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Comprehensive review on cardiac manifestation of scrub typhus

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Scrub typhus, a zoonotic disease caused by *Orientia tsutsugamushi* and transmitted by chiggers, predominantly affects the Asia-Pacific region. Complications of Scrub Typhus involve multiple systems, including cardiovascular (pericarditis, arrhythmia, myocarditis), respiratory (acute respiratory distress syndrome), hepatic (hepatitis), and renal (azotemia). In this review, we comprehensively focused on the cardiac manifestations caused due to scrub typhus. Scrub typhus-induced pericarditis should be suspected in patients residing in endemic regions presenting with fever, thrombocytopenia, and pericardial effusion. If undetected, it frequently leads to cardiomegaly, pericardial effusion, and congestive heart failure. Heart failure with scrub typhus commonly occurs following myocardial inflammation, particularly in patients with pre-existing cardiac disorders. Scrub typhus myocarditis is a relatively rare, but serious cardiac complication with a high mortality rate of up to 24.0%. Arrhythmias arise due to the involvement of the interventricular septum, coronary artery, or cardiac valves causing variable ECG findings including sinus arrhythmia, T wave changes, and QTc interval prolongation. Atrial fibrillation due to scrub typhus is associated with a 1.3 fold increase in 3-month mortality. These cardiac complications are mainly assessed using electrocardiography (ECG) and echocardiography. Serology is the primary diagnostic tool for *O. tsutsugamushi*. While the Scrub Typhus Detect IFA test offers 100% sensitivity, the Weil Felix test is specific and cost-effective. Nested PCR and ELISA are effective for early detection but are limited to resource-rich settings. Diagnostic difficulties arise from nonspecific symptoms and current testing limitations. Vaccine development using extracellular vesicles, nanoparticles, and subunit vaccines shows promise. Combined therapy with

doxycycline and azithromycin is recommended for cardiac complications, alongside guideline-directed therapy. The review underscores the need for heightened clinical awareness and prompt management of scrub typhus, especially in endemic regions. It also highlights the necessity for further research into the pathogenesis of cardiac involvement and the development of more effective diagnostic tools and treatments.

KEYWORDS

scrub typhus (tsutsugamushi disease), *Orientia* species, cardiac complications, pericarditis, myocarditis, arrhythmia, heart failure, mite-borne disease

Introduction

Scrub typhus is a zoonotic illness caused by *Orientia tsutsugamushi*, transmitted by the bite of trombiculid mite larvae (chiggers). The disease is most frequently reported within the "tsutsugamushi triangle" of the Asia-Pacific region, which encompasses more than 8 million km², including Thailand, India, China, Korea, Philippines, Taiwan, Sri Lanka, and extending from the Russian Far East in the north, to Pakistan in the west, Australia in the south, and Japan in the east (1). The pathophysiology of O tsutsugamushi involves vasculitis due to the infection of endothelial cells, leading to perivascular infiltration of T cells and monocytes or macrophages. Subsequently, a wide range of inflammatory responses occurs, with endothelial and non-endothelial cells producing various cytokines. These cytokines can have both beneficial effects, such as antimicrobial activity, and detrimental effects, causing tissue destruction in the infected host. This immune response's dual nature can lead to severe complications such as hepatitis, renal failure, meningoencephalitis, and respiratory failure, including acute respiratory distress syndrome (ARDS) and myocarditis (2). The bacterial dissemination and replication during early infection need to be better understood (3). Vascular involvement is hypothesized to occur by downregulating the expression of glycoprotein-96 in the endothelial cells and phagocytes and by neutralizing the host's immune response (4). *Orientia* infection is also proposed to affect CCR2, which drives blood monocytes into the lung, accelerating bacterial replication and the development of inflammation of the pulmonary interstitium (5). C-type lectin receptors, CCR7/dendritic cell-mediated mechanisms are also involved in immune dysregulation in scrub typhus (3, 6, 7). TNF receptors played an intrinsic role in CD8+ T cell activation, revealing the protective immunity of TNF against *O. tsutsugamushi* infection (8). Scrub typhus can increase several interleukins (IL-1alpha/beta, IL-4, IL-6, IL-7, IL10, IL-11, IL-18, and IL-24), chemokines (CXCL8, CCL2/MCP1, CCL5/RANTES, and CCL17), growth factors (NODAL, CNTF, and CSF2/GM-CSF), and TNFSF13B (9, 10). This can disrupt the B/T cell microenvironment and dysregulation of B cell responses during active infection (11). The cytokine signatures can be used as depletion targets in future experiments (12).

Scrub typhus is known to produce several cardiotoxic, hepatotoxic, and nephrotoxic metabolites (13). The incidence of complicated scrub typhus increases with delayed diagnosis and treatment of scrub typhus (14). Untreated infections can cause multi-organ involvement and raise the cost of treatment (15). The most common complications are hepatitis (40.5%), thrombocytopenia (28.4%), acute respiratory distress syndrome (20.5%), acute kidney injury (19.2%), meningitis (16.4%), shock (16.2%), and myocarditis (15.5%) (16, 17). Scrub typhus can persist for longer durations in the affected organs (18). Cardiac involvement due to scrub typhus is attributed to macrophage activation, immune-mediated or direct invasion of cardiomyocytes, and electrolyte alterations (4, 19–22). Table 1 depicts the Cardiac manifestation of scrub typhus.

