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Scrub typhus update: A re-emerging global threat beyond the Tsutsugamushi Triangle and the physiological ramifications of scrub typhus infection (Review)

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Abstract. Scrub typhus, caused by *Orientia tsutsugamushi*, is a mite-borne zoonotic disease endemic to Asia and the Pacific. The prevalence of *Orientia tsutsugamushi*, originating from the Tsutsugamushi Triangle, is increasingly globally. The present review delves into the intricate mechanisms of bacterial-host interactions and immune responses within dendritic cells, blood vessels and kidney endothelial cells. Additionally, it provides an in-depth discussion of the scrub typhus life cycle and its associated organ system diseases. It presents with flu-like symptoms, including fever, rash and eschar formation, but can progress to severe complications, such as acute encephalitis syndrome and multi-organ dysfunction syndrome. The disease has affected vulnerable populations in India, China, Thailand, Malaysia, Korea, and other countries with outbreaks reported across various states. The diagnosis of scrub typhus has evolved from the outdated Weil-Felix test to more accurate techniques, such as the use of PCR and ELISA. Early treatment with antibiotics, such as doxycycline or azithromycin markedly reduces morbidity and mortality rates. The disease remains neglected in a number of regions despite the growing number of cases. Preventive measures, primarily the use of insect repellent, are essential, as no licensed vaccine exists to date.

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1. Introduction

The World Health Organization (WHO) classifies scrub typhus as a neglected tropical disease and a major public health issue in the Asia-Pacific region. Scrub typhus markeldy impacted the Allied forces during World War II and in the Vietnam War. In the Pacific theater, >18,000 troops contracted the disease, while in Vietnam, it remained a prominent cause of severe, undifferentiated febrile illness (1). *Orientia tsutsugamushi* is a Gram-negative bacterium that depends on living cells for survival and causes scrub typhus, a disease transmitted by mites (2) Fig. 1. The earliest known cases of scrub typhus were documented in China in 313 A.D. The bacterium *Orientia tsutsugamushi* was initially discovered in Guangzhou, China. It is estimated that globally, more than a billion individuals are at risk of contracting scrub typhus, with approximately one million cases being reported annually (3,4). This disease, prevalent in regions of Asia, the Pacific and the Indian Ocean islands, is spread to humans through the bites of larval trombiculid mites, commonly referred to as chiggers (5,6). Scrub typhus has become a pressing global health issue, characterized by increasing cases and inadequate medical resources to address the problem. Some countries, including China, Korea, Japan and Thailand, have implemented preventive measures, such as rodent control, insecticide use and surveillance systems. A system for monitoring scrub typhus cases was introduced in 1952.

These mites primarily infest rodents and were first identified as carriers of scrub typhus by Hashimoto in Japan in 1899 (7,8). Following a bite from an infected mite, the incubation period for scrub typhus generally lasts between 10 and 12 days, although it may vary from 6 to 21 days (9). The symptoms resemble those of the flu and may include fever, rash, a dark eschar at the bite site, headache, muscle pain, cough, swollen lymph nodes, nausea, vomiting and abdominal discomfort (10). In severe cases, the infection can cause life-threatening complications affecting the lungs, heart, liver, brain or kidneys (11,12). Research from Assam has also shown that infection with *Orientia tsutsugamushi* can cause acute encephalitis syndrome, a severe condition that can be fatal (13).

The bacterium is primarily transmitted through the bite of red-colored, microscopic chigger larvae, which are more abundant in dense, vegetated areas during the rainy season, giving rise to names, such as river fever or flood fever. These mites typically lay their eggs between July and December. While mites and rodents inhabit various ecosystems, 'scrub' refers to the vegetation type that facilitates chigger-host interactions. The hallmark of scrub typhus is the formation of an eschar at the bite site, which develops into a black, crusty lesion resembling a cigarette burn. While studies from Korea have identified a notable number of cases with eschar, data from Thailand and Taiwan indicate a markedly lower prevalence (14).

2. Scrub typhus in various regions in Asia

Scrub typhus in India. Scrub typhus has been documented in India since at least 1917 with notable outbreaks occurring during World War II and the 1965 Indo-Pakistani War, primarily along the border with Burma. The disease resurfaced in the 1990s near the Pakistan border (15). Despite decades of awareness, scrub typhus remains a frequently underdiagnosed health concern in India The disease presents a serious health risk, with a substantial number of cases reported from various parts of the country (16). Recent data indicate that scrub typhus is prevalent in various regions of India, including the southern states, northern regions, northeastern states, eastern areas and western states (17). The extensive distribution of this disease is largely due to the vast geographic expanse and varied ecological landscapes of India.

In Odisha in India, scrub typhus was identified in four districts, with two experiencing the most significant impact (18). One of these heavily affected districts also faced a substantial number of dengue cases, further straining local health services. However, no cases of co-infection were reported in the region at that time. The likelihood of simultaneous infections with diseases, such as typhoid, dengue, or influenza in patients with scrub typhus can obscure clinical presentations, complicating diagnosis and treatment efforts (19). This situation places additional pressure on healthcare systems, particularly since vulnerable groups such as children, individuals with weakened immune systems, and the elderly are at increased risk (20). Studies conducted in hospitals in Southern India have found that a number of children with scrub typhus exhibit acute, nonspecific symptoms (21). Neurological complications (meningitis, meningoencephalitis, encephalopathy and seizures) have been shown to be associated with high mortality rates in these patients (22-24).

Scrub typhus has drawn considerable attention after thousands of confirmed cases and several fatalities were reported in India during 2023 (17). This zoonotic infection affected multiple regions, leading to 17 deaths. The Indian states of Himachal Pradesh, Odisha and Rajasthan were among the hardest hit, with Himachal Pradesh reporting nine deaths and Odisha recording eight. In Himachal Pradesh, all the fatalities occurred in the Shimla district, while deaths in Odisha were limited to two districts. Furthermore, Telangana State recorded cases of scrub typhus co-occurring with dengue and influenza.

Scrub typhus in China (Yunnan Province). Scrub typhus is a common infectious disease in China, with Yunnan Province being a hotspot. According to official records, >41,000 cases were reported in Yunnan between 2010 and 2019, with a significant concentration in the western regions, particularly Baoshan, Lincang and Dehong (25). The first recorded case of scrub typhus in China appeared in 1948 (26). A cluster of scrub typhus cases has been reported in Yunnan Province, particularly in Longling City, which has reported the highest number of cases over the past decade. The diverse climate of Yunnan Province, particularly the tropical and subtropical conditions in Baoshan City, creates favourable environments for small mammals and mites, potential hosts and vectors of scrub typhus. This region has consistently reported the highest number of cases in the province. Recent studies have identified additional mite species, beyond *Leptotrombidium deliense*, as potential vectors in Yunnan. A total of 182,991 cases and 186 reated deaths were documented over a period of 51 years in China (27). The geographical location of scrub typhus cases in China is illustrated in the map presented in Fig. 2.

