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Scrub Typhus in a Malaysian cohort: Epidemiological Trends, Clinical Outcomes, and Molecular Diversity

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Abstract

Background:

Scrub typhus, a potentially fatal infection spread by chiggers' bites carrying *Orientia tsutsugamushi*, is under-reported in Malaysia.

Methods:

This retrospective, descriptive study analyzed 28 PCR-confirmed *Orientia tsutsugamushi* cases presented in Hospital Tuanku Ja'afar Seremban between 2023–2025, focusing data on socio-demographic data, clinical spectrum and outcome, and molecular analysis of the cohort.

Results:

The study found most patients were male (82%), Malaysian (68%), and from the Seremban area (71%). A significant portion (43%) worked in plantations, and 46% had rodent exposure. Initial diagnoses often missed scrub typhus, with common symptoms including acute fever (93%). About 21% of subjects had detectable eschars on physical examinations. Common laboratory abnormalities include elevated transaminases (89%), and renal impairment (36%). Severe complications like the need for mechanical ventilation (25%) were observed, with a mortality rate of 11%. PCR analysis showed Karp genotype formed the largest cluster of the cohort.

Conclusions:

The findings highlight the need for more local data and increased awareness among healthcare professionals and the public to better manage this neglected tropical disease. Delayed recognition of the disease likely contributes to severe complications as illustrated from the study.

INTRODUCTION

Scrub typhus is a neglected tropical disease, frequently a cause for undifferentiated fever. Malaysia is known for its endemicity of scrub typhus, one of the countries located in Tsutsugamushi Triangle. Recent studies had shown scrub typhus has been detected globally beyond the traditionally known endemic countries [1] and increasingly seen at urban areas [2]. Scrub typhus is caused by obligate intracellular, gram-negative coccobacillus bacteria known as *Orientia tsutsugamushi* (OT) [3, 4]. Transmission to human is caused by the bite of larval stage of infected *Leptotrombidium* mites [3] commonly known as Chigger [5]. Local socio-demographic and clinical spectrum data on scrub typhus is lacking with no clear representation of disease burden in Malaysia. In Southeast Asia, it was estimated 1 million of scrub typhus cases occurred annually [6]. A study published in 1976 estimated a prevalence

scrub typhus among oil-palm worker to be 400 cases annually in a population of 10,000 people living on one plantation in Malaysia [7]. Another study done at northern region of Malaysia, Teluk Intan, showed rickettsioses (19%) was the cause of acute febrile illness with scrub typhus was identified in 24 subjects (11%) [8]. Comparing to a study done in India, scrub typhus was the cause of undifferentiated fever in at least 25.3% of cases [9].

The aims of this study were to characterize the Polymerase Chain Reaction (PCR) confirmed cases in our tertiary center, to better understand the socio-demography, risk factors, clinical presentations, complications, outcome, and to raise local awareness about the burden of the disease.

MATERIALS AND METHODS

We conducted an observational, retrospective, cross- sectional study on the scrub typhus cases which were positive for OT PCR from the year 2023 to 2025 presented at Hospital Tuanku Ja'afar, Seremban, Negeri Sembilan. List of positive PCR subjects were compiled by the Institute Medical of Research (IMR) and microbiology team Hospital Tuanku Ja'afar, Seremban. Data of each of the subjects were traced via digital records. Data was compiled and analyzed with Microsoft Excel. Blood samples and eschar biopsies in study subjects were sent to IMR for rickettsia PCR test.

DNA Extraction and PCR

The DNA was extracted from buffy coat (EDTA blood) or tissue using a QIAmp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Both real-time quantitative PCR (qPCR) and nested PCR (nPCR) were performed. In qPCR, the 47 kDa periplasmic serine protease *htr*A (47 kDa) is targeted and prepared as described[10, 11, 12]. Briefly, a total of 25 µL reaction per tube was prepared, including 6.25 µL master mix and 1 µL of each primer (forward: 5'-AACTGATTTTATTCAAAC TAATGCTGC T-3', reverse: 5'-TATGCCTGAGTAAGATACRTGAATRGAATT-3') and probe (6FAM-TGGGTAGCTTTGGTGGACCGATGTTTAATCT-TAMRA). The two-step qPCR reaction was performed on a QuantStudio™ 6 Flex Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, USA) at initial denaturation; 94°C for 5 min followed by 40 cycles of denaturation at 94°C, 5 s, and annealing at 60°C, 30 s using a 4X CAPITAL™ qPCR Probe Master Mix (Biotech Rabbit, Berlin, Germany) according to the manufacturer's protocol.

The qPCR-positive DNA were proceeded for nested PCR or conventional PCR targeting 56 kDa type specific antigen (TSA56) to determine the genotypes of the OT strains [12, 13]. Two rounds of PCR were performed in nested PCR using primer pairs: (i) primer 34: 5'-TCAAGCTTATTGCTAGTGCAATGTCTGC-3', primer 55: 5'AGGGATCCCTGCTGCTGTGCTTGCTGCG3'); (ii)primer10:5'GATCAAGCTTCCTCAGCCTACTATAATGCC-3',primer11:5'CTAGGGATCCCGACAGATGCACTATTAGGC-3'). First, the outer fragments of *tsa56* were amplified followed by the amplification of the inner fragments using a DNA template and the first PCR product, respectively. The PCR was run using PCRBIO Ultra mix (PCR Biosystems, Wayne, PA, USA) and

the same conditions for both rounds of PCR: initial denaturation at 94°C for 2 s, denaturation at 94°C for 30 s, annealing at 57°C for one min and extension at 72°C for one min for 35 cycles and final extension at 72°C for 10 min. The PCR product was run on a 2% agarose gel and viewed on Bio-Rad ChemiDoc Touch Image System (Bio-Rad, Hercules, CA, USA).