Several studies are being published in the native languages of endemic areas and studies that rely on national surveillance systems often face missing data due to unreported cases. Most studies are conducted on a small sample size with a single-center design. Due to a paucity of literature on cardiovascular compilations, there is a delay in timely diagnosis (23, 24). Scrub typhus is a rapidly emerging public health threat but no national protocols for prevention exist in Southeast Asia (25).

This article aims to provide a comprehensive review of the literature on the cardiac manifestation of scrub typhus to aid clinicians in timely diagnosis by ordering the ideal tests and optimal treatment modalities.

Epidemiology

Scrub typhus is endemic to a vast area known as the "tsutsugamushi triangle," encompassing over 8 million km² of the Asia-Pacific region. This includes countries like Thailand, India, China, Korea, the Philippines, Taiwan, and Sri Lanka, stretching from the Russian Far East in the north to Pakistan in the west,

TABLE 1 Cardiac manifestation of scrub typhus.

Cardiac Complication	Pathogenesis	Presentation	Diagnostic Methods	Risk Factors	Treatment
Myocarditis	Inflammation of the cardiac muscle.	Chest pain, palpitations, heart failure; ECG changes, elevated inflammatory and cardiac biomarkers. Incidence varies from 4.3% to 14%; high mortality rate (up to 24%).	ECG, echocardiography, endomyocardial biopsy, cardiac MRI, and cardiac biomarkers (Troponin T).	Paroxysmal atrial fibrillation, elevated bilirubin, short duration of symptoms before presentation, liver disease, elevated C-reactive protein, and creatinine levels.	Doxycycline, IV chloramphenicol, and low-dose glucocorticoids in severe cases. VA ECMO for fulminant cases.
Pericarditis	Leads to cardiomegaly, pericardial effusion, and congestive heart failure.	Often presents with fever, thrombocytopenia, and pericardial effusion.	Immunofluorescence assay (IFA), IgM ELISA, PCR-based detection, inflammatory markers, ST-elevation in ECG, and CT angiography.	Endemic region, fever, and thrombocytopenia.	Doxycycline, and azithromycin.
Endocarditis	Blood culture-Negative infective endocarditis (BCNIE) is extremely rare.	N/A	Clinical suspicion, endemicity workup, and PCR-based detection.	N/A	Chloramphenicol.
Heart Failure	Follows myocardial inflammation	Moderate reduction in ejection fraction in younger patients; high risk in predisposed cardiac disorders	Chest X-ray, BNP (or NT-proBNP), echocardiogram, and endomyocardial biopsy.	CK-MB>25U/L, predisposed cardiac disorders.	Hemodynamic monitoring, fluid resuscitation, vasopressor support, GDMT-guided therapy, antibiotics (doxycycline), low-dose glucocorticoids, and ECMO.
Arrhythmia (Atrial Fibrillation)	Involvement of interventricular septum, coronary artery, and cardiac valves.	Poor prognostic factor with a 1.3-fold increase in 3-month mortality.	Serial ECG monitoring, baseline cardiac enzymes (CK MB), and echocardiogram.	Older age, male sex, fever, leukocytosis, abnormal renal/liver functions, cardio-vascular disease, hyper-tension, ischemic heart disease, and heart failure.	Hemodynamic stability (ICU admission), rate control (calcium channel blockers, beta-blockers), rhythm control (cardioversion, antiarrhythmic agents), antibiotics, and metabolic panel monitoring.
Myocardial Ischemia	Endothelial dysfunction, systemic vasculitis, proinflammatory mediators; can cause ischemic events.	N/A	Coronary angiography, elevated cardiac enzymes, and EKG changes.	Diabetes, hypertension, previous coronary artery disease, male sex, and age 35-49 years.	Appropriate antibiotics, and further management based on coronary angiography findings.

ECG, Electrocardiogram; VA ECMO, Veno-Arterial Extracorporeal Membrane Oxygenation; IGM ELISA, Immunoglobulin M Enzyme-Linked Immunosorbent Assay; PCR, Polymerase Chain Reaction; CT, Computed Tomography; N/A, Not Applicable; BNP, B-type Natriuretic Peptide; NT PRO BNP, N-terminal Pro B-type Natriuretic Peptide; CK-MB, Creatine Kinase-MB; GDMT, Guideline-Directed Medical Therapy; ICU, Intensive Care Unit; EKG, Electrocardiogram (alternate abbreviation); MRI, Magnetic Resonance Imaging.

Australia in the south, and Japan in the east. Within this region, specific locations like the northeastern states of India, Fujian province in China, the northern region of Thailand, and the southernmost part of Taiwan exhibit higher disease prevalence (1, 16). In South Korea, scrub typhus is most common in the southern and western regions, with a peak incidence in the later months of the year (26). The disease is also concentrated in the western province of Sri Lanka and limited to northern Queensland, Australia (27, 28). Epidemiology across various countries with respective prefectures and seasonal pattern have been described in the below.

The disease poses a significant public health threat, with an estimated one million cases annually. While primarily concentrated within the tsutsugamushi triangle, sporadic cases have been identified outside this region, underscoring the importance of understanding its global distribution (1) (Table 2).

Pericarditis

Pericarditis is an additional noteworthy cardiac complication linked with scrub typhus. It frequently leads to cardiomegaly and, if untreated, can progress to pericardial effusion and congestive heart failure (26). Recent studies by Karthik et al. and Chin et al. have observed pericardial effusion in 51% and 20% of their respective patient populations. However, there is no mention of the

development of cardiac tamponade in the studies (27, 28). In patients residing in endemic regions presenting with fever, thrombocytopenia, and pericardial effusion, clinical suspicion of scrub-typhus pericarditis should be taken into consideration (29). In certain cases, perimyocarditis can also occur and poses a diagnostic challenge due to its nonspecific presentation (30).

