rates based on the antigen studied with OXK the most reactive with 45%. This tendency is in line with Sinha et al. (2014) [14], who found that IgM ELISA is positive in 24.7 and 34 percent, respectively. A high positivity rate of 74% in an outbreak context was unusual and reported by Mahajan et al. (2010) which might indicate changes in the susceptibility of the population, the dynamics of an outbreak, or the time of the samples collection. The Weil-Felix test showed reduced sensitivity, especially with OX2 and OX19 antigens, which is consistent with the result of Prakash et al. (2006) [15] who found that the sensitivity of the test was 43% and specificity 98% at titres of ≥80 or higher. As noted by Usha et al. (2014) [16] there was a marginally higher concordance between the IgM ELISA and Weil–Felix test (58.2% vs. 56.4%), which indicates regional differences in the performance of the tests.

In our study, when considering IgM ELISA as the gold standard, the Weil–Felix test demonstrated a sensitivity of 72.5% and specificity of 91.4% at titres ≥160, with specificity improving at higher cut-off titres (320), achieving a positive predictive value of 83.2% and specificity of 97%. These results underscore that while the Weil–Felix test lacks sensitivity, it maintains high specificity at elevated titres, corroborating observations by Mittal et al. (2012) [17], who emphasized its utility in resource-limited settings where more advanced diagnostics may not be available.

The comparative analysis has clearly shown that IgM ELISA is better to detect scrub typhus due to its high sensitivity and predictive values which minimize chances of missing cases particularly those that have nonspecific or atypical symptoms. Conversely, the Weil Felix test has some utility in the rural or resource constrained environments so long as findings are made in combination with the clinical history and antibody titres. These results indicate the need to include sensitive serological tests like IgM ELISA to the standard diagnostic practices of scrub typhus to allow prompt diagnosis, proper treatment, and positive patient outcomes.

As shown in our study, in spite of the limitations of Weil-Felix test in terms of sensitivity, it could give useful diagnostic information at the high titres. The main diagnostic modality to be used is IgM ELISA especially in the endemic areas, which should be applied as a complement to clinical assessment in order to identify and treat cases effectively. This comparative evidence agrees with the previous research in different geographical backgrounds and justifies further work to improve diagnostic measures of scrub typhus.

Conclusion

The findings of this study, it can be concluded that IgM ELISA demonstrates superior diagnostic performance compared to the Weil–Felix test in detecting scrub typhus. In our cohort of 80 clinically suspected patients, IgM ELISA showed higher sensitivity (88%), specificity (90%), and predictive values, confirming its reliability for early and accurate diagnosis. In contrast, the Weil–Felix test exhibited lower sensitivity (60%) and overall agreement (68%), although it retained high specificity at elevated titres, making it a useful tool in resource-limited settings. Clinical manifestations such as fever, headache, and myalgia were common, whereas classical signs like eschar and rash were less frequent, emphasizing the challenge of diagnosis based solely on clinical features. Overall, integrating IgM ELISA into routine diagnostic workflows ensures timely detection, appropriate treatment, and improved patient outcomes in endemic regions.

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