For conventional PCR, the single-run PCR protocol used one set of primers, TSA1F (5'-AGTTTAGAATGGTTACCACTA-3') and TSA1R (5'-CTGCATGACGCTGCAATTT-3'). Briefly, a total of 25 µL reaction mix including 12.5 µL of Q5® High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, MA, USA) and 10 pmol of each primer was run under conditions: 98°C for 30 s (polymerase activation), 40 cycles of denaturation, annealing and extension at 98°C for 10 s, 56°C for one min, and 72°C for 30 s with a final extension at 72°C for 2 min. The PCR products were run on a 1.5% agarose gel at 80 V, 45 min and viewed on Bio-Rad ChemiDoc Touch Image System (Bio-Rad, Hercules, CA, USA).

Orientia tsutsugamushi (OT) Genotyping

The PCR products of 16 confirmed OT samples were available for genotype analysis by Sanger sequencing (1st Base, Seri Kembangan, Malaysia). The sequences were first blasted against NCBI databases to check completeness and the correct CDS region. All the *tsa56* partial genes from nested PCR and TSA1 sequences were aligned together with other known OT genotype strains from Malaysia [12] and neighboring countries in Southeast Asia [14, 15] (**Table S1**), and a phylogenetic tree was generated using the maximum likelihood method conducted in MEGA 11[16]. Bootstrap analysis with 1000 repetitions was performed to assess the robustness and reliability of the tree branching. The sequences have been deposited in GenBank (PZ379605-PZ379616).

RESULTS

Demographic and epidemiological characteristic

The cohort comprised of 28 subjects with the median age of 41(range 15–72). The majority were male (n = 23,82%) and five (18%) were female. Nineteen subjects (68%) were local Malaysians while nine (32%) subjects were non- citizens originating from various countries including Indonesia, Bangladesh, Nepal and Vietnam.

Geographically, within Negeri Sembilan and its vicinity, most subjects resided in Seremban (20,71%), followed by Port Dickson (2, 7%) Jelebu (1, 4%),Jempol(1, 4%), Lenggeng(1,4%). One subject was from the neighboring state of Sungai Pelek, Selangor (1,4%), while the residential locality was unknown for two subjects (7%). The map of Negeri Sembilan is showed in Fig. 1.

Majority of the subjects (20, 71%) did not have concurrent co-morbidities. Among those with underlying illnesses, the most common was hypertension (5, 18%), followed by diabetes mellitus (3, 11%), dyslipidaemia(1, 4%), end stage renal failure (1, 4%), benign prostatic hyperplasia (1, 4%) and

thalassemia carrier (1, 4%). Two subjects had history of recent travelling prior to illness, one subject to Bangladesh and another to Thailand.

Regarding occupational exposure, 12 subjects (43%) worked in areas of outdoor and potential vector exposure including farming, gardening, palm oil worker and rubber tapper. One subject worked as construction worker, while another worked as electrical technician. Others had no known outdoor occupation exposure or the occupations were not stated.

Nearly all of the eleven subjects who has risk of vector exposure did not wear personal protective clothing such as fully covered top and bottom clothing, during outdoor working. Thirteen subjects had potential exposure to rodents during occupational activities (Table 1).

Table 1
Socio-demographic data and risk factors

Socio-demographic data	Description	N = 28(%)
Gender	Male	23(82)
	Female	5(18)
Locality	Seremban	20(71)
	Port Dickson	2(7)
	Jelebu	1(4)
	Jempol	1(4)
	Lenggeng	1(4)
	Sungai Pelek (Selangor)	1(4)
	Unknown	2(7)
Citizenship	Malaysian	19(68)
	Non-Malaysians	9(32)
Co-morbidities	No co-morbidities	20(71)
	Hypertension	5(18)
	Diabetes mellitus	3(11)
	Dyslipidemia	1(4)
	End Stage Renal Failure	1(4)
	Benign Prostatic Hyperplasia	2(7)
	Thalassemia carrier	1(4)
Occupation	Farmer	6(21)
	Palm oil plantation worker	4(14)
	Rubber Tapper	2(7)
	Student	2(7)
	Gardener	1(4)
	Electrician	1(4)
	Construction worker	1(4)
	Lorry driver	1(4)
	Homemaker	1(4)

Socio-demographic data	Description	N = 28(%)
	Cook	1(4)
	Unknown	8(29)
Personal protective measures (insect repellent or protective clothing)	Yes	1(4)
	No	27(96)
Potential exposure to rodents during occupational activities	Yes	13(46)
	No	15(54)

Clinical Manifestation and Initial Diagnoses:

Clinically, the predominant symptoms of the subjects were fever (26, 93%). Other common symptoms were vomiting (12,43%), diarrhea (12, 43%), cough (9,32%), abdominal pain (6, 21%), myalgia (6, 21%), lethargy (3, 11%), headache (3, 11%), shortness of breath (2, 7%), seizure (1, 4%), chest pain (1, 4%). On physical examination, a total of 6 subjects (21%) had clinical findings of eschars (Fig. 2,3,4).

