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Scrub Typhus in Northeastern Thailand: Eschar Distribution, Abnormal Electrocardiographic Findings, and Predictors of Fatal Outcome

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Abstract. Scrub typhus is endemic in Thailand. Of the 495 patients with acute undifferentiated fever studied in Maharat Nakhon Ratchasima Hospital, Nakhon Ratchasima, Thailand, from June 1, 2011, to December 31, 2012, 146 patients (29.5%) had confirmed scrub typhus. The majority of cases were male, farmers, with the mean (±standard deviation) age of 54.1 ± 15.2 years. A total of 59 patients (40.4%) had eschar lesion. The commonest sites for an eschar in male patients were the perineum, inguinal, and buttock area; whereas in females, it was the head and neck area. Abnormal electrocardiogram was found in 39 of 79 patients (49.4%) with sinus tachycardia being the most frequent finding (17, 21.5%). A total of 73 patients (50%) had at least one complication. Myocarditis was the cause of complete heart block in a scrub typhus patient, and he fully recovered after receiving intravenous chloramphenicol treatment. The case fatality rate was 6.2% (nine deaths).The independent predictors for fatal outcome were age over 65 years (odds ratio [OR] = 14.49, 95% confidence interval [CI] = 1.26–166.44, $P = 0.03$), acute kidney injury (OR = 12.75, 95% CI = 1.77–92.07, $P = 0.01$), and hyperbilirubinemia (OR = 24.82, 95% CI = 2.12–286.61, $P = 0.01$). Early diagnosis and prompt appropriate treatment can improve the patient's outcome.

INTRODUCTION

Scrub typhus is a mite-borne rickettsial infection caused by the gram-negative intracellular bacteria, *Orientia tsutsugamushi*. The disease is prevalent in Asia and northern Australia.[1,2] The disease spectrum can range from self-limiting acute febrile illness to fatal disease. Common signs and symptoms are fever, headache, muscle pain, lymphadenopathy, eschar, and rash.[1] Presence of an eschar is a useful sign for diagnosing scrub typhus infection. Complications usually develop after the second week of illness, particularly in untreated cases, with systemic involvement[3] and disseminated vasculitis,[4] including septic shock,[5] acute respiratory distress syndrome (ARDS),[6,7] pneumonitis,[7] acute renal failure (ARF),[8,9] meningitis or meningoencephalitis,[10] myocarditis,[11] disseminated intravascular coagulation (DIC),[12] upper gastrointestinal bleeding,[13] and multiorgan dysfunctions (MODS).[1,4] Myocarditis is a rare complication, few reports showed abnormal electrocardiographic (ECG) findings in scrub typhus patients. Serology is the mainstay for diagnosing scrub typhus.[2] Patient outcome varies depending on pathogen factors (strain virulence[14] and high bacterial load[15]), severity of the disease,[16] time to diagnosis,[17] commencement of appropriate treatment,[3] and the presence of drug resistance.[18] This study aims to demonstrate the clinical and laboratory manifestation, particularly eschar distribution and abnormal ECG findings, and the predictors of fatal outcome in scrub typhus patients.

MATERIALS AND METHODS

Patients and study site. This study is a subanalysis of a prospective etiological study of patients with acute undifferentiated fever (AUF) admitted to the Maharat Nakhon Ratchasima Hospital (MNRH), a 1,300-bed tertiary-care hospital in northeastern Thailand, between June 1, 2011, and December 31, 2012. Nakhon Ratchasima is the largest province in Thailand, with an area about 20 million square kilometers and an estimated population of 2,600,000. Most of the population are agricultural workers and live in rural areas. Patients in this cohort were from rural areas throughout Nakhon Ratchasima Province.

Adult (≥ 18 years of age) patients with AUF (oral temperature ≥ 38.0°C, ≤ 14 days) who were diagnosed with scrub typhus were recruited in the analysis. Scrub typhus was confirmed based on one of these criteria: 1) a 4-fold increase in IgM or IgG antibody against pooled antigens of *O. tsutsugamushi* strain Karp, Kato, and Gilliam in paired sera (7–14 days apart) using the indirect immunofluorescence assay (IFA),[2] 2) a single IFA with IgM or IgG antibody titer ≥ 1:400,[19–21] or 3) positive real-time polymerase chain reaction (PCR) targeting 47-kDa genes and/or nested PCR targeting 56-kDa genes in *O. tsutsugamushi* using DNA extracted from patient's buffy coat.[22,23] The screening dilution for IFA was 1:50, and positive samples on screening were further serially diluted (2-fold) from 1:50 to 1:6,400. Scrub typhus patients who had other coinfections were excluded from the analysis. The study was approved by the Ethical Review Subcommittee of MNRH, the Public Health Ministry of Thailand, and the Faculty of Medicine at Siriraj Hospital, Mahidol University. Written informed consent was obtained from all participants.