Scrub typhus in Thailand. Data from the national surveillance system in Thailand reveal a marked increase in reported scrub typhus cases between 2003 and 2018 (28). Males were more commonly affected than females, and agricultural workers accounted for the majority of cases, primarily among adults. The disease is seasonal, peaking during the rainy season. Northern provinces, particularly Chiangrai, experienced the highest number of cases. Factors, such as agricultural activity, elevation, rainfall, temperature and land cover contribute to the disease burden in Chiangrai. These findings enhance the current understanding of scrub typhus in Thailand and help identify potential risk factors.

Scrub typhus in the Chungcheong region of Korea. To understand the trends in scrub typhus cases within the Chungcheong region, epidemiological data were collected from 14,379 cases reported to the Korea Centers for Disease Control and Prevention between 2012 and 2022 (29). Geographical analysis revealed an association between areas with a high scrub typhus incidence and a larger elderly

Figure 1. *Orientia tsutsugamushi* mite vector.

Figure 2. Reported cases of scrub typhus in China Province during 2006-2018; the map was created by data wrapper with data from China Center for Disease Control and Prevention.

population. The elderly population and changes in agricultural practices were significantly linked to the occurrence of scrub typhus. Although the overall number of cases and agricultural activity in the Chungcheong region has declined since 2012-2013, recent data indicate a possible increase in the number of cases due to more individuals spending time outdoors (30,31). Older individuals are at a greater risk of infection and may also experience repeated infections or infections with other illnesses that cause fever. Scrub typhus cases were documented in Korea as early as the Korean War in 1951. Studies conducted between 2001 and 2006 revealed a higher incidence of the disease among females compared to males (~65 vs. 35%). Researchers attributed this disparity to differences in agricultural practices, suggesting that the more crouched working posture of females may increase their exposure to the mites that transmit the disease (32). These findings underscore the need for updated prevention and promotion strategies tailored to the changing demographics and risk factors for scrub typhus in the region.

3. Seasonal variations and factors controlling the spread of scrub typhus

Scrub typhus cases exhibit seasonal variations across different regions. In Korea, outbreaks typically occur in the fall (October to November) (33), while in India, they peak during the monsoon and post-monsoon seasons (July to February) (21). Nepal and Thailand report peak cases in the summer and early fall (34). In China, particularly in the southern regions, scrub typhus cases often peak in the summer, with a recent trend of additional peaks in September and October in Yunnan Province (35). Environmental factors, such as temperature, humidity, and precipitation play crucial roles in the transmission of scrub typhus in China. Research suggests that higher temperatures and rainfall can increase mite populations, leading to a greater risk of transmission (36). However, the specific environmental conditions conducive to mite abundance and transmission vary across different regions. Additionally, humidity levels must be within a suitable range to support both mite and small mammal populations, which are essential for the transmission cycle of the disease.

The Tsutsugamushi Triangle. The term 'Tsutsugamushi' comes from two Japanese words: 'tsutsuga', meaning illness, and 'mushi', meaning insect (37). The primary endemic region for scrub typhus is referred to as the 'Tsutsugamushi Triangle', covering over eight million square kilometers (6). This region stretches from the Russian Far East in the north to Pakistan in the west, Australia in the south and Japan in the east (Fig. 3). Approximately one billion individuals are at risk of infection in the highly populated area. The Tsutsugamushi Triangle encompasses over half of the global population, placing more than one billion individuals at risk (16).

Globalization and increased travel have contributed to the spreading of infected individuals to areas outside the endemic regions, heightening concerns about the broader reach of *s*scrub typhus (38). The diverse antigenic and genetic properties of *Orientia tsutsugamushi* strains, coupled with the uncertain link between these differences and the severity of human illness, hinder our epidemiological understanding of scrub typhus. A more in-depth understanding of its epidemiology is essential for creating effective prevention and control measures (39). This section of the review explores the geographic distribution of scrub typhus and the associated risk factors, both in endemic areas and among travelers from non-endemic regions. In India, scrub typhus has been reported across a wide range of geographical regions as shown in Fig. 4 and as previously demonstrated (13,40-43).