Upon presentation, majority initial diagnosis by attending clinicians upon presentation were leptospirosis (36%, 10), dengue (9,32%), community acquired pneumonia (5,18%), viral fever (2,7%), necrotizing fasciitis of the arm (1,4%) and sepsis unknown source (1,4%). (Table 2)

Table 2
Clinical manifestations and complications of scrub typhus cases

Clinical manifestations:	**n = 28(%)**
Fever	26(93)
Diarrhea	12(43)
Vomiting	12(43)
Cough	9(32)
Abdominal pain	6(21)
Myalgia	6(21)
Lethargy	3(11)
Headache	3(11)
Shortness of Breath	2(7)
Seizure	1(4)
Chest pain	1(4)
Eschar	6(21)
Complications:	n = 28 (%)
Hypoxaemia	10(36)
Acute kidney injury	10(36)
ICU admission	8(29)
Mechanical ventilation	7(25)
Septic shock	4(14)
Meningoencephalitis	1(4)
Infective Endocarditis	1(4)
Dialysis	4(14)
Mortality	3(11)

Laboratory Findings and Diagnostics

Upon initial presentation the median of total white cell count was 9x 10^9/L, median haemoglobin level for male was 125 g/L (IQR 109–137), and female 127g/L (IQR 109–141). Seventeen subjects (61%) had thrombocytopenia with platelet count of less than 150 x10^9/L with median value of platelet 116 (IQR

83–191). Most of the subjects (n = 23, 82%) had elevated transaminases with elevation of either aspartate aminotransferase (AST), alanine aminotransferase (ALT) level or both. The degree of liver impairment was further classified based on the degree of upper limits for AST and ALT (Table 3). For creatinine level, the median value was 103 umol/L (IQR 71–193) and the median glomerular filtration rate (GFR) was 82 mL/min/1.73m^2 (IQR 36–108). The GFR was estimated based on the 2021 CKD-EPI formula estimation. The degree of renal impairments was further classified based on KDIGO 2012 classification and showed that 13(46%) subjects had normal or high GFR while 15(54%) subjects had reduced GFR with various degree of impairments. (Table 3).

All subjects had at least one blood culture taken however no clinically significant growth. One subject was positive for NS1 antigen for dengue, however subsequent dengue PCR was negative. All subjects included in this study were confirmed *Orientia tsutsugamushi* via serum rickettsia PCR. Three subject's eschar tissues were sent for PCR testing and were positive for *Orientia tsutsugamushi*.

Table 3
Laboratory characteristics of scrub typhus cases

Laboratory indices	**Median value (n = 28)**	**Interquartile Range Q1-Q3**	**Normal range(unit)**
Haemoglobin	Male:125 Female:127	Male:109–137 Female:109–141	Male:120–170 g/L Female:120–150 g/L
Total white count	9	5.9–10.9	4–10 x10^9/L
Platelet	116	82.8–190.5	150–400 x10^9/L
Aspartate aminotransferase(AST)	121	82.5–176	< 34 U/L
Alanine aminotransferase	88.5	62.3–204.8	10–49 U/L
Creatinine	103 umol/L(median)	70.8 -192.5	49–90 umol/L
*GFR	82		> 90 mL/min/1.73m^2
Severity of AST impairment	n = 28(%)		
Normal AST level < 34U/L	2(7)		
Mild (< x3 upper normal limit) < 102 U/L	6(21)		
Moderate (x3 to x5 upper normal Limit 103 to 170 U/L	8(29)		
Severe (above x5 upper normal limit) > 171 U/L	6(21)		
AST Not available	5(18)		
Severity of ALT impairment	n = 28(%)		
Normal ALT Level 10-49U/L	5(18)		
Mild ALT (< x3 upper normal limit) < 147 U/L	14(50)		
Moderate ALT (x3 to x5 upper normal) 147 to 245 U/L	3(11)		
*GFR was calculated using 2021 CKD-EPI Creatinine			

Laboratory indices	Median value (n = 28)	Interquartile Range Q1-Q3	Normal range(unit)
Severe ALT (> x5 upper normal)> 245U/L	5(18)		
Both AST and ALT elevated	17(61)		
Severity of GFR impairment based on KDIGO 2012 classification			
G1 Normal or high (above 90)	13(46)		
G2 Mildly decreased (60–89)	4(14)		
G3a Mildly to moderately decreased (45–59)	3(11)		
G3b Moderately to severely decreased (30–44)	2(7)		
G4 Severely decreased (15–29)	2(7)		
G5 Kidney Failure (< 15)	4(14)		
*GFR was calculated using 2021 CKD-EPI Creatinine			

All subjects had at least one blood culture taken however no clinically significant growth. One subject was positive for NS1 antigen for dengue, however subsequent dengue PCR was negative. All subjects included in this study were confirmed via OT PCR. Three subject's eschar tissues were sent for PCR testing and were positive for OT.

Clinical course, Treatment and Outcome

Regarding disease complications, four subjects suffered from septic shock upon initial presentation (14%) and thirteen subjects (36%) had renal impairment, with four subjects (14%) required dialysis support. One subject was an end stage renal failure patient on regular dialysis. Respiratory failure requiring oxygen supplements occurred in ten subjects (36%) and 7 subjects needed mechanical ventilation support. Intensive care management was required for eight subjects (29%). Majority of the subjects (25, 89%) were discharged and well while mortality was three subjects (3,11%). Notable rare and atypical systemic involvement from this subject cohort includes one case of possible scrub typhus infective endocarditis and one subject with presumed meningoencephalitis due to scrub typhus infection (Table 2).