The diagnosis entails confirming the presence of pericardial effusion and serological tests such as the immunofluorescence assay (IFA) or the IgM enzyme-linked immunosorbent assay (ELISA) (26). The recently developed tool of PCR-based detection of tissue or tissue fluid can also aid in the diagnosis of scrub typhus (26, 29). Other supportive findings include an increase in inflammatory markers (erythrocyte sedimentation rate/C-reactive protein), ST-elevation in the anterior leads of the ECG, and a hypervascular epicardial region observed in CT angiography (30). Treatment options consist of doxycycline and azithromycin as an alternative if any side effects develop with doxycycline (29). Timely suspicion and appropriate management are crucial for achieving clinical improvement and reducing mortality (26).

Heart failure

Cardiac failure in patients with scrub typhus commonly occurs following myocardial inflammation. Younger patients often had a moderate reduction in ejection fraction and patients with predisposed cardiac disorders had a higher risk of developing heart failure. The presentation of heart failure characterized by dyspnea and features of fluid overload, may or may not be present (31–36). Even rare presentations such as Takotsubo cardiomyopathy and acute cor pulmonale with predominant lung involvement are seen with scrub typhus (31, 37). Due to atypical presentation and absence of physical signs, a high index of suspicion is necessary to diagnose heart failure before the development of cardiogenic shock (14, 38, 39). This warrants the identification of scrub typhus, chest X-ray, BNP (or NT-proBNP), and echocardiogram workup in suspected patients (28, 36, 40). 18.5% of patients with scrub typhus had detectable features of congestive heart failure on Chest X-ray (40). An echocardiogram is recommended to assess cardiac chamber size, wall thickness, and ventricular function in suspected myocarditis (41). Patients with CK-MB >25 U/L had a significantly longer length of hospital stay (36). If the diagnosis is uncertain, an endomyocardial biopsy can be obtained, but we exercise caution in patients with heart failure to further prevent the development of cardiogenic shock (42).

Management of acute heart failure from scrub typhus includes hemodynamic monitoring, stabilization with fluid resuscitation, vasopressor support, GDMT-guided therapy, and appropriate antibiotics such as doxycycline (31, 34, 42–44). As glucocorticoid is often used in severe sepsis, low doses of the same can be used in life threatening infections (45). One study reported a dramatic improvement in scrub typhus when steroids and chloramphenicol were used together (46). In our hospital, we have observed prompt improvements with the use of steroids for refractory shock (severe myocarditis), impending adult respiratory distress syndrome, or repeated seizures (severe encephalitis). However, as steroids inhibit the development of immunity against scrub typhus, the duration of use should be minimized (47). ECMO has also been effective in treating patients with complications (14). A repeat echo, 2 weeks following recovery is recommended to assess the resolution of Ejection Fraction and heart enlargement (31, 35, 44).

Arrhythmia

During acute infection, arrhythmia occurs due to the involvement of the interventricular septum, coronary artery, or cardiac valves (48). Table 3 depicts different ECG findings encountered in Scrub Typhus.

Atrial fibrillation

We focused on atrial fibrillation due to scrub typhus because it is a poor prognostic factor, with a 1.3-fold increase in 3-month mortality associated with new-onset AF (49). About 1% of patients with scrub typhus are found to have new-onset AF and the majority (87.2%) were >60 years of age. Regardless of occurrence, these patients were also more likely to have a previous diagnosis of cardiovascular disease, with a 1.67 times higher risk in patients with prior hypertension, a 1.9 times higher risk in ischemic heart disease, and a 1.5 times higher risk of heart failure in developing AF (49–51). Atrial fibrillation was seen in patients with an older age, male sex, fever, leukocytosis, and abnormal renal and liver functions (50, 51). However, the management of atrial fibrillation remains the same. In patients with the acute phase of infection, it is important to perform serial ECG monitoring (52) and in patients with suspected atrial fibrillation baseline cardiac enzymes (mainly CK MB) and Echocardiogram are needed. These will aid in identifying myocarditis as Paroxysmal AF is a strong predictor of myocarditis (70% sensitivity and 84% specificity) (28).

For treatment of atrial fibrillation, we recommend taking appropriate measures to maintain hemodynamic stability including ICU admission (52). Achieving rate control is key in the treatment through the use of agents like calcium channel blockers and/or beta-blockers. If rate control is hard to achieve or the patient is symptomatic, attain rhythm control through cardioversion or antiarrhythmic agents (amiodarone, flecainide, propafenone, dofetilide, and ibutilide) (53). Prompt treatment of scrub typhus with oral or intravenous antibiotics (doxycycline, azithromycin, or rifampicin) prevents serious cardiac complications (48, 52, 54). In patients with hemodynamic instability, intravenous antibiotics are preferred to achieve therapeutic levels (55). In addition to this, monitoring the metabolic panel is essential to prevent further complications (52).

Myocarditis

Myocarditis is defined as the inflammation of the cardiac muscle that causes chest pain, palpitations, and heart failure and is often accompanied by changes in the electrocardiogram, and elevated levels of inflammatory and cardiac biomarkers (56). Myocarditis is relatively rare and has been reported in only a few cases of scrub typhus and varies in severity from mild to fulminant,

TABLE 2 Epidemiological distribution of scrub typhus.