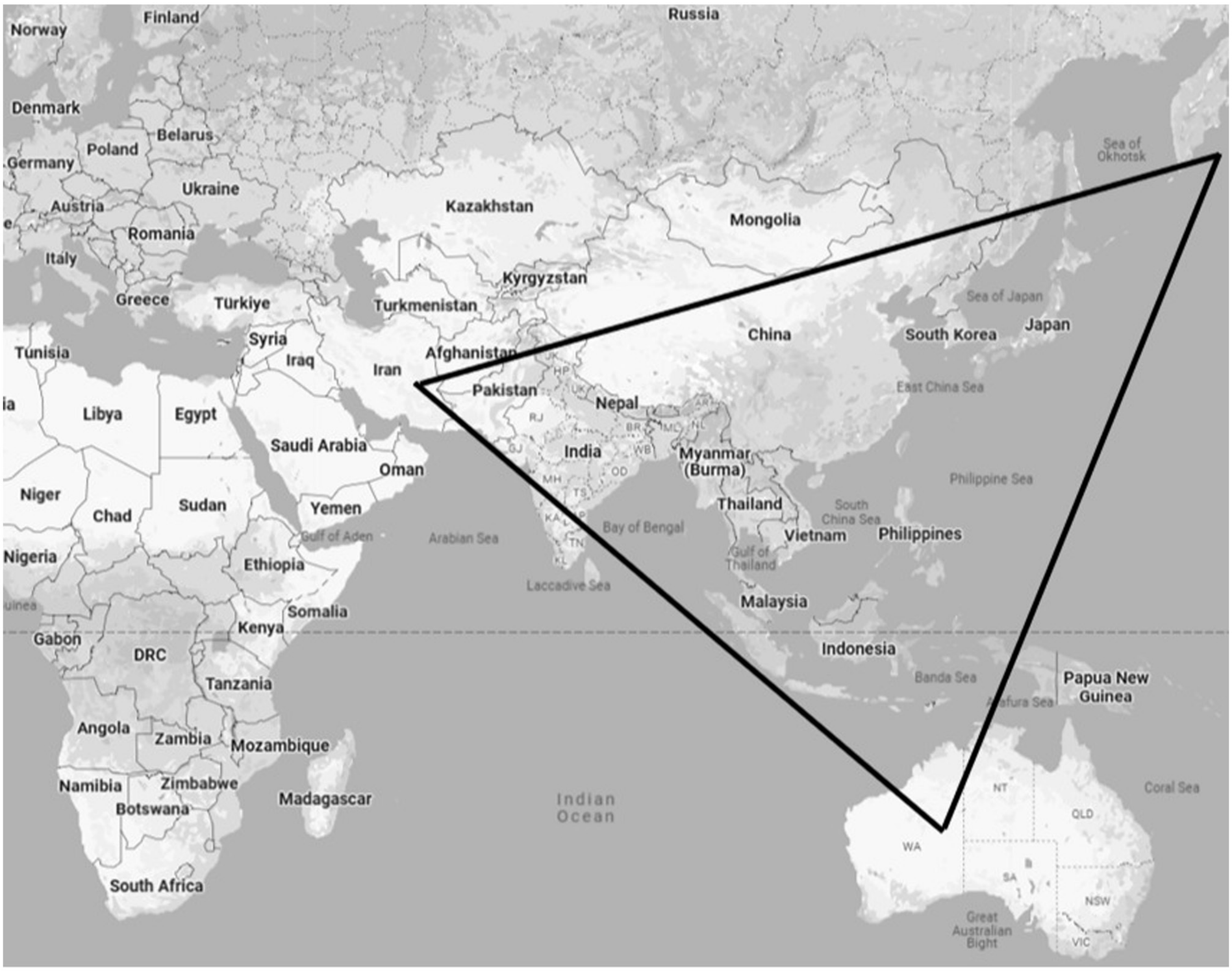

Figure 3. Endemic to the 'Tsutsugamushi Triangle', this acute febrile illness spans from Pakistan to Japan and southward to Australia illustrated in the map.

Previous studies, primarily conducted in rural areas of India, have indicated that scrub typhus is widespread and disproportionately affects vulnerable populations, including impoverished farmers and children (44,45). In some regions, the increasing number of cases has led to scrub typhus being recognized as a re-emerging infectious disease, raising serious public health concerns. In certain areas, ~20 to 24% of patients with unexplained fever have been diagnosed with scrub typhus, with 53% of these cases exhibiting signs of acute kidney injury (44).

4. Life cycle and transmission of *Orientia tsutsugamushi*

Orientia tsutsugamushi targets a variety of cells throughout its life cycle. Initially, it infects dermal dendritic cells and activates monocytes at the bite site, using these cells to spread to lymph nodes. In mice, the bacteria prefer macrophages, while in humans, it primarily infects endothelial cells in multiple organs, including the skin, heart, lungs, kidneys and brain. Other target cells include mouse fibroblasts, neutrophils and polymorphonuclear leukocytes in various infection models (16,46). Cell invasion and the intracellular life cycle of *Orientia* involve complex interactions with host cells. The bacteria attach to extracellular matrix components, particularly fibronectin, which interacts with *Orientia* proteins to facilitate entry into nonphagocytic cells. Integrins and heparan sulfate proteoglycans also play roles in this process (47).

Once it has gained entry into the host, *Orientia* utilizes clathrin-mediated endocytosis to enter host cells and rapidly escapes the phagosome to avoid the defense mechanisms of the host. The bacteria replicate in the cytoplasm and may accumulate in high densities before being released from the host cell, potentially via membrane budding. Despite notable advancements being made, several aspects of intracellular life cycle and escape mechanisms of *Orientia* remain unclear (37).

The larvae of trombiculid mites, also known as chiggers, are primary vectors for rickettsial pathogens, particularly *Orientia* spp., which cause the zoonotic disease scrub typhus. Chiggers are the only known vectors for *Orientia* bacteria, which cause scrub typhus. Chiggers are primarily parasitic on wild vertebrates, including small mammals, reptiles and birds. While humans are not their preferred hosts, they can be incidentally parasitized by chiggers. Chiggers of the genus *Leptotrombidium* are the primary vectors for transmitting *Orientia tsutsugamushi* to humans; a recent study in Chile identified *Herpetacarus antarctica* as another potential

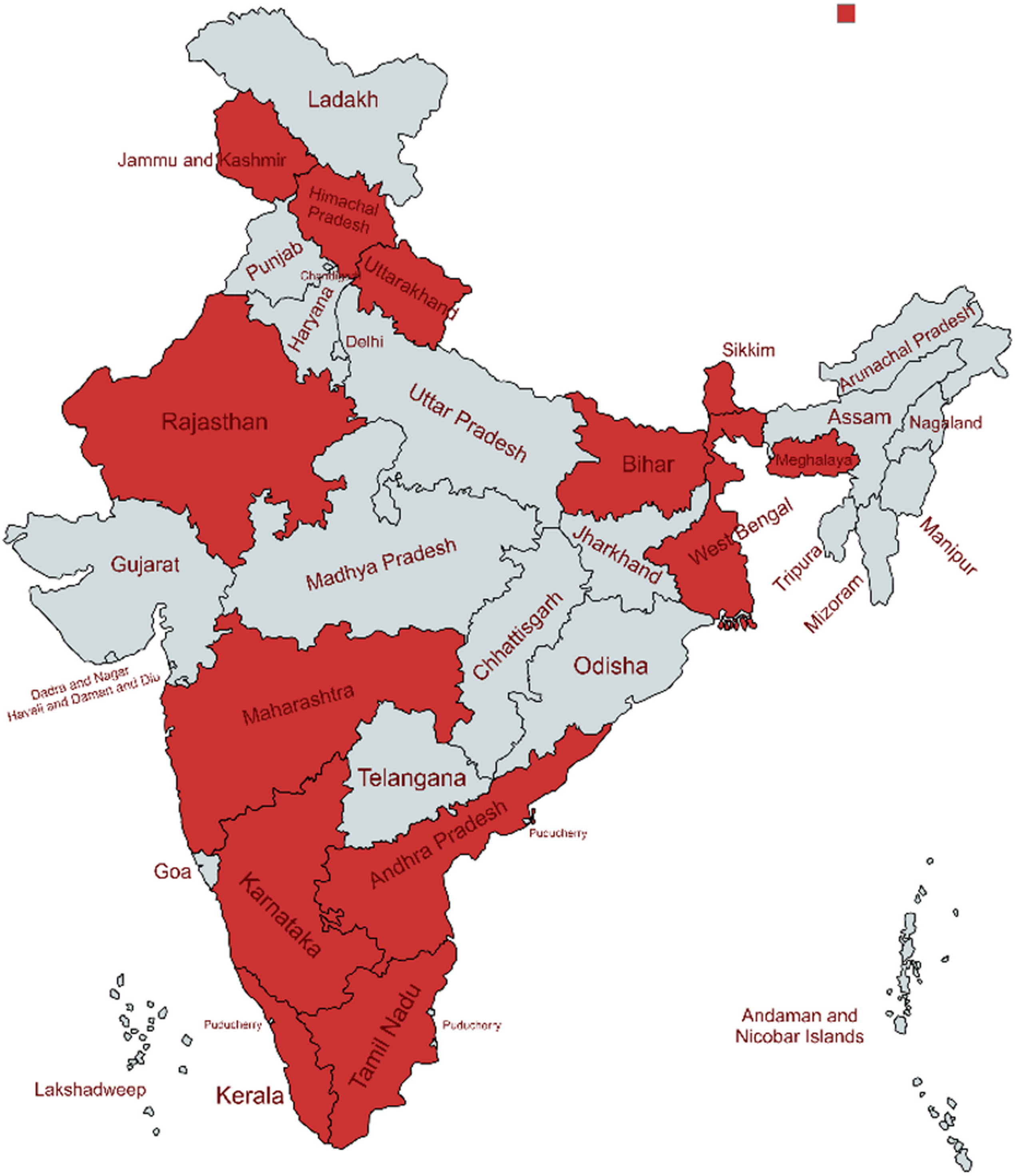

Figure 4. Map highlighting the geographical distribution of scrub typhus in India, represented by the red-shaded regions.