Therapeutically, 18 subjects (64%) had received monotherapy doxycycline. Four subjects (14%) received combination therapy of doxycycline and azithromycin. While one subject (4%) received azithromycin only. Total of 5 subjects received no appropriate antibiotics that can target scrub typhus, including 3 subjects received amoxicillin- clavulanate, while one patient received piperacillin- tazobactam and

clindamycin for upper limb necrotizing fasciitis and one subject who was not given any antibiotic as was managed for dengue fever.

Genotyping of OT in Scrub typhus Cases in Negeri Sembilan

The phylogenetic analysis demonstrated that the isolates from Negeri Sembilan were distributed into three major genotypic clusters: Karp, Gilliam and TA763 (Fig. 5, Table S1). Among these, the Karp genotype formed the largest cluster, indicating that it may be the predominant circulating genotype in the study population. The bootstrap values observed at several nodes (ranging up to 1000) indicate strong statistical support for the grouping of these genotypes, confirming the reliability of the phylogenetic relationships.

DISCUSSIONS

Scrub typhus remains an important neglected tropical disease whereby the lack of local epidemiology data and awareness may further worsen the consequences of these infections. From the above collection of cases, male and working age predominance were observed with occupational exposure remains an important factor in leading to scrub typhus infection. Negeri Sembilan is a state with various agriculture activities such as palm oil, rubber, lemon grass plantation, and agricultural activities. Behavior of outdoor exposed workers such as sitting on grass are associated with scrub typhus infections [17]. Low usage of personal protective clothing remains an important risk factor to be address in Negeri Sembilan. A case control study done at India showed wearing top clothing and shoes during outdoor occupational activities were potentially protective against scrub typhus infection [18]. Other known risk factors which may played a role in transmission were demonstrated in cross- sectional study done in India showed exposure to animals including domestic pets or peridomestic rodent and bushy or scrub vegetation in surrounding area may further enhance the risk of scrub typhus transmission [19].

The differential diagnosis of acute undifferentiated fever may encompass various tropical diseases such as dengue, leptospirosis, malaria, and bacterial infection for example typhoid fever [20]. In this cohort of subjects there was a high number of missed diagnoses, with subjects treated as leptospirosis (36%) and dengue (32%). This highlights the importance of considering scrub typhus as differential diagnosis in acute undifferentiated fever. The low rate of eschars (21%) may hamper the diagnosis of scrub typhus. In comparison with published data, prevalence of eschar finding in Southeast Asian region was 41% [21] and with the highest frequency at the inguinal region 29% [21]. In endemic areas with high incidence of scrub typhus, diagnosis of scrub typhus should be considered even in the absence of eschars. Another possible explanation to this low finding of eschars may be due to lack of awareness and clinical examination to search for presence of eschars, in addition to obscure areas where eschar present, therefore contributing to delay or missed diagnosis. When appropriate antimicrobial treatment is administered untimely, potentially will lead to mortality [22, 23].

Regarding clinical manifestation, a study done Vietnam showed the commonest symptoms includes fever, headache and myalgia [24]. This differs from our cohort, where we observed fever, vomiting and diarrhea were almost as common as myalgia and or headache. These differing clinical manifestations may confused clinicians and mimic other conditions such as acute gastroenteritis or food poisoning, potentially leading to misdiagnosis. Laboratory abnormalities that were notable as well were thrombocytopenia, elevated transaminases, and acute kidney injury. Therefore, in Negeri Sembilan, scrub typhus should be strongly suspected if patients presenting with undifferentiated fever, these specific constellation of symptoms, and an occupational risk of exposure.

Clinical presentation of scrub typhus can be potentially severe, requiring oxygen support, dialysis and ICU care as demonstrated in this cohort. Similarly, a study conducted in rural India, acute respiratory syndrome, shock and acute kidney injury were the top three complications noted in their cohort [25]. Therefore, it is imperative to include multidisciplinary team management especially intensive care for severe scrub typhus cases with organ dysfunction to improve clinical outcomes [26]. Modest data showed mortality was high in complicated scrub typhus infection involving central nervous system (13.6%) and multiorgan dysfunction (24.1%) [2, 27].

There is lacking of standardized guideline on treatment of severe scrub typhus. In the INTREST trial, combination therapy of azithromycin and doxycycline for the treatment of severe scrub typhus showed better clinical outcome in terms of mortality, complications and fever defervescence in comparison with monotherapy alone [28]. Regimen used in INTREST trial included loading dose of intravenous doxycycline 200mg twice daily for day 1, maintained by 100mg twice daily from day 2 to day 7 of treatments. For intravenous azithromycin, loading dose of 500mg twice daily for day 1, followed by maintenance of azithromycin 500mg daily from day 2 to day 7 of treatment. For the severe cases managed in intensive care unit, we use intravenous azithromycin in combination with oral doxycycline due to unavailability of intravenous doxycycline.

Unusual presentations such as infective endocarditis and meningoencephalitis are possible complications from scrub typhus, therefore, in culture negative infective endocarditis, diagnostic workup should be considered for scrub typhus as a possible etiology [29]. However, guidance on antimicrobial approach remains limited. Our single case of infective endocarditis has favorable clinical outcome with 4 weeks of oral doxycycline. A separate case report demonstrated a good clinical response with intravenous chloramphenicol for 10 days duration [30], while another utilized a prolonged 6 weeks course of doxycycline [31]. For the case of presumed meningoencephalitis, the subject responded favorably with one week of combination therapy. Similarly, a published case report of meningitis from China responded well with one week of doxycycline [32].