Country	Peak Months	Region	Prefecture
China	May, June and July	Southwest region Southeast Coastal region Eastern region	6 Clusters 1: Guangdong, southern Fujian, Jiangxi, Guangxi 2: Yunnan, Sichuan 3: Jiangsu, Anhui, Shandong 4: Shaanxi 5: Beijing, 6: Zhejiang, northern Fujian Other provinces: Hunan, Tibet
Japan	November and May	Almost all areas except Okinawa and Hokkaido	Kyushu (51%) Tohoku-Hokuriku (27%) Kanto (19%)
South Korea	October and November	Southern and Western Regions	NA
India	August to October	All over India (South India > North India)	Tamil Nadu (37.6%), Himachal Pradesh (11%), Karnataka (8.8%), and Uttrakhand (8.5%)
Thailand	June to November	Northern and Northeastern region	Northern region provinces (83.99%)– Chiangrai, Chiangmai, Tak, Nan Mae Hong Son
Vietnam	May-August (Summer)	Northern Vietnam	Khanh Hoa
Australia	March-July	Tropical coastal periphery of northeastern Queensland, Tropical region of the Northern Territory, Kimberly region of Western Australia	-Ingham -Cooktown
Taiwan	June to July September to October	Island region (Penghu) Mountainous region Eastern region	NA
Srilanka	NA	NA	Kurunegala district, North Western province Anuradhapura district, North Central Province Ratnapura district, Sabaragamuwa province Matara district, Southern province

NA, not available.
Table References: (1, 16, 29–31).

with some cases requiring mechanical circulatory support (14, 30, 44–46). The incidence of myocarditis in scrub typhus varies and has been reported to range from 8% to 14% (27, 28, 57–63) and a recent systematic review indicated an incidence of 4.3% (23, 27, 28, 62). Scrub typhus myocarditis is a serious cardiac complication with a mortality rate as high as 24.0% (23). Certain risk factors have been identified that can increase the risk of acute myocarditis, including paroxysmal atrial fibrillation (OR= 2.85; p= 0.02), elevated levels of total bilirubin (OR= 1.2; p= 0.04), and a shorter duration of symptoms before presentation (mean duration of illness= 6.8d vs 8.3d OR= 0.69; p= 0.04) (28, 30). The other risk factors that contribute to a poor prognosis with scrub typhus include liver disease (OR= 62.70; p=0.011), elevated C-reactive protein (OR= 122.69; p= 0.002), elevated bilirubin levels (RR= 9.28; p= 0.02), and elevated creatinine levels (RR= 43.9; p= 0.003) (28, 64). Identifying and acknowledging these risk factors promptly facilitates risk stratification and enables timely management (28, 64). The exact mechanism of myocarditis in scrub typhus is not well understood, and further research is needed for better understanding (14).

Patients with myocarditis can present with nonspecific symptoms like fever, myalgia, palpitations, and exertional dyspnea or even with cardiogenic shock and sudden cardiac death (65, 66). Thus, in cases of systemic infection with scrub typhus and concomitant new cardiovascular dysfunction or elevated cardiac enzymes, myocarditis should be suspected (45). Various tests like ECG, echocardiography, endomyocardial biopsy, and cardiac MRI aid in the diagnosis of acute myocarditis in scrub typhus (27, 44, 45). ST abnormalities on ECG may serve as an initial manifestation in the majority of patients (44). In a prospective study, ECG changes such as T wave inversion (p= 0.02) and QRS changes (p< 0.001) correlated with the occurrence of myocarditis (27). Cardiac biomarker- Troponin T is utilized as the most sensitive biomarker to indicate myocyte injury in patients with suspected myocarditis. The diagnosis of myocarditis is considered if myocardial

injury coincides with global myocardial dysfunction (27). Echocardiography to detect impaired left ventricular function is needed in suspected cases of myocarditis (27). Endomyocardial biopsy has proven helpful in establishing the diagnosis in certain cases, as highlighted in a recent case report by Park et al. (14, 42, 67). A special indication of endomyocardial biopsy includes patients who develop acute decompensated heart failure of unknown etiology (less than 2 weeks in duration) (68). This technique has its limitations, such as invasiveness in hemodynamically unstable patients, difficulties in acquiring adequate specimens, and low sensitivity (45). Recently, cardiac MRI has been increasingly utilized for the diagnosis and prognostic assessment of myocarditis. This imaging modality offers three key advantages- it indirectly assesses cardiac function, aids in guiding myocardial biopsy at suitable sites, such as focal regions identified on the cardiac MRI, and facilitates the distinction between myocarditis and myocardial infarction based on the delayed gadolinium enhancement pattern in the myocardium (45, 69). Treatment for scrub typhus-induced myocarditis is doxycycline 100 mg IV or orally twice daily for 7-14 days and it has demonstrated efficacy in multiple cases (14, 44, 45). Interestingly, in certain severe cases, the addition of intravenous chloramphenicol to doxycycline is necessary (66). Low-dose glucocorticoids have been used in conjunction with antibiotics for severe myocarditis in a few cases with improvement (17, 47). However, this remains controversial as steroids inhibit the development of immunity against scrub typhus, and increase the risk of scrub relapse, and post-typhus asthenia. Also, *in-vitro* studies have shown steroids do not increase bacteria counts yet further *in vivo* research is warranted to support this line of management (70). In cases of fulminant progression that does not respond to medical management, the successful and prompt implementation of VA ECMO has proven to be beneficial (14, 46).