vector for scrub typhus caused by *Candidatus Orientia chiloensis* (48). Chiggers do not suck blood from the host, but produce a specialized feeding organ known as a stylostome, which is comprised of glycoprotein and forms after attaching to the host (49). The digestive enzyme secreted from the mouth is released into the deepest layer of the skin, known as the dermis and digests dermis tissue protein (50). Chiggers are known to feed only once on their host before detaching to molt through three nymphal stages: Protonymph, deutonymph and tritonymph (51). Trombiculid mites undergo a complex life cycle with several stages: Egg, prelarva, larva (chigger), protonymph, deutonymph, tritonymph and adult. After 6 days of laying the egg, when the shell breaks down, eggs hatch into inactive prelarvae, which then develop into active, six-legged larvae (12 days). These larvae feed on a host for several days before dropping off to become inactive protonymphs. Protonymphs transform into active, eight-legged deutonymphs, which then enter a quiescent tritonymph stage.

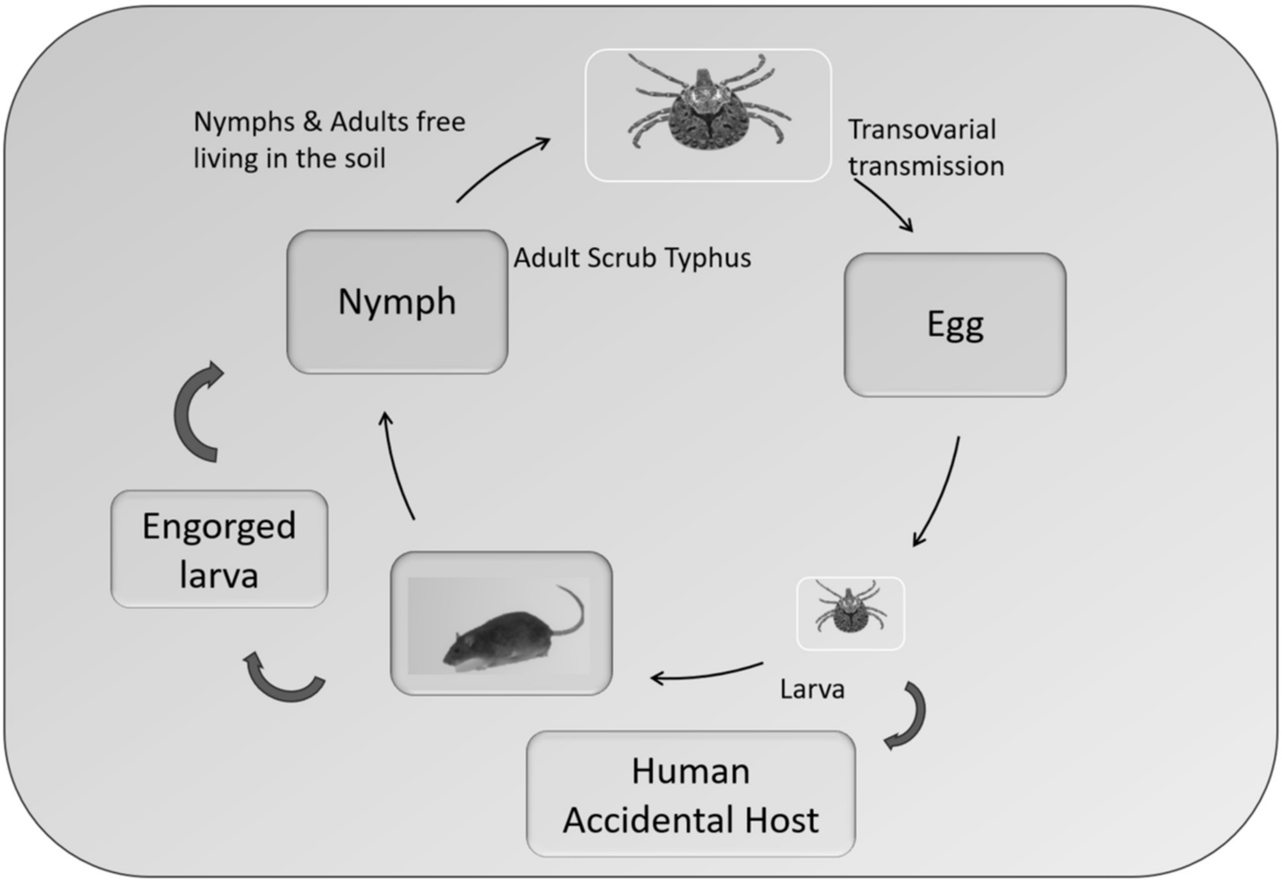

Figure 5. Lifecycle of the *Leptotrombidium* mite. Scrub typhus is spread from one individual to another by the bite of a mite (termed a 'chigger') infected with *Orientia tsutsugamushi*. Only the larval stage, commonly termed the chigger, is capable of transmitting the disease. This is due to the fact that in the nymph and adult stages, the mites do not feed on vertebrate animals.

Finally, tritonymphs develop into eight-legged adults. Mites in the prelarval, protonymphal and tritonymphal stages are inactive and do not feed. By contrast, mites in the deutonymphal and adult stages are non-parasitic and typically feed on arthropod eggs or small arthropods (52). After reaching adulthood, these mites become free-living predators in the soil, primarily feeding on the eggs of other arthropods. The life cycle of chiggers (genus *Leptotrombidium*) is illustrated in Fig. 5.

5. Clinical manifestations of scrub typhus

The symptoms of scrub typhus can vary from mild fever to severe illness, life-threatening cases involving multi-organ dysfunction syndrome (MODS) (53). Common symptoms include fever, gastrointestinal issues, fatigue, cough, muscle pain and headache. A maculopapular rash typically develops by the end of the first week, beginning on the trunk and spreading to the limbs. Regional lymph node swelling is often observed, and an eschar at the bite site is considered highly indicative of scrub typhus. The reported prevalence of eschars in patients varies from 7 to 80%, which may be attributed to the difficulty in identifying small eschars in individuals with darker skin, differences in the eschar-forming ability of various *Orientia tsutsugamushi* strains, and atypical eschar appearance in moist or damp areas of the skin (21).