The phylogenetic tree of *tsa*56 gene of OT in Negeri Sembilan illustrated distinct separation of the three genotype clusters suggests genetic diversity among the circulating strains in Negeri Sembilan. Additionally, the outgroup strain (Shimokoshi from Japan) was clearly separated from the main clusters, supporting the robustness of the tree topology and evolutionary divergence of the analyzed isolates. The

clustering pattern showed high genetic similarity between the local isolates and reference strains from neighboring countries such as Thailand, Cambodia, and Vietnam, suggesting regional genetic relatedness and possible shared evolutionary origins or regional transmission patterns.

Strengths and limitations:

This is a first cohort of PCR confirmed scrub typhus cases analyzed in Malaysia with sociodemographic details, clinical insights and molecular analysis.

Small sample size(n = 28) and limited data analysis for association therefore limits generalizability.

Recommendations and future research:

The authors recommend larger, prospective study to better characterized the clinical manifestations, risk factors for severe diseases and outcome stratified by timeliness of empiric therapy. Molecular tests such as PCR should be widely utilize to characterize virulence and geographical association in Malaysia. Interventional studies on personal protective equipment for at risk occupational exposure, rodent or vector control, and clinician and public education on awareness of scrub typhus will potentially address the gaps in Malaysia.

CONCLUSIONS

Scrub typhus is potentially a severe and fatal disease, often mis-diagnosed affecting predominately male outdoor workers in Negeri Sembilan. Clinical characteristics include high rate of gastrointestinal manifestation, with significant thrombocytopenia, hepatic and renal impairment. A high index of clinical suspicion, supported by a meticulous history and physical examination, is warranted, and empirical treatment should be considered in selected cases. Furthermore, public education to raise awareness and promotion of personal protective clothing usage should be implemented for at-risk outdoor workers.

Abbreviations

PCR: Polymerase Chain Reaction

qPCR: Quantitative Polymerase Chain Reaction

nPCR: Nested Polymerase Chain Reaction

OT: Orientia tsutsugamushi

ALT : alanine aminotransferase

AST : Aspartate aminotransferase

GFR: Glomerular Filtration Rate

KDIGO: Kidney Disease: Improving Global Outcomes

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

IQR: Interquartile Range

ICU: Intensive Care Unit

Declarations

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Registration and protocol

This study is registered with National Medical Research Register (NMRR ID 25-00987-EXV (IIR)), Malaysia. Protocol is available upon request to the authors.

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Ethics approval and consent to participate

This study was conducted in accordance to principle of the declaration of Helsinki and the Malaysian Good Clinical Practice Guideline. Ethical approval was granted by Medical Research and Ethics Committee (MREC) Malaysia however consent from subjects were not required due to descriptive and observational nature of the study.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

Data, Code, and material availability

All data collected were included in this paper. Protocol for this descriptive study is available upon request to the authors. The sequences of genetic analysis have been deposited in GenBank (PZ379605-PZ379616).

Use of generative AI: Gemini Pro was used during manuscript preparation for language refinement and editorial assistance. All outputs were reviewed, revised, and verified by the authors, who take full responsibility for the final content.

Authors Contribution

All authors have met the ICMJE authorship requirements. Kok Soon Lee, Aisyah Md Nor, Mohd Sofi Hafizi Sarip@ Ma'arof, Rheshara Sundram, Yee Ann Tan, Nor Zanariah Zainol Abidin, Shirley Yi Fen Hii, Siti Roszilawati Ramli, Tiang Koi Ng

contributed equally to this work. KSL, AMN, MSHS, RS, YAT, NZZA, SYFH, SRR and TKN were responsible for the study's design and data collection, including proposing the manuscript framework, developing the search strategy, conducting the literature review, and designing the data extraction form. KSL drafted initial manuscript and editing. All authors contributed to the writing, and critical review of the manuscript. SYFH and SRR contributed on molecular data and analysis. SRR and TKN provided supervision and critical feedback throughout the process. All authors have reviewed and approved the final draft and agreed to be held responsible for the work.

References

1. Jiang J, Richards AL. Scrub Typhus: No Longer Restricted to the Tsutsugamushi Triangle. Trop Med Infect Dis. 2018;3:11. https://doi.org/10.3390/tropicalmed3010011.
2. Bonell A, Lubell Y, Newton PN, Crump JA, Paris DH. Estimating the burden of scrub typhus: A systematic review. PLoS Negl Trop Dis. 2017;11(9):e0005838. https://doi.org/10.1371/journal.pntd.0005838.
3. Luce-Fedrow A, Lehman ML, Kelly DJ, Mullins K, Maina AN, Stewart RL, et al. A Review of Scrub Typhus (Orientia tsutsugamushi and Related Organisms): Then, Now, and Tomorrow. Trop Med Infect Dis. 2018;3(1):8. 10.3390/tropicalmed3010008. . PMID: 30274407; PMCID: PMC6136631.
4. Watt G, Parola P. Scrub typhus and tropical rickettsioses. Curr Opin Infect Dis. 2003;16(5):429–36.
5. Alexander L, Chen RJ, Buckley CJ. Chigger Bites and Trombiculiasis. [Updated 2024 Feb 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538528/
6. Saraswati K, Day NP, Mukaka M, Blacksell SD. Scrub typhus point-of-care testing: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12:e0006330. 10.1371/journal.pntd.0006330.
7. Brown GW, Robinson DM, Huxsoll DL, Ng TS, Lim KJ. Scrub typhus: a common cause of illness in indigenous populations. Trans R Soc Trop Med Hyg. 1976;70(5–6):444-8. 10.1016/0035-9203(76)90127-9. PMID: 402722.
8. Yuhana MY, Hanboonkunupakarn B, Tanganuchitcharnchai A, Sujariyakul P, Sonthayanon P, Chotivanich K et al. Rickettsial Infections Are Neglected Causes of Acute Febrile Illness in Teluk Intan, Peninsular Malaysia. Trop Med Infect Dis. 2022;7(5):77. doi: 10.3390/tropicalmed7050077.