Statistical analysis. Data were collected and analyzed using SPSS (Version 21.0, IBM Corp., Armonk, NY). χ^2 test was used to compare the proportion of eschar lesion between males and females. Continuous variables were transformed into categorical variables using cutoff values based on clinical significance. Fisher's exact test and stepwise exact logistic regression were used to determine factors associated with fatal scrub typhus. All variables with P value < 0.05 in the univariate analysis were included in the multivariate analysis. All tests were two sided; a P value < 0.05 was considered statistically significant.

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RESULTS

Of the 495 AUF patients who were enrolled, 146 patients (29.5%) had confirmed scrub typhus. The criteria diagnosis of scrub typhus is shown in Table 1. PCR was performed in 128 scrub typhus patients, whereas the other 18 patients had insufficient samples. The majority of scrub typhus patients were male (63.7%), farmers (71.2%), with the mean (±standard deviation) age of 54.1 ± 15.2 (range 18–97) years. The median duration of fever and duration of admission were 7 (range 3–14) and 3 (range 1–21) days, respectively. Most cases (57.7%) occurred in the rainy season (late May to mid-October). Signs and symptoms were fever (100%), headache (60.9%), and myalgia (65.8%). In all, 59 patients (40.4%) had eschar lesion and no patients presented with more than one eschar (Table 2). None of them underwent eschar biopsy for PCR.

Abnormal laboratory findings, including acute kidney injury (AKI), chest radiograph, and ECG, are shown in Table 3. ECG of a 53-year-old male who presented with a 7-day history of fever and dyspnea demonstrated complete heart block. His cardiac enzyme was increased (creatine kinase-MB = 199.2 IU/L, troponin I = 28.2 ng/mL), whereas cardiac catheterization demonstrated normal coronary arteries. Myocarditis was identified as the cause of complete heart block in the patient with scrub typhus and his ECG returned to normal after 7 days of intravenous chloramphenicol. A total of 73 patients (50%) had at least one complication (Table 4). Four of 15 patients with ARF required renal replacement therapy and all of them recovered. Nine patients died, resulting in case fatality rate of 6.2% (Table 5). Autopsy was not performed in any of the fatal cases due to refusal by their relatives. Prognostic factors associated with fatal outcome are shown in Tables 6 and 7.

DISCUSSION

Scrub typhus is a common cause of AUF in Thailand with varying incidence (4.6–23.4%) across geographic regions.[24,25] The incidence of scrub typhus in MNRH (29.5%) during the study period was higher when compared with a previous study at the same institute in 2001–2002 (23.4%). This was in keeping with the report from the Ministry of Public Health of Thailand that the incidence of scrub typhus has been increasing over the past 10 years.[26] Serology with a 4-fold increase in antibody titer in paired sera using IFA is the gold standard for diagnosis of scrub typhus.[2,27] The IFA against combined Karp, Kato, and Gilliam strains of *O. tsutsugamushi* was performed in our study, and it covers the majority of the strains in Thailand.[28] There is no consensus on the positive cutoff titer for a single acute serum sample.[27] In addition, false-positive antibody test for scrub typhus at low titer has been reported in two patients with leptospirosis.[29] In the present study, we used a positive single cutoff IgM/IgG titer ≥ 1:400, same as the surveillance criteria for scrub typhus, Thai Ministry of Public Health, 2001.[20] The majority of antibody titers against *O. tsutsugamushi* in seroprevalence studies in healthy Thai rural villagers,[30,31] Thai soldiers,[32] and febrile illness patients[33] were less than 1:400. The sensitivity and specificity of cutoff IgM titer of ≥ 1:400 in Thai patients were 81.0% and 94.1%, respectively.[34] Lim and others suggested a single IgM cutoff titer of ≥ 1:3,200; however, the sensitivity was only 67.5% despite 100% specificity.[34] Moreover, scrub typhus patients coinfected with other diseases were excluded from the analysis because of the difficulty in interpretation of the serology result, severity of disease, and patient's outcome. PCR method improves the