Eschars typically begin as small papules that gradually enlarge and develop central necrosis, forming a distinctive black lesion. Common areas for eschar development include the groin, armpits, waist and other exposed body parts (54). Eschars were commonly found on the chest and within 30 cm of the navel in both males and females. However, males more commonly had eschars on their lower limbs, while females often had them on their backs. These patterns may differ across populations due to variations in gender-related clothing and outdoor activities (24).

Systemic symptoms of scrub typhus may emerge within the second week of infection, particularly in untreated cases. Multiple organ systems can be involved, including the heart, kidney, lungs and brain.

6. Association of various body systems with scrub typhus

Central nervous system and scrub typhus. Scrub typhus, a rickettsial disease, can cause neurological symptoms such as tremors, nervousness and delirium (55). The central nervous system is frequently affected in patients with Rocky Mountain spotted fever and other rickettsial illnesses. A small number of patients also exhibit symptoms of encephalitis and meningitis. In a previous study on 1,650 febrile American servicemen in Vietnam, direct fluorescent antibody testing identified scrub typhus in 109 cases; Berman and Kund (56) found mononuclear cells in cerebrospinal fluid samples from several patients with scrub typhus, who also exhibited generalized lymphadenopathy and lymphocytosis. Meningitis or meningoencephalitis has been observed in 14 to 83% of individuals with scrub typhus (57). Acute disseminated encephalomyelitis was also reported in patients with Scrub typhus (58). An elderly individual with scrub typhus exhibited ptosis (drooping of the upper eyelid) and ophthalmoplegia (ophthalmoplegia) (59). Ptosis can be present from birth (congenital) or later in life (acquired) suggesting involvement of multiple cranial nerves in this disease. Hearing loss accompanied by fever is a common symptom in up to one-third of individuals with scrub typhus (60). Research has shown that cerebellitis can occur in individuals with scrub typhus involving the nervous system; Rana *et al* (61) reported cerebellitis in 11% of such cases, either in isolation or alongside generalized neurological symptoms.

Cardiovascular system. Potential issues include rhythm disturbances, myocardial involvement leading to congestive heart failure and vasculitis. Scrub typhus has been linked to acute myocardial infarction (55). However, heart failure due to this infection is less common. In another study, Ray *et al* (62) documented a case of acute heart failure in a female patient. The patient exhibited elevated NT-proBNP levels, right heart enlargement and bilateral pulmonary edema. A Weil-Felix test and IgM antibodies for scrub typhus both yielded positive results, confirmed by the diagnosis. Previous literature may have underestimated the prevalence of heart failure syndrome in acute scrub typhus infections (63). Of note, two prospective cohort studies, employing echocardiography and cardiac biomarker analysis, found that a significant proportion of patients (30.9-42.8%) exhibited a reduced ejection fraction and elevated levels of troponin T or creatine kinase-muscle/brain isoenzyme (61.7-72.8%) (64-65). Furthermore, the severity of systolic dysfunction was associated with elevated cardiac biomarker levels (64-65). These findings emphasize the significance of recognizing heart failure as a possible complication of scrub typhus.

Scrub typhus can increase the risk of developing atrial fibrillation, a common heart rhythm condition. This is likely due to the inflammation it causes. The inflammation can damage heart muscle cells, leading to cell death and scarring. It can also disrupt the electrical signals in the heart, causing irregular rhythms (66,67).

Renal system. The link between scrub typhus and acute kidney failure has also been observed. In India, Between September, 2011 and November, 2012, 49 of 201 patients tested were diagnosed with scrub typhus through nested PCR (68). The average age of these patients was 34 years (range, 11-65 years). The majority of the patients were male, and the majority of the cases were found in the rainy season. In addition, 82% of the patients experienced renal complications, including acute kidney infection (53% of patients). Urinalysis abnormalities included albuminuria (55%) and microscopic hematuria (16%) (68). Jaundice was associated with acute kidney infection. A total of 8 patients succumbed, including 3 patients requiring dialysis. Oliguria, acute respiratory distress syndrome (ARDS) and acute kidney injury were linked to mortality (68).

Scrub typhus can lead to acute kidney injury through several mechanisms (69-72). In another study, the new biomarker link with acute kidney injury was shown with 138 patients being included (73). Among these 138 patients, 25 patients developed scrub typhus-associated acute kidney injury. Several novel biomarkers for acute kidney injury were evaluated, including neutrophil gelatinase-associated lipocalin and kidney injury molecule 1. These results suggest that these biomarkers may be valuable tools for diagnosing and monitoring acute kidney injury in individuals with scrub typhus (73).

Respiratory system. Complications can include interstitial pneumonia and ARDS. The case study of a 52-year-old female patient with ARDS was published in 2015 in the 'Journal of Clinical and Diagnostic Research' (74). The patient was admitted to the intensive care unit with clinical symptoms of high fever (39˚C), chills, cough and shortness of breath, a heart rate of 134/min and a respiratory rate of 36/min. The patient presented with decreased breath sounds and crackles in both lungs, indicative of respiratory distress. Blood gas analysis confirmed type I respiratory failure. Chest imaging revealed bilateral alveolar shadows consistent with ARDS. A physical examination identified a characteristic eschar on the right thigh, a key diagnostic sign for scrub typhus. A positive Weil-Felix test, along with the exclusion of other febrile illnesses, confirmed the diagnosis of scrub typhus complicated by ARDS. Doxycycline treatment was initiated (74).

Gastrointestinal system. Gastrointestinal manifestations of scrub typhus are less commonly described in the literature. Typical symptoms include abdominal pain, the vomiting of blood, and diarrhea. Of note, 2 cases were reported of peritonitis associated with scrub typhus, which highlights the importance of considering scrub typhus in the differential diagnosis of peritonitis in regions where *Orientia tsutsugamushi* is endemic. Until 2009, no case was reported of scrub typhus associated with peritonitis. In 2010, A 71-year-old male patient complained of abdominal pain with a high temperature (39˚C), pulse rate, 110/min; respiration rate, 26/min. Indirect immunofluorescence assay and clinical diagnostic test and eschar in the left axillar confirmed the diagnosis of scrub typhus. The patient was treated with doxycycline for scrub typhus and antibiotics for peritonitis (75).

In severe cases, MODS may occur. Due to the wide range of possible symptoms, diagnosing scrub typhus can be difficult, often leading to delays or missed diagnoses.