Erratum in:. Trop Med Infect Dis. 2022;7(7):134. 10.3390/tropicalmed7070134. PMID: 35622704; PMCID: PMC9143963.

9. Devasagayam E, Dayanand D, Kundu D, Kamath MS, Kirubakaran R, Varghese GM. The burden of scrub typhus in India: A systematic review. PLoS Negl Trop Dis. 2021;15(7):e0009619.
10. Jiang J, Chan TC, Temenak JJ, Dasch GA, Ching WM, Richards AL. Development of a quantitative real-time polymerase chain reaction assay specific for Orientia tsutsugamushi. Am J Trop Med Hyg. 2004;70:351–6.
11. Ramli SR, Arifin N, Ismail MF, Hii SYF, Sulaiman NS, Lah EFC. Nik Abdul Aziz, N.A.H. Severe Scrub Typhus with Acute Kidney Injury: Urine PCR Evidence from an East Coast Malaysian Cluster. Trop Med Infect Dis. 2025;10:208.
12. Hii SYF, Zaidi M, Kassim MN, Hashim WN, R., Ramli SR. Genotype Analysis on Orientia tsutsugamushi Causing Scrub Typhus in Malaysia: A Re-Emerging Disease. Trop Med Infect Dis. 2025;9:252.
13. Furuya Y, Yoshida Y, Katayama T, Yamamoto S, Kawamura A Jr. Serotype-specific amplification of Rickettsia tsutsugamushi DNA by nested polymerase chain reaction. J Clin Microbiol. 1993;31:1637–40. [Google Scholar] [CrossRef].
14. Blacksell SD, Luksameetanasan R, Kalambaheti T, Aukkanit N, Paris DH, McGready R, Nosten F, Peacock SJ, Day NP. Genetic typing of the 56-kDa type-specific antigen gene of contemporary Orientia tsutsugamushi isolates causing human scrub typhus at two sites in north-eastern and western Thailand. FEMS Immunol Med Microbiol. 2008;52(3):335–42. 10.1111/j.1574-695X.2007.00375.x. . Epub 2008 Feb 27. PMID: 18312580.
15. Kim G, Ha NY, Min CK et al. Diversification of Orientia tsutsugamushi genotypes by intragenic recombination and their potential expansion in endemic areas. PLoS Negl Trop Dis. 2017;11(3):e0005408. Published 2017 Mar 1. 10.1371/journal.pntd.0005408
16. Tamura K, Stecher G, Kumar S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evolut. 2021;38:3022–7.
17. Zangpo T, Phuentshok Y, Dorji K, Dorjee C, Dorjee S, Jolly P, et al. Environmental, Occupational, and Demographic Risk Factors for Clinical Scrub Typhus, Bhutan. Emerg Infect Dis. 2023;29(5):909–18. 10.3201/eid2905.221430. . PMID: 37081000; PMCID: PMC10124658.
18. Samal C, Das PP, Mishra DK, Sahu DK, Kar PK. Risk Factors Associated with Adult Scrub Typhus Cases in a Tertiary Care Hospital - A Case Control Study. Indian J Community Med. 2026 Jan-Feb;51(1):83–9. 10.4103/ijcm.ijcm_897_24. . Epub 2025 Jul 29. PMID: 41836712; PMCID: PMC12981415.
19. Dasgupta S, Petchiammal P, Rajathi R, Gopinath KR, Asish PR, Sindhuja V, et al. Prevalence and risk factors of scrub typhus infection: Findings from a cross-sectional study in a rural setting in southern Tamil Nadu, India. Clin Epidemiol Glob Health [1]. 2025;36:102199. 10.1016/j.cegh.2025.102199.
20. Singh OB, Panda PK, Scrub Typhus. [Updated 2024 Mar 17]. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026. Jan-.