TABLE 1
Criteria for diagnosis 146 scrub typhus patients*

Criteria diagnosis for scrub typhus	Patients (%)
A 4-fold increase in antibody titer using IFA and positive PCR for *Orientia tsutsugamushi*	60 (41.1)
A 4-fold increase in antibody titer using IFA	38 (26.0)
A single antibody titer ≥ 1:400 using IFA and positive PCR for *O. tsutsugamushi*	21 (14.4)
A single antibody titer ≥ 1:400 using IFA	
1:400	3 (2.1)
1:800	2 (1.4)
1:1,600	2 (1.4)
1:3,200	5 (3.4)
≥ 1:6,400	5 (3.4)
Positive PCR for *O. tsutsugamushi* despite nondiagnostic IFA	10 (6.8)

IFA = indirect immunofluorescence assay; PCR = polymerase chain reaction.
*PCR was performed in 128 scrub typhus patients, whereas the other 18 patients had insufficient samples.

TABLE 2
Distribution of eschar lesions in 59 scrub typhus patients*

Eschar locations	Male (%)	Female (%)	*P* value
Total	36 (61)	23 (39)	0.60
Head and neck	0	4 (17.4)	0.02
Chest and abdomen (including axillar)	9 (25.0)	5 (21.7)	0.06
Perineum, inguinal, and buttock	16 (44.4)	4 (17.4)	0.048
Back	4 (11.1)	5 (21.7)	0.29
Upper extremities	1 (2.8)	3 (13.1)	0.29
Lower extremities	6 (16.7)	2 (8.7)	0.46

*No patients had more than one eschar lesion.

TABLE 3
Laboratory findings of scrub typhus cases

Laboratory findings	Patients (%)
AKI* (increase SCr > 1.5 times of normal level; *N* = 146)	28 (19.2)
Renal at risk (increase SCr × 1.5 times of normal level)	6 (4.1)
Renal injury (increase SCr × 2 times of normal level)	7 (4.8)
Acute renal failure (increase SCr × 3 times of normal level or if baseline SCr > 4 mg/dL and increase SCr > 0.5 mg/dL)	15 (10.3)
Abnormal chest radiograph findings (*N* = 142 cases)	62 (43.6)
Bilateral interstitial infiltration	36 (25.4)
Bilateral patchy consolidation	6 (4.2)
Pulmonary edema	6 (4.2)
Localized infiltration	4 (2.8)
Unilateral pleural effusion	2 (1.4)
Cardiomegaly	8 (5.6)
Abnormal ECG findings (*N* = 79 cases)	39 (49.4)
Sinus tachycardia	17 (21.5)
Atrial fibrillation (new onset)	9 (11.3)
Supraventricular tachycardia	3 (3.8)
Nonspecific ST-T change	3 (3.8)
Right bundle branch block	3 (3.8)
Left anterior fascicular block	1 (1.3)
Premature ventricular complex	1 (1.3)
First degree AV block	1 (1.3)
Complete heart block	1 (1.3)

AV = atrioventricular; AKI = acute kidney injury; ECG = electrocardiographic; SCr = serum creatinine.
*For patients with unknown baseline creatinine level, the modification of Diet in Renal Disease equation was used to estimate renal function based on age, sex, and race.

TABLE 4
Complications of 73 scrub typhus cases*

Complications	Cases (%)
Septic shock	27 (18.5)
Pulmonary involvement	
ARDS	13 (8.9)
Pneumonitis	33 (22.6)
ARF	15 (10.3)
Meningitis or meningoencephalitis	5 (3.4)
Upper GI bleeding	4 (2.7)
DIC	3 (2.1)
Myocarditis	1 (0.7)
Pericardial effusion	1 (0.7)
MODS	27 (18.5)

ARDS = acute respiratory distress syndrome; ARF = acute renal failure; DIC = disseminated intravascular coagulation; GI = gastrointestinal; MODS = multiorgan dysfunction.
* Each patient can have more than one complication.

diagnostic yield during the rickettsemia period when antibodies are not detectable.[35] Likewise, 10 patients (6.8%) with nondiagnostic IFA were positive in PCR for scrub typhus. In this study, we performed PCR targeting 47- and 56-kDa genes in *O. tsutsugamushi* using DNA extracted from buffy coat because these two targets were highly specific for *O. tsutsugamushi*,[2] and using buffy coat provided higher sensitivity of PCR when compared with whole blood.[36]

To the best of our knowledge, this is the first report of eschar distribution and abnormal ECG findings in scrub typhus patients in northeastern Thailand. The incidence of eschar lesion in our study (40.4%) was higher than the previous study in adult Thai patients (20.9%),[21] but lower than studies in Thai children (68%)[37] and other Asian countries.[38,39] The commonest locations for eschar in males were the perineum, inguinal, and buttock areas, similar to the report from Korea[39]; whereas in females, the eschar predominantly occurred in the head and neck area, as opposed to the chest as described in the study by Kim and others.[39] Eschar lesion is a pathognomonic sign of scrub typhus infection. In previous studies, the absence of an eschar was a predictor for severe[13] and fatal scrub typhus.[16] In our study, absence of eschar was predictive of death in univariate analysis, but did not reach statistical significance in multivariate analysis.