Myocardial ischemia

Scrub typhus can cause ischemic events through endothelial dysfunction and increase proinflammatory mediators which further worsens this event. It can also cause systemic vasculitis or can also cause plaque stability (71). Patients who have high-risk factors for acute coronary syndrome can also develop myocardial ischemia during the illness. Men and patients of 35-49 years of age had a 2-fold increased risk of developing acute coronary syndrome. The risk of ACS was also associated with diabetes (adjusted HR =2.77, 95% CI 2.04 to 3.76), hypertension (adjusted HR 1.88, 95% CI 1.38 to 2.76), and previous coronary artery disease (adjusted HR=1.53, 95% CI 1.03 to 2.27). However, they also remain independent risk factors of ACS after adjusting for covariates (72). During 6 month follow-up, patients who had scrub typhus had a 3-fold significant increase in the risk of developing ACS (95% CI 1.47 to 7.70) (72). As the pathogenesis involves systemic vasculitis, young patients have presented with no atherosclerotic blockage on coronary angiography but exhibit symptoms of acute coronary syndrome. Additionally, they show elevated cardiac enzymes and EKG changes (73). There are also cases of scrub typhus where thrombotic occlusion was identified and percutaneous intervention where necessary to stabilize the patient (74, 75). We recommend treating patients with appropriate antibiotics and depending on coronary angiography findings further management should be planned.

Endocarditis

BCNIE (blood culture-negative infective endocarditis) can manifest in up to 31% of all patients with infective endocarditis with significant diagnostic and therapeutic difficulties. Scrub typhus-induced endocarditis is extremely rare and presents as blood-culture negative IE (76). However, based on clinical suspicion and endemicity in the region, clinicians should consider workup for scrub typhus as a possible etiology (77). In a case report by Yu et al. in 2016, chloramphenicol (1g q12h iv drip for 10 days) may be appropriate for scrub typhus-associated infective endocarditis. However, further large-scale prospective randomized controlled trials are necessary to validate this finding (77).

Diagnostic methods

Serology is the primary diagnostic tool for *O. tsutsugamushi* infection. IgM antibody titer increases by the end of the 1st week of the infection, while IgG antibody peaks by the end of the 2nd week. The Weil-Felix test is the most affordable serological test to detect antibodies for various *Proteus* species. A titer of 1:320 or above, or a fourfold increase from 1:50, is considered positive (76). However, the test has a low sensitivity of 33% at a breakpoint titer of 1:80 but with 100% specificity and positive predictive value (78, 79). Indirect immunofluorescence antibody (IFA) is an expensive, specialized test to confirm infection before seroconversion (80, 81). The lateral flow rapid test and Scrub Typhus Detect use recombinant 56-kDa antigen and have shown a strong potential for diagnosing scrub typhus patients with 100% sensitivity and 92% specificity for IgM. This prototype product can help clinicians diagnose patients rapidly, accurately, and easily, allowing them to provide timely care (82). Some commercial laboratories in India offer the immunochromatographic test (ICT) that serves as a rapid diagnostic test, with the advantage of being inexpensive (83). Replacing fluorescein with peroxidase, indirect immunoperoxidase eliminates the need for a fluorescent microscope, making it beneficial in a resource-poor setting (84). Western immunoblot assay is an effective serodiagnostic tool for large-scale screening and confirming serologic diagnosis. It uses sodium dodecyl sulfate-gel electrophoresed and electro-blotted antigens and helps analyze cross-reactive strains (80). The most abundant and immunodominant protein is employed in a recombinant protein-based enzyme-linked immunosorbent assay, which has been developed to detect *Orientia*-specific antibodies in serum. Four prototype strains of *Karp* were used to generate three recombinant protein antigens, namely TA763 (r56C1), Kato (r56Kt), and Gilliam (r56Gm) *Orientia*. Chimeric proteins were compared to titers of serum samples against Karp, Kato, Gilliam, and TA763 strains. These new proteins had similar reactivity to parent proteins and were recognized by 14 *Orientia* strains, making them useful for

diagnosis and vaccination. Chimeric C1 was identified as a potential substitute for parent proteins to diagnose *Orientia* infection and as a vaccine candidate with broad protective efficacy (81). It can be regarded as an enhanced, practical, and affordable substitute for the gold standard IFA in rapid diagnosis and seroprevalence (85). A cell-based ELISA technique was used to test serum samples from ST-positive rats and monoclonal antibodies. The accuracy, sensitivity, and specificity of this technique were 96.3%, 98.6%, and 84.6%, respectively. The results were consistent with those of immunofluorescence assays. This technique is safe, easy to operate, and does not require specialized equipment (86). *Rickettsial culture* can be obtained from buffy coats of heparinized blood, defibrinated whole blood, plasma, and skin biopsy. Different methods, such as MRC 5 cells, Vero cell culture, BHK21, etc., are used for this purpose, with Vero or L929 cells enabling quicker and more effective isolation (87). The average time required for culture isolation of *Rickettsia* species is four weeks (88). However, bacterial culture for *O. tsutsugamushi* is a tedious process requiring biosafety level 3 containment and significant technical expertise. Due to its complexity, it is primarily used for research purposes in reference laboratories.