7. Laboratory methods for the detection of scrub typhus

Historically, the diagnosis of scrub typhus relied on serological methods; however, the integration of DNA-based pathogen detection, particularly during the early stages of infection, has led to the combined use of PCR and serology for the diagnosis of the majority of rickettsial diseases. The outdated Weil-Felix OX-K agglutination test, which was developed in 1916 was replaced by the indirect immunofluorescence assay (IFA) in the 1960s (76-78). Over time, the IFA was refined with new positivity criteria during the 1970s and 1980s, and it remained the gold standard for decades (79). However, inconsistencies in standardizing antigens and criteria led to the introduction of dynamic titer increase (a fourfold rise in IgM or IgG levels) as a more reliable marker for diagnosis. ELISAs using either culture-derived *Orientia tsutsugamushi* antigens or recombinant proteins have improved the diagnostic accuracy and allow for higher throughput compared to the more labor-intensive IFA and immunoperoxidase tests (80). Rapid diagnostic tests (RDTs) targeting anti-Orientia IgM and IgG antibodies are currently under evaluation, with hopes that future advancements will include antigen-capture-based RDTs for early detection at the point of care (81). However, pre-existing IgM and IgG antibodies in populations residing in endemic areas can complicate serological diagnoses during the acute phase by causing false-positive results. This is particularly problematic when a convalescent serum sample is unavailable to confirm a dynamic rise in antibody titers (82).

The introduction of PCR testing has revolutionized the diagnostic landscape for rickettsial diseases. PCR provides greater diagnostic accuracy and has expanded the diagnostic window to earlier stages of infection (83). Commonly targeted genes in PCR assays include the 56-kDa, 47-kDa, 16S rRNA, and groEL genes. This combined diagnostic approach has led to improved treatment outcomes by ensuring the timely administration of anti-rickettsial therapy, reducing both morbidity and mortality rates. It has also provided stronger evidence for diagnostic and vaccine research. The time from symptom onset to medical presentation, often referred to as 'days of fever before admission', is a critical indicator of disease progression and informs the choice of diagnostic methods. Bacteraemia in scrub typhus can persist for up to 10 days after the onset of fever, while the antibody response in individuals with no prior exposure typically takes 7 to 10 days to develop. For optimal diagnosis, both PCR and serological tests (such as RDT or ELISA) are recommended (84).

When accessible, eschar swabs or crust samples serve as valuable non-invasive diagnostic specimens for scrub typhus. These samples typically contain high bacterial loads, which are less influenced by the bloodstream, allowing for accurate molecular detection, even in the later stages of the disease and after treatment has begun. Culturing *Orientia* species from blood is challenging due to the fastidious growth requirements of the bacteria, often taking several weeks to yield results. This process necessitates the use of specialized cell culture techniques and biosafety level 3 laboratories for safety and accuracy (85).

Loop-mediated isothermal amplification (LAMP) is a DNA amplification technique that employs three specially designed primer pairs and Bst DNA polymerase (86). Unlike PCR, LAMP does not require complex DNA extraction or temperature cycling. The entire reaction occurs at a constant temperature, thus rendering it suitable for simple equipment such as a water bath or heating block. The LAMP results can be visually assessed, with a positive reaction indicated by a white pellet, eliminating the need for specialized equipment. A small preliminary study involving 9 patients demonstrated the sensitivity of LAMP in detecting DNA levels as low as 14 copies/μl, compared to 3 copies/μl for real-time PCR (84). However, further validation through prospective clinical trials is necessary to establish the reliability of LAMP in a clinical setting.

Multi-locus sequence typing (MLST) is a technique used to analyze multiple genetic loci, providing a more comprehensive understanding of evolutionary associations between isolates compared to single-gene genotyping methods like the 56-kDa genotype analysis. MLST employs 'housekeeping' or conserved genes to minimize the impact of selective pressure and recombination on phylogenetic relationships. Notably, MLST analyses were not performed on the genomes of *Orientia tsutsugamushi* Boryong and Ikeda, despite their sequencing (87,88). While MLST is commonly used to classify other microorganisms, its application to *Orientia tsutsugamushi* has been relatively limited (51).

8. Immune response in scrub typhus

The immune defense against *Orientia* involves both humoral and cell-mediated responses. While the humoral response mainly offers protection against the same strain with minimal cross-protection against others, the cell-mediated response provides short-term protection against different strains; however, this protection diminishes over time. The spreading of the pathogen throughout the body is a process likened to a 'Trojan horse' effect (89). This bacteremia phase aligns with the onset of fever and typically lasts ~10 days. However, the immune response to scrub typhus is short-lived and offers limited cross-protection between different strains. Immunity acquired after infection can diminish within months, rendering individuals vulnerable to symptomatic illness upon re-exposure to different strains, although immunity to the same strain may last for >1 year (90).

Understanding the balance between these immune mechanisms and how they influence bacterial spread is essential for the development of an effective vaccine (91). With timely treatment, the majority of patients with scrub typhus experience fever resolution within 48 h. However, regions such as northern Thailand and southern India have reported delayed fever clearance and high fatality rates of 12-13%. Ongoing research is investigating the potential contribution of antibiotic resistance in *Orientia* species to adverse clinical outcomes in affected regions (92).

Orientia tsutsugamushi, the causative agent of scrub typhus, initiates infection by binding to the heparin sulfate proteoglycan receptor, specifically syndican-4, expressed on dermal cells (93). Upon entry into the dermis, the bacteria are phagocytosed by dendritic cells (macrophages), leading to the upregulation of MHC class II and co-stimulatory markers (CD40, CD80, CD86 and CD83) (94). This triggers mononuclear cell infiltration in the dermis.

Once in the bloodstream, *Orientia tsutsugamushi* activates the type-1 interferon pathway and MAPK signaling, resulting in the production of IFN-β. Additionally, NF-κB nuclear translocation suggests its involvement in inducing chemokine production. MAPK pathways also contribute to the upregulation of TNF-α. Notably, *Orientia tsutsugamushi* can manipulate host immune responses by increasing the production of immunosuppressive molecules, which inhibit TNF-α production and enhance bacterial survival within phagocytic cells (95). Dendritic cells and monocytes act as vehicles for spreading *Orientia tsutsugamushi,* while circulating to the lymph nodes (89).