21. Yoo JS, Kim D, Choi HY, Yoo S, Hwang JH, Hwang JH, et al. Prevalence Rate and Distribution of Eschar in Patients with Scrub Typhus. Am J Trop Med Hyg. 2022;106(5):1358–62. 10.4269/ajtmh.21-1129. . Epub 2022 Mar 28. PMCID: PMC9128708.
22. Walker DH. Scrub Typhus - Scientific Neglect, Ever-Widening Impact. N. Engl. J. Med. 2016;375(10):913-5. 10.1056/NEJMp1608499. PMID: 27602663.
23. Ramli SR, Hii SYF, Zainal Abidin S, Lee KS, Ng TK, Misnan NA, Abdad MY. Neglected and lethal: Case series of fatal scrub typhus in Malaysia. PLoS Negl Trop Dis. 2025;19(12):e0013156. 10.1371/journal.pntd.0013156. . PMID: 41433254; PMCID: PMC12725589.
24. Trung N, Hoi L, Dien V, Huong D, Hoa T, Lien V, et al. Clinical Manifestations and Molecular Diagnosis of Scrub Typhus and Murine Typhus, Vietnam, 2015–2017. Emerg Infect Dis. 2019;25(4):633–41. https://doi.org/10.3201/eid2504.180691.
25. Devamani C, Alexander N, Chandramohan D, Stenos J, Cameron M, Abhilash KPP, et al. Incidence of scrub typhus in rural south India. N Engl J Med. 2025;392:1089–99.
26. Griffith M, Peter JV, Karthik G, Ramakrishna K, Prakash JA, Kalki RC, et al. Profile of organ dysfunction and predictors of mortality in severe scrub typhus infection requiring intensive care admission. Indian J Crit Care Med. 2014;18:497–502.
27. Peter JV, Sudarsan TI, Prakash JAJ, Varghese GM. Severe scrub typhus infection: Clinical features, diagnostic challenges and management. World J Crit Care Med. 2015;4(3):244–50. 10.5492/wjccm.v4.i3.244]. [PMID: 26261776.
28. Varghese GM, Dayanand D, Gunasekaran K, Kundu D, Wyawahare M, Sharma N, et al. INTREST Trial Investigators. Intravenous Doxycycline, Azithromycin, or Both for Severe Scrub Typhus. N Engl J Med. 2023;388(9):792–803. 10.1056/NEJMoa2208449. . PMID: 36856615; PMCID: PMC7614458.
29. Sivasubramanian BP, Abdul Khader AHS, Ravikumar DB, Dominic Savio FV, Thirupathy U, Thiruvadi V, et al. Comprehensive review on cardiac manifestation of scrub typhus. Front Trop Dis. 2024;5:1375087. 10.3389/fitd.2024.1375087.
30. Yu S, Yu X, Zhou B, Liu D, Wang M, Zhang H, et al. Tsutsugamushi disease presenting with aortic valve endocarditis: a case report and literature review. Am J Cardiovasc Dis. 2016;6(4):185–90. PMID: 28078179; PMCID: PMC5218851.
31. Devi S, Das D, Acharya D, Das T, Singh S, Gupta JD, et al. Scrub typhus presenting as mitral valve infective endocarditis. Natl Med J India. 2024;37:203–4. 10.25259/NMJI_295_21].
32. Zhang BC, Yang ZB, Liao RL, Ma ZQ, Zhang QJ, He QK et al. A case of scrub typhus with meningitis as the onset: Case report and literature review. Med 103(25): p e38613, June 21, 2024. | 10.1097/MD.0000000000038613