Polymerase Chain Reaction (PCR) molecular detection using skin rash, lymph node, or blood samples is an efficient and sensitive method for diagnosing scrub typhus. Real-time PCR assays based on GroEL provide a more quantitative result, while nested PCR is one hundred times more sensitive than single PCR (76, 89, 90). Combined with IFA, nested PCR has a sensitivity of 82.2% and specificity of 100% (91) and other studies also show similar findings (87, 92, 93).

A tool was created to distinguish severe fever with thrombocytopenia syndrome (SFTS) from scrub typhus. It uses low CRP, thrombocytopenia, and leukopenia as variables. A score of >=2 has a specificity of 96.1% and a sensitivity of 93.1% for SFTS (89). Recently, the highly conserved *O. tsutsugamushi* 60 kDa GroEL chaperonin produced by *E. coli* was the focus of an immunochromatographic antigen detection test kit (ICT AgTK). Polyclonal antibodies, including a rGroEL-specific monoclonal antibody, were used as antigen detection reagents. In in-house validation studies, the test showed potential with a sensitivity, specificity, and accuracy of 85%, 100%, and 95%, respectively, compared to the combined clinical characteristics and standard IFA. This test holds promise for on-the-spot and early diagnosis of scrub typhus (90). In a recent report by Zuan Zhan et al, metagenomic next-generation has been used to diagnose scrub typhus in a patient with fever, multiorgan dysfunction, and negative serology (94). It has the advantage of having the ability to diagnose scrub typhus early in the course of illness (95). The investigations are summarized in Table 4.

TABLE 3 ECG findings encountered in Scrub Typhus.

S. no	ECG abnormality	Incidence/Comments	Recovery with antibiotics	Citations
1.	Sinus arrhythmia	Incidence rate is 43%	Yes	(48, 50)
2.	Sinus or relative Bradycardia	Incidence of 53%.	Yes	(96, 97)
3.	Sinus tachycardia	Incidence of 46%.	Yes	(48, 55, 98)
4.	Inverted T waves	Incidence rate is 12.7%.	Yes	(48, 50)
5.	Other T wave abnormalities (Prominent, Notched, Tall and Peaked)	–	Yes	(48)
6.	U waves	Incidence rate is 7%.	Yes	(48, 99)
7.	PR depression	–	Yes	(48)
8.	ST elevations	Incidence rate is 0.6%.	Yes	(48, 50)
9.	Heart blocks (1st degree, Mobitz type 1, 3rd degree heart blocks)	–	Yes	(48, 54, 55, 100)
10.	Q-Tc interval prolongation	Incidence rate is 19%. Be watchful of drugs prolonging the QT interval.	Yes	(48, 50)
11.	Torsade de pointes	–		(50)
12.	AV junctional escapes	–	Yes	(48)
13.	Premature ventricular contractions	Incidence is 4%.	Yes	(48, 50, 99)
14.	Incomplete right bundle branch block	Incidence of 3%.	Yes	(48, 50)
15.	Left bundle branch block	Incidence of 0.6%.		(50)
16.	Right axis deviation	Incidence of 2%.	Yes	(48)
17.	Atrial Fibrillation	Incidence rate of 1%.	Yes	(50)
18.	Atrial Flutter	Incidence of 1%.		(50)
19.	Atrial premature beat	Incidence of 3%.		(50)

Treatment

The increase in outbreaks both within and outside the Tsutsugamushi Triangle, the emergence of antibiotic-resistant strains of *Orientia*, and the emergence of new *Orientia* species (101). During the 1990s, genotypes in Thailand that were resistant to chloramphenicol and doxycycline were identified (101, 102). The strains AFSC-3 and AFSC-4 of the pathogen are identified as resistant to doxycycline (102). Proposed mechanisms to explain resistance include dormant organisms within patients subjected to repeated or long-term exposure to antibiotics. Additionally, the supplementation of poultry feed in Thailand with antibiotics may result in the development of drug-resistant strains of chiggers. The ingestion of antibiotics by mites during their feeding on their rodent host, which frequently consumes grain designated for poultry, is a plausible consequence (101). The occurrence of treatment failures has also prompted reports of potential doxycycline resistance in India and South Korea (103). It is important to note that the prophylactic administration of doxycycline has failed to prevent breakthrough infections and can lead to the emergence of resistant strains (101). Despite this, there is no evidence of extensive presence or prevalent distribution of scrub typhus strains that are resistant to antibiotics (101).

Monotherapy vs combination therapy?

When it comes to treating cardiac complications, our recommendation aligns with Varghese et al. The study by Varghese et al. was a prospective, multicenter, randomized trial with a large sample size, and concluded that combination therapy with azithromycin and doxycycline is better than monotherapy when it comes to severe sepsis and organopathies (104). We recommend combination therapy, especially in patients with multiple organ involvement and in those with multiple comorbidities that further increase their risk for cardiac complications (104). In patients who have not yet developed cardiac complications but are from endemic regions of scrub typhus and have an eschar on presentation, we recommend empirical therapy (103) as either a monotherapy or as a combination therapy (47, 104). In regions where doxycycline resistance is not a concern, drugs such as doxycycline, rifampicin, or azithromycin can be used (47). Rifampicin and doxycycline combination therapy showed more effectiveness and longer fever clearance than monotherapy (105). Since rifampicin is an integral part of antitubercular therapy, it should be kept as a last resort. In pregnant women, azithromycin is preferred over doxycycline. For the treatment of nonpregnant adults, oral doxycycline is comparable to iv azithromycin in treating scrub typhus (106). When treating an undifferentiated fever in clinic practice, cefotaxime is not a preferred therapy for scrub typhus. This is because when used in combination therapy, it has demonstrated an antagonistic effect with azithromycin and doxycycline. The reason behind the antagonism is not well understood (47). Table 5 depicts commonly used antimicrobial agents in scrub typhus infection.