Abnormal ECG findings in the present study was more prevalent and more severe than the previous study.[40] Watt and others reported that ECGs were mainly normal or only showed minor nonspecific changes, such as ST segment/T wave change, prominent U wave, and premature ventricular contraction.[40] Myocarditis is a rare complication in scrub typhus patients.[1] To the best of our knowledge, this is the first report of a complete heart block resulting from scrub typhus complicated with myocarditis. Pulmonary complication and bilateral interstitial opacity on chest radiograph were the most frequent findings in our study, consistent with the studies in Korea.[13,41] The incidence rate of ARDS in this study was 8.9%, which was lower than previous studies.[6,42] None of our patients had acute hearing loss, although it is a common presentation.[43] The incidence of MODS (18.5%) was higher than the previous study in Thailand (11.9%).[21]

The case fatality rate in our study (6.3%) was higher than the previous study in Thailand between 2000 and 2003 (2.3%).[21] Six of nine fatal cases (66.7%) developed severe disease within the first week of illness, in contrast with literature reporting that complications usually appear after the second week.[3] Sonthayanon and others demonstrated

TABLE 6
Univariate analysis of prognostic factors for fatal outcome

Factors	Death (N = 9)	Survive (N = 137)	P value
Age > 65 years	6 (66.7)	29 (21.2)	0.01
Sex: male	5 (55.6)	88 (64.2)	0.72
Cirrhosis	2 (22.2)	2 (1.5)	0.02
Diabetes	2 (22.2)	6 (4.4)	0.08
Abdominal pain	2 (22.2)	23 (16.8)	0.65
Alteration of consciousness	2 (22.2)	7 (5.1)	0.09
Hypotension	6 (66.7)	28 (20.4)	0.005
Tachypnea (RR > 20 breaths/min)	8 (88.9)	67 (48.9)	0.03
Tachycardia (HR > 100 beats/min)	1 (11.1)	15 (33.3)	0.28
Absence of eschar	9 (100)	78 (56.9)	0.01
Hepatomegaly	1 (11.1)	18 (13.1)	1.00
ARDS	2 (22.2)	12 (8.8)	0.15
Investigation			
Hemoglobin < 10 g/dL	4 (44.4)	21 (15.3)	0.047
White blood cell > 10,000 cell/mm^3	7 (77.8)	73 (53.3)	0.18
Lymphocyte < 1,500/mm^3	6 (66.7)	54 (39.4)	0.16
Platelet < 100,000/μL	6 (66.7)	37 (27)	0.02
AKI	7 (77.8)	21 (15.3)	< 0.001
Blood urea nitrogen > 20 mg/dL	6 (66.7)	38 (27.7)	0.02
Serum bicarbonate level < 20 mEq/L	7 (77.8)	30 (21.9)	0.001
Serum albumin < 3.0 g/dL	6 (66.7)	33 (24.1)	0.01
Total bilirubin > 3 mg/dL	5 (55.6)	12 (8.8)	0.001
ALT > 120 IU/L	3 (33.3)	43 (31.4)	1.00
AST > 120 IU/L	7 (77.8)	74 (54)	0.29
Bilateral lung infiltration in CXR	4 (44.4)	37 (27)	0.27
Not received proper ATB within 24 hours after admission	3 (33.3)	8 (5.8)	0.02

AKI = acute kidney injury; ALT = alanine aminotransferase; ARDS = acute respiratory distress syndrome; AST = aspartate aminotransferase; ATB = antibiotic; CXR = chest X-ray; HR = heart rate; RR = respiratory rate.