Figures

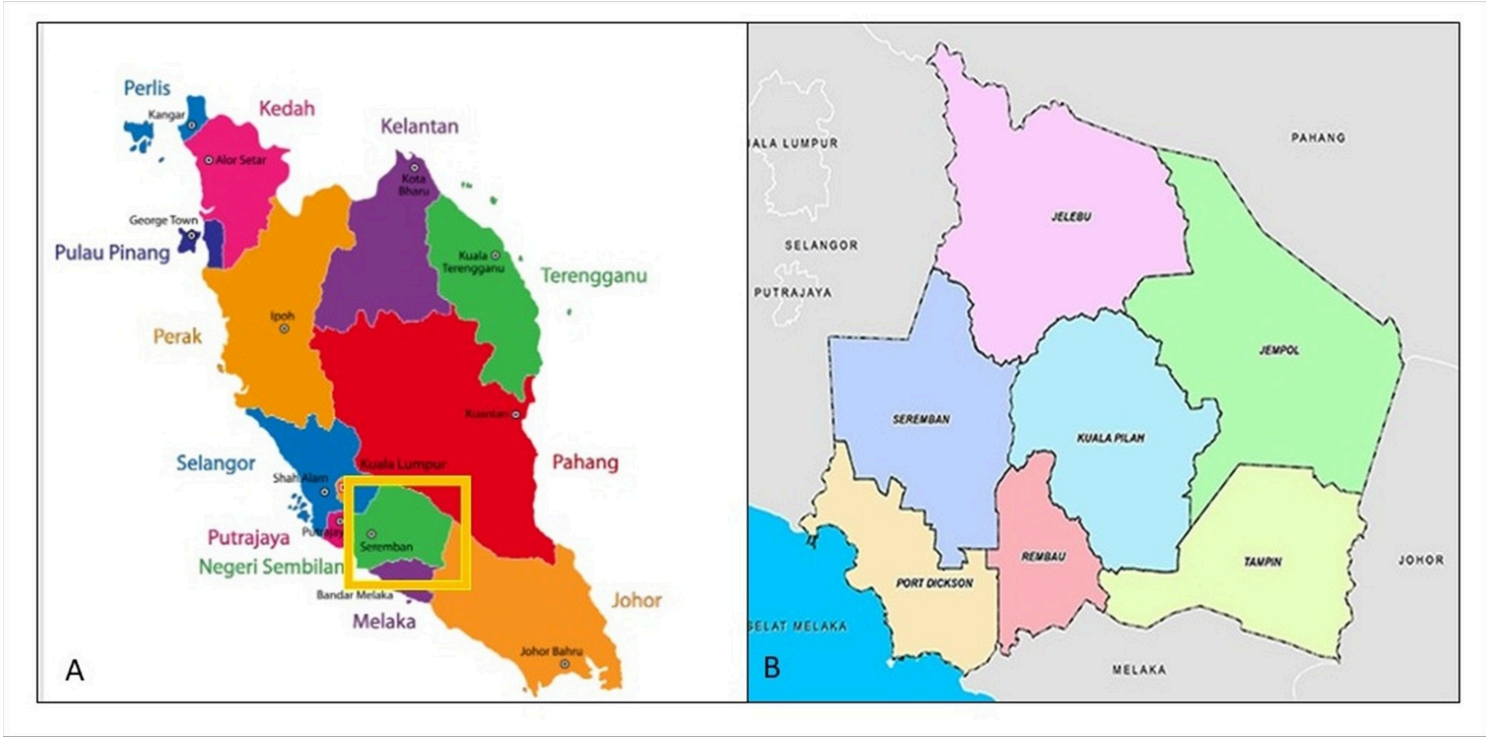


Figure 1

(A) Map of West Malaysia. The box indicate state of Negeri Sembilan (B) Map of 7 districts in Negeri Sembilan state.



Figure 2

Two eschars noted over left inguinal region of a subject



Figure 3

Single eschar found over the left neck region



Figure 4

Single eschar over the right posterior neck region

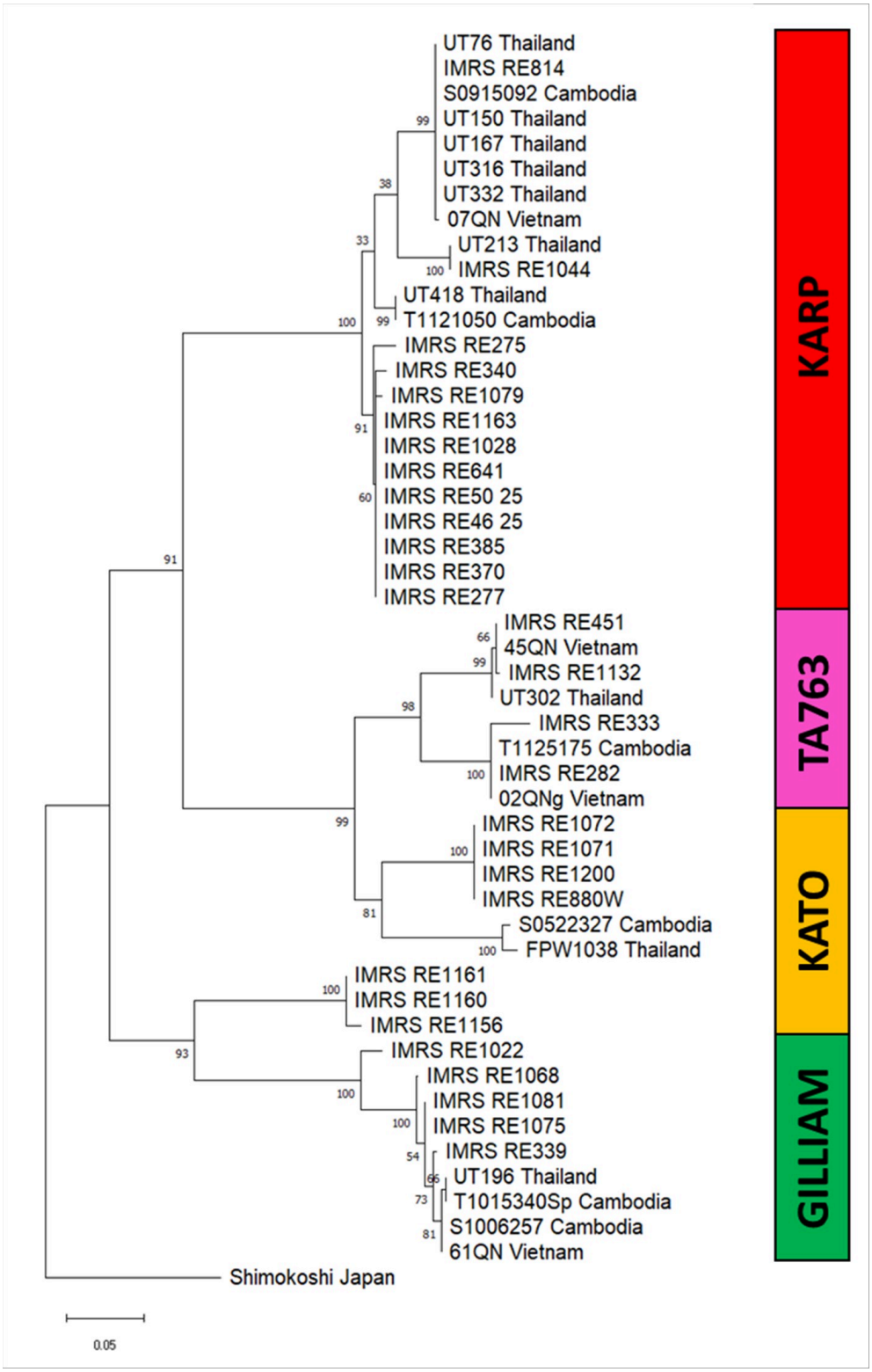


Figure 5

Phylogenetic tree based on *tsa*56 gene of strains in Negeri Sembilan and Southeast Asia showing the genetic relationships of isolates from Negeri Sembilan, grouped into three major genotypes: Karp, Gilliam and TA763. The tree demonstrates the clustering of local isolates with reference strains from Southeast Asia, with bootstrap values indicating the confidence of each branch.

Supplementary Files

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- TableS1NS.xlsx