The next step in treatment and prevention

Traditional ayurvedic and Chinese phytochemicals containing compounds (ZINC8214635, ZINC32793028, ZINC08101133, ZINC85625167, ZINC06018678, and ZINC13377938) have demonstrated successful inhibition of *Orientia tsutsugamushi* (107). Newer compounds and antibiotic discovery are underway. The CDC recommends avoiding contact with infected chiggers (25). General measures against vectors include minimizing exposure through clothes avoiding walking through dense vegetation, sitting in bare ground or grass, and use of insecticides and insect repellants. Appropriate clothing to avoid getting bitten in

TABLE 4 Summary of the investigations.

S. No	Investigation	Sensitivity	Specificity	Accuracy	Advantages	Disadvantages	Biomarker (gene/ antibody)	References
1.	IFA	100%	92%	-	Gold standard - High sensitivity	Expensive, requires specialized laboratory	IgM	(88)
2.	Cell based ELISA	98.6%	84.6%	96.3%	Rapid diagnosis and seroprevalence	Practical and affordable	IgM	(86)
3.	nPCR	97%	100%	-	Early diagnosis (as early as Day 3, before antibody formation)	—	56 kDa tsa	(111)
4.	ICT AgTK	85%	100%	95%	On-the-spot testing and early diagnosis	—	60 kDa GroEL chaperonin	(90)
5.	Weil Felix	33%	100%	-	Inexpensive, readily available	Low sensitivity	IgM	(88)

IFA, Immunofluorescence Assay; ELISA, Enzyme-Linked Immunosorbent Assay; nPCR, Nested Polymerase Chain Reaction; ICT AgTK, Immunochromatographic Test Antigen Test Kit; IgM, Immunoglobulin M; kDa tsa, Kilodalton type-specific antigen; kDa GroEL, Kilodalton GroEL.

sun-exposed areas. Personal hygiene is advised and removal of clothing and thorough cleaning of skin and clothes with detergent can reduce the risk of infection. Postexposure prophylaxis with doxycycline is currently not recommended (1, 108). However, prophylactic medications of doxycycline, chloramphenicol, or tetracycline are provided in the endemic areas (single dose every 5 days for a total of 35 days) (1, 109). Measures to control rodents that attract these vectors are also recommended (108).

Vaccines

The existence of numerous strains of *Orientia tsutsugamushi* hinders the development of a broad and lasting immune response. Efforts to prepare a vaccine have been ongoing since World War II. A recent study has highlighted that the CD-1 outbred mice model proves valuable for understanding host susceptibility and facilitating future vaccine studies (110). Table 6 depicts the evolution of vaccines for scrub typhus. Field trials and large-scale attempts, such as the Tyburn operation and studies by the Japanese, were not fruitful, each demonstrating setbacks, particularly the waning of immunity or the acquisition of a fatal infection post-vaccination (112–115). Vaccine preparation has been attempted through formalin-inactivated pathogens, live attenuated strains, irradiated strains, and using antibiotics and live vaccines together. However, all these strategies have failed to provide either long-term immunity for homologous strains or any immunity against heterologous strains (115–122). This encouraged a shift towards the development of subunit vaccines (112).

Over the last two decades, the focus on scrub typhus subunit vaccine involved the production of recombinant proteins (kDa antigens) and their incorporation into DNA vaccine candidates through cloning (124, 125). Research on the 56-kDa protein of *Rickettsia tsutsugamushi* explored TSA56 (56-kDa type-specific antigen), a key outer membrane protein, as a potential vaccine candidate for generating immunity against scrub typhus. Kp r56 showed strong immunogenicity, triggering immune responses and IFN-γ production (126). The plasmid DNA vaccine *pKarp56, pKarp110*, 47 kDa gene fragment, and the fused antigen Sta56-47 demonstrated the ability to elicit robust immune responses (124, 127–129). Additionally, a novel recombinant antigen derived from the conserved regions of the 56 kDa type-specific antigen (cTSA56) provided superior protection against both homologous and heterologous genotypes (130). Recent studies also explored the protective effects of ScaA immunization and Zinc oxide nanoparticles (ZNPs) as a novel vaccine carrier system for *O. tsutsugamushi* (131, 132). Subsequently, dual-antigen subunit vaccine nanoparticles (47 kD and 56 kD) also highlight their potential as a promising vaccine strategy (133).

The intranasal rec56 vaccines elicited a higher IgG response than the intramuscular route (134). Supporting this, Park et al. demonstrate the development of an intranasal vaccine targeting *Orientia tsutsugamushi*'s outer-membrane protein (OMPOT), intranasal vaccination boosts cell-mediated and protective immunity in pulmonary compartments (135). A model vaccine, utilizing extracellular vesicles (EVs) derived from *Salmonella* expressing DNA sequences of full-length Ot proteins (TSA56, ScaA, ScaC, ScaD, and ScaE), showed promising results. Inoculation with EVs from TSA56-expressing cells protected mice from *Salmonella*-induced illness, suggesting the potential for scrub typhus immunizations based on T-cell immune response (136).