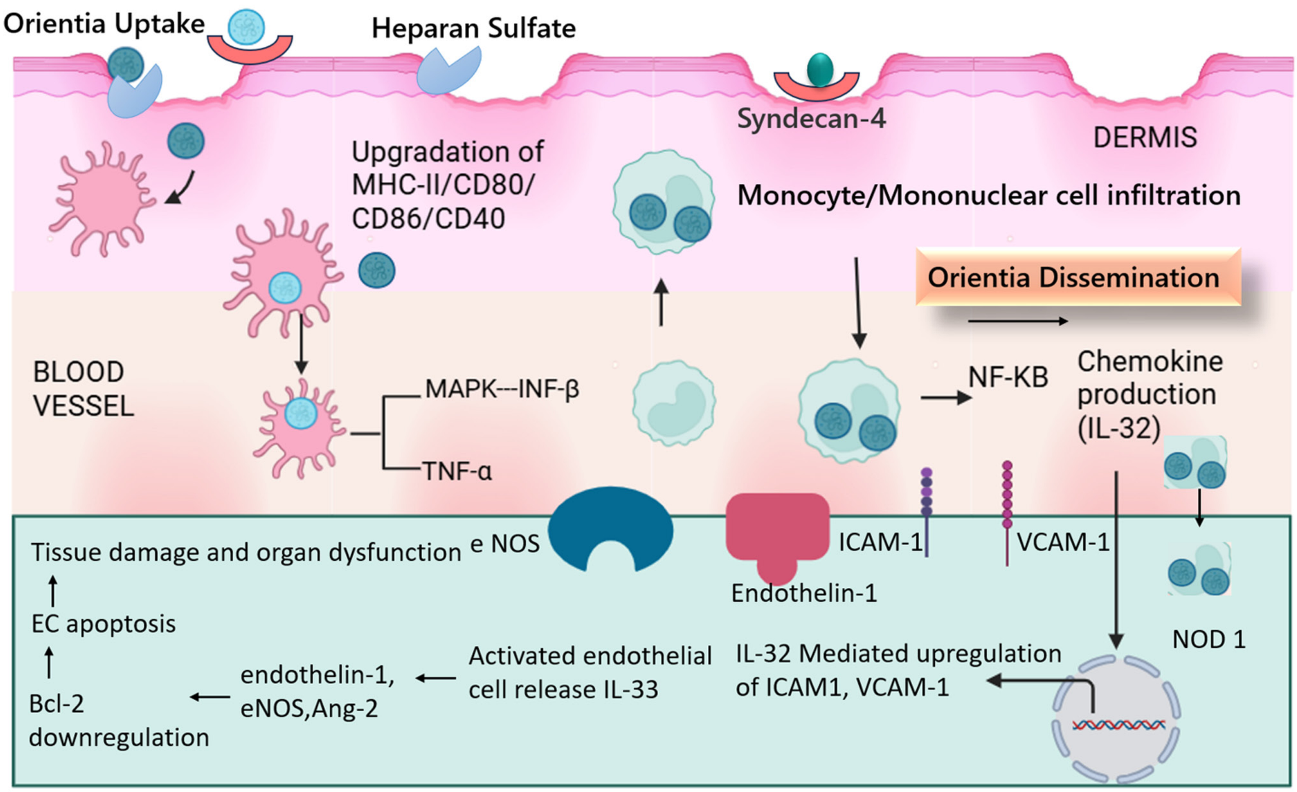

Figure 6. The proposed mechanisms of *Orientia tsutsugamushi* infection in human and organ dysfunction. *Orientia tsutsugamushi* infection of endothelial cells triggers intracellular recognition by NOD1. This activation stimulates the infected endothelium, leading to IL-32-mediated upregulation of inflammatory and adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin. The enhanced expression of adhesion molecules and endothelial cell activation alters the balance of angiogenic factors, increasing the Ang2/Ang1 ratio. Additionally, endothelial nitric oxide synthase and endothelin-1 levels are dysregulated. This complex interplay is further influenced by the intranuclear expression and release of IL-33, which contributes to increased endothelin-1 and Ang-2 levels. Ultimately, these cellular events culminate in the downregulation of the anti-apoptotic protein Bcl-2, inducing apoptosis of renal endothelial cells and subsequent renal tissue damage. EC, endothelial cell; eNOS, endothelial nitric oxide synthase.

The bacteria subsequently spread to various organs, including the kidneys and liver, heart and lungs, where they enter endothelial cells. The recognition of *Orientia tsutsugamushi* by nucleotide-binding oligomerization domain-like receptors in kidney endothelial cells triggers a cascade of events. The upregulation of adhesion molecules, such as ICAM-1, VCAM-1 and ALCAM, facilitates leukocyte adhesion and trafficking, contributing to disease severity (96). Furthermore, the increased expression of angiopoietin (Ang)-2 and the elevated Ang-2/Ang-1 ratio, along with the production of endothelin-1 and endothelial nitric oxide synthase, promote endothelial activation (97). The upregulation of IL-33 and its receptor in the liver and kidneys further exacerbates endothelial cell activation and dysfunction, leading to significant tissue damage and organ impairment (98). The proposed mechanisms of *Orientia tsutsugamushi* infection in humans and the subsequent immune response to renal cell damage are depicted in Fig. 6.

9. Therapeutic and prevention strategies for scrub typhus

Tetracycline, doxycycline, azithromycin and rifampicin are effective treatments for scrub typhus with low rates of treatment failure. Limited evidence suggests a minimal to no difference between tetracycline, doxycycline and azithromycin. Due to low-certainty evidence and the risk of inducing resistance in undiagnosed tuberculosis, rifampicin should not be a first-line treatment. It may be considered a second-line option after ruling out active tuberculosis (99).

Scrub typhus is effectively treated with antibiotics, such as doxycycline (preferred), azithromycin or chloramphenicol. Early empirical therapy is recommended based on clinical suspicion, as fever reduction is often observed within 48 h of initiating doxycycline. The standard treatment regimen consists of doxycycline 100 mg taken orally twice a day for 7 days. Tetracycline, although less commonly used, may be an alternative treatment at a dose of 500 mg every 6 h for 7 days (99).

Azithromycin, which has comparable effectiveness to doxycycline, is commonly prescribed as a 3-day regimen. This typically involves an initial dose of 1,000 mg or 500 mg, followed by 500 mg or 250 mg for the following 2 days. Azithromycin is the preferred option for pregnant women and children, due to concerns over the side-effects associated with doxycycline and tetracyclines. Chloramphenicol, another effective alternative to doxycycline, is administered at a dose of 500 mg every 6 h for adults, or 50 to 75 mg/kg/day in children for 7 days. However, its use has declined due to its potential for hematological side-effects (1 in 21,600 cases) and the rare occurrence of gray baby syndrome in premature infants, which can cause circulatory collapse (100).

For delayed responses to treatment, the combined use of doxycycline and either azithromycin or chloramphenicol may be effective, as it can shorten the fever clearance time and reduce complications, particularly in pregnant women. There have been no reported negative interactions between these drug combinations. When combining treatments with rifampicin, dosage adjustments should be made to prevent reduced drug levels.

For patients with suspected typhoid fever or typhus, high-dose azithromycin (in the typhoid treatment range) or chloramphenicol can be considered, as it covers both infections. For uncomplicated cases of scrub typhus, shorter treatment regimens have been explored; 3-day courses of doxycycline have shown similar effectiveness to the traditional 7-day regimens, with comparable fever resolution and no treatment failures. In areas such as northern Thailand, where doxycycline resistance has been reported, azithromycin remains an effective alternative, despite slower fever clearance times.