TABLE 5
Characteristic of nine fatal scrub typhus cases

	Age, /sex	DOF, /LOS	IFA titer: IgM/IgG†	PCR (copies)*	Complications	Antibiotic treatment within first 24 hours after admission
1	73, F	7, 6	6,400/800	Positive (< 100)	Septic shock, ARDS, ARF	Chloramphenicol, ceftriaxone
2	73, M	7, 2	100/800	Positive (< 100)	Septic shock, ARF	Chloramphenicol, ceftriaxone
3	87, M	3, 8	800/50	Negative	Septic shock, ARF	Ceftriaxone, levofloxacin
4	57, F	5, 1	3,200/50	Negative	Septic shock, ARF	Ceftriaxone
5	41, M	10, 4	1,600/3,200	Positive (< 100)	Septic shock	Ceftriaxone
6	87, F	3, 5	400/< 50	Negative	Septic shock, pericardial effusion	Oral doxycycline, ceftazidime, clarithromycin
7	83, F	4, 2	400/100	Negative	ARDS	Oral doxycycline, ceftazidime, clarithromycin
8	51, M	3, 3	< 50/< 50	Positive (24,415)	Pneumonitis, ARF	Chloramphenicol, ceftriaxone
9	67, M	3, 17	1,600/100	Negative	Pneumonitis	Oral doxycycline, ceftazidime, clarithromycin

ARDS = acute respiratory distress syndrome; ARF = acute renal failure; DOF = days of fever; F = female; IFA = indirect immunofluorescence assay; LOS = length of hospital stay; M = male.
*Polymerase chain reaction result was demonstrated in copies number per 500 ng of human genomic DNA.
†IFA titer expressed as the reciprocal of the dilution.

TABLE 7
Multivariate analysis of prognostic factors for fatal outcome

	P value	Odds ratio	95% CI
Age ≥ 65 years	0.03	14.49	1.26–166.44
Creatinine > 1.5 times of normal level	0.01	12.75	1.77–92.07
Total bilirubin > 3 mg/dL	0.01	24.82	2.12–286.61

CI = confidence interval.

positive correlation between disease severity and high *O. tsutsugamushi* DNA by performing PCR targeting on 16S ribosomal RNA gene of *O. tsutsugamushi* using DNA extracted from admission ethylenediaminetetraacetic acid blood samples,[15] whereas in the present study, four fatal cases had positive PCR targeting on 47- and/or 56-kDa genes in *O. tsutsugamushi* using DNA extracted from buffy coat. Three of them had *O. tsutsugamushi* DNA less than 100 copies, whereas the other one had 24,415 copies per 500 ng of human genomic DNA. Antibiotic treatment of severe scrub typhus is doxycycline[44] or chloramphenicol.[1,45] However, only the oral formulation of doxycycline is available in Thailand. The use of oral doxycycline may not allow the achievement of therapeutic levels due to hemodynamic instability[1] as seen in patient 6 (Table 5). Three fatal cases received inappropriate antibiotic treatment because of misdiagnosis. Treatment failure from doxycycline resistance was suspected in deceased cases who received doxycycline. In 1996, Watt and others reported doxycycline resistance in *O. tsutsugamushi* in northern Thailand[18]; however, in the present study, we did not perform scrub typhus culture and drug resistance testing. Therefore, further studies in patients' isolates in this region are needed to examine this question.

The significant predictors for fatal outcome in multivariate analysis were age over 65 years, AKI, and hyperbilirubinemia. Elderly people are vulnerable to infection due to impairment of both humoral and cell-mediated immunity and increased severity in some diseases, such as scrub typhus.[46] Jang and others reported that scrub typhus patients over 65 years of age had higher morbidity and mortality compared with younger patients.[47] Also, age over 65 years was an independent predictor of fatal outcome in their study; our result supported that finding. AKI is a consequence of multiple factors like hypotension, direct renal invasion by *O. tsutsugamushi*, vasculitis, or rhabdomyolysis.[8,9,47,48] Half of patients with AKI were related to hypotension. AKI was a statistically significant predictor of mortality in our study and the study from India.[42] Liver involvement; hypoalbuminemia; and elevated alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and bilirubin levels have been described in scrub typhus patients.[13,21,42,49] Elevated liver enzymes is the most frequent finding in a previous report[50] and also in our study. Hyperbilirubinemia was an independent predictor of fatal outcome in our study, in accordance with a study from Varghese and others.[42] The possible reason was patients' underlying liver diseases; all 17 patients with hyperbilirubinemia had history of liver conditions: liver cirrhosis (four cases), alcoholic liver disease (11 cases), and chronic hepatitis B (two cases).

There were several limitations in this study. First, lack of *O. tsutsugamushi* strains and scrub typhus isolates for drug resistance testing. Second, our hospital being a tertiary-care hospital, complications of scrub typhus cases may be overestimated. Third, this study was performed in a single hospital; therefore, it might not be a representative of the epidemiology of the whole of northeastern Thailand. In conclusion, scrub typhus is a major cause of AUF in Thailand, and early diagnosis and prompt accurate treatment can improve the outcome.

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