Conclusion

We conducted a review of the literature to raise awareness on cardiac complications of scrub typhus. Our review is limited to strain-specific epidemiological patterns and treatment options based on disease severity. The early identification of at-risk cases is key to managing these complications, particularly patients from endemic regions presenting with eschars. IFA is the gold standard for investigation and rapid ICT kits are invaluable in resource-limited areas. The approach to the individual cardiac complications is decided based on the presentation and investigations such as ECG and echocardiography findings of the patients. We recommend combined therapy with doxycycline and azithromycin for treating

TABLE 5 Commonly used antimicrobial agents in scrub typhus infection (123).

Name of drug	Dose and administration in adults	Comments
Doxycycline (70, 72)	100 mg twice daily for 7 days.	Drug of choice; Intravenous preferred for sicker patients.
Tetracycline	500 mg four times daily.	No difference between doxycycline and tetracycline.
Azithromycin	Mild infections: 500 mg single dose Severe infections: 500 mg once daily for 3 to 5 days; 1 g loading dose may be given.	Preferred drug in pregnancy; recommended when doxycycline resistance is present.
Telithromycin	800 mg daily for 5 days.	As effective as doxycycline.
Chloramphenicol	500 mg every 6 h for 7 days.	Alternative to tetracycline; contraindicated in pregnancy; risk of aplastic anemia.
Rifampicin	600 to 900 mg daily for 7 days.	Rifampicin or doxycycline in mild scrub typhus; shorter duration of fever with Rifampicin in Northern Thailand when compared with Doxycycline; caution in tuberculosis endemic areas.

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TABLE 6 Evolution of vaccines for scrub typhus.

S.No	Type/Method	Participants	Immunogen/Antigen	Disadvantages	Citation
1.	Killed vaccine	Humans	Formalin-fixed homogenized lungs from *Orientia*-infected cotton rats	Not effective in humans	(137)
2.	Killed vaccine	Humans	Formalin-killed *Volner strain of O. tsutsugamushi*	Incidence was not reduced Occurrence of severe and nearly fatal cases. Failed to provide long lasting immunity	(115)
3.	Killed vaccine	Mice	Formalin-killed *O. tsutsugamushi*	Provided protection against homologous strains and not heterologous strains.	(116)
4.	Live vaccine	Humans	*Pescadores strain of O. tsutsugamushi*	Solid protection in humans	(138)
5.	Live vaccine + antibiotic treatment	Humans	Live *O.tsutsugamushi* strain followed by antibiotics	Provided protection against homologous and not heterologous protection	(117, 139, 140)
6.	Attenuated vaccine	Mice	Live *O.tsutsugamushi* irradiated with 300 krads	Protected against homologous strains and poor protection against heterologous strains	(119–121)
7.	Subunit vaccine	Mice	56KDa protein fused with maltose binding protein of *E. Coli* (mBP- Bor56)	Protection against homologous strains	(141)
8.	Subunit vaccine	Mice	Recombinant 56-kDa protein	Protection against homologous strains	(142)
9.	Subunit vaccine	Monkeys	Recombinant fragment of 56-kDa protein	Only partial protection against homologous strain in monkeys	(126)
10.	Subunit vaccine	Mice	DNA encoding 56-kDa protein	Partial protection against homologous strain in mice	(127)
11.	Subunit vaccine	Mice	Recombinant fusion of 56-kDa and 47-kDa proteins	Partial protection against homologous strain in mice	(129)
12.	Subunit vaccine	Mice	40kDa Fragment of the 47kDa Recombinant Protein and DNA Vaccine from *Karp Strain of Orientia tsutsugamushi*	Combined immunization with DNA and protein provided stronger immunogenicity	(128)
13	Subunit vaccine	Mice	DNA vaccine- *pKarp47*	Protection against homologous strains	(143)
14.	Subunit vaccine	Mice	Recombinant fusion protein (sta56-47) 56 kDa and 47 kDa	Stronger protection against homologous strains	(129)
15.	Subunit vaccine	Mice	Conserved regions of 56 kDa type-specific antigen (cTSA56)	Protection against homologous and heterologous strains	(130)
16.	Vaccines generated using extracellular vesicles from *Salmonella*	Mice	TSA56, ScaA, ScaC,ScaD, ScaE	Provided T cell mediated immunity. Mice were protected from *Salmonella* induced mortality.	(136)
17.	Zinc oxide - nanoparticles (ZNP) induces inflammatory responses when injected. ZNP can act as antigen carrier and adjuvant system	Mice	ZBP-ScaA	Its protective action, if included in a multiplex subunit vaccine is also unknown augment antigen-specific adaptive immunity.	(132)
18.	Intranasal vaccination made using the outer membrane protein of *O. tsutsugamushi*	Mice	47 kDa Ag from outer membrane protein of *Boryong strain* was used for preparation of the vaccine.	Induces strong Th1 and Th17 responses in the both spleen and lungs to the *O tsutsugamushi* Antigen.	(135)

E. Coli, Escherichia coli; mBP-Bor56, Maltose Binding Protein-Borrelia 56; kDa, kilodalton; pKarp47, Protein Karp 47; TSA56, 56 kDa type-specific antigen; ScaA, Surface cell antigen A; ScaC, Surface cell antigen C; ScaD, Surface cell antigen D; ScaE, Surface cell antigen E.

cardiac complications in addition to the guideline-directed therapy for the cardiac event. Vaccine development using extracellular vesicles, nanoparticles, and subunit vaccines containing recombinant proteins has demonstrated potential. However further research is needed to design a vaccine that can provide a long-lasting immunity against scrub typhus.

Author contributions

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