Antibiotic resistance. Scrub typhus has historically received limited clinical attention due to the availability of effective treatments (99). While chloramphenicol and tetracyclines, particularly doxycycline, remain effective, concerns about potential side-effects in specific populations, such as pregnant women and children, have prompted the exploration of alternative therapies (101,102). Macrolides, quinolones and rifampicin have emerged as promising options, although their efficacy may vary. A study in 1996 reported that scrub typhus patients from Chiangrai, Thailand, responded poorly to standard doxycycline treatment compared to those from western Thailand and Vietnam (92). This slower response was not attributed to poor drug absorption, but was hypothesized to be due to either more virulent or resistant strains of *Orientia tsutsugamushi*. Subsequent analysis identified a doxycycline-resistant isolate (C3) and a partially resistant isolate (C27) from Chiangrai. While the resistant strains exhibited reduced virulence in the absence of the drug, the strains maintained their ability to invade cells even in the presence of doxycycline (92).

Of note, two potential mechanisms have been proposed for the emergence of resistant strains. The first, though less likely, involves the persistence of dormant organisms in patients undergoing prolonged antibiotic therapy. However, the transmission of such resistant strains to chiggers, the primary reservoir of infection, remains unclear. The second mechanism involves the potential development of drug resistance in chiggers due to antibiotic supplementation in poultry feed. Chiggers, feeding on rodents that consume this contaminated grain, could inadvertently ingest the antibiotics. If *Orientia tsutsugamushi* can be transmitted transovarially, this could lead to the emergence of drug-resistant strains in subsequent generations of chiggers, ultimately infecting humans.

A second *Orientia tsutsugamushi* strain, designated AFSC-4, was isolated from a patient in Kanchanaburi, western Thailand, in 1990. This strain, geographically distant from the Chiangrai isolates, exhibited reduced susceptibility to doxycycline (103). A study in 1995 investigated azithromycin's efficacy against Orientia strains, including a doxycycline-resistant isolate. It demonstrated that AFSC-4 (O. tsutsugamushi strain showing reduced doxycycline susceptibility) required a significantly higher concentration of doxycycline to inhibit growth compared to the reference strain Karp. The strain AFSC-4 required a 32-fold higher doxycycline concentration (0.25 mg/ml) to achieve an equivalent level of growth inhibition as observed in the control strain (Karp). This suggests a significant difference in drug susceptibility between the two strains (104). A study in 2000 conducted in northern Thailand investigated the efficacy of rifampicin in treating scrub typhus patients who responded poorly to standard doxycycline therapy (105). Previous research has demonstrated the superior activity of rifampicin against northern *Thai Orientia tsutsugamushi* strains compared to doxycycline (92). The results indicated that rifampicin was more effective than doxycycline, with more rapid fever clearance times and a lower relapse rate. These findings suggest that rifampicin may be a valuable alternative treatment option for scrub typhus infections in regions with drug-resistant strains.

Prevention of scrub typhus. Scrub typhus is one of the top 10 causes of acute, potentially life-threatening illnesses among travelers returning from tropical regions. With improved awareness among healthcare professionals and the availability of more effective diagnostic tools, there has been an increase in the identification of scrub typhus cases, including severe and fatal outcomes, among travelers. The majority of cases are contracted in Southeast Asia, where the disease-transmitting chigger mites are commonly found in scrublands and tall grasses of rural areas. This puts tourists, locals, and military personnel at risk of exposure (106,107).

The following precaution can be taken to avoid the bites of chiggers: Wearing clots having long sleeves, keeping clothes off the grass during sitting on the grass, using insect repellents applied to the skin, and clothing containing dibutyl phthalate, benzyl benzoate, diethyl toluamide, clearing the vegetation where rodents live, provide health education of the people regarding the modes of transmission.

Preventive measures primarily involve the use of insect repellents, such as N,N-diethyl-meta-toluamide, which offers effective but short-term protection against mites and ticks during travel in areas where the disease is endemic. Currently, no licensed vaccines are available for scrub typhus, rendering personal protective measures essential for reducing the risk of infection (108).

Scrub typhus symptoms often mimic other illnesses, rendering diagnosis challenging. In the case that an individual recently traveled to an endemic region and has experienced fever and an eschar (a small, dark scab), that individual should seek immediate medical attention. Early diagnosis and treatment are crucial for a full recovery. To prevent infection, travelers to endemic areas should take preventive measures to avoid mite bites. Some strategies to increase the awareness of scrub typhus and which may help to prevent scrub typhus are the following: i) The creation of informative brochures, posters and videos that explain the symptoms, causes, prevention and treatment of scrub typhus; ii) the organization of community events and workshops to educate the population about the disease, particularly in rural areas and regions with high incidence rates; iii) the use of social media platforms to share information, raise awareness and dispel myths about the disease; iv) the organization of CME programs to educate healthcare providers about the clinical presentation, diagnosis and management of scrub typhus; v) following the clinical guidelines for the diagnosis and treatment of scrub typhus.

10. Strategies to mitigate the impact of socioeconomic factors

The following strategies may be used to mitigate the impact of socioeconomic factors: i) Enhanced healthcare accessibility: This involves expanding healthcare infrastructure and services in rural regions which can facilitate the timely diagnosis and treatment of scrub typhus. ii) Public health education and awareness: This includes raising public awareness about the disease, its symptoms, preventive measures and treatment options, which can empower individuals to take proactive steps to reduce their risk of infection. iii) Vector control strategies: These involve implementing effective vector control measures, such as clearing vegetation and using appropriate insecticides, which can greatly reduce chigger populations and consequently, the transmission of scrub typhus. iv) Socioeconomic development and disease control: This involves addressing poverty, improving sanitation and promoting sustainable development, which can indirectly contribute to reducing the burden of scrub typhus. By addressing these underlying socioeconomic factors, a more resilient environment can be created and the impact of the disease can be minimized.

By comprehending the intricate association between socioeconomic factors and the prevalence of scrub typhus, comprehensive strategies can be formulated to prevent and control this disease, especially among vulnerable populations.

11. Conclusion and future perspectives

The incidence of scrub typhus and *Orientia tsutsugamushi* infection is continuously spreading globally. Scrub typhus, a potentially life-threatening illness presents several challenges in both diagnosis and treatment. The initial symptoms of scrub typhus, such as fever, headache and muscle aches, are often indistinguishable from other common illnesses such as flu or dengue fever. This can lead to misdiagnosis and delayed treatment. In a number of regions where scrub typhus is endemic, healthcare providers may not be aware of the disease or may not consider it in their differential diagnosis, further delaying appropriate care. While serological tests and PCR-based assays can confirm scrub typhus, these tests may not be readily available in all healthcare settings, particularly in resource-limited areas. There is growing concern about the emergence of drug-resistant strains of *Orientia tsutsugamushi*, which could render current antibiotics less effective. In some cases, scrub typhus can lead to severe complications such as encephalitis, pneumonia and multi-organ failure, which can be difficult to manage and may have long-term consequences. As aforementioned, delayed diagnosis can lead to delayed treatment, which can increase the risk of severe complications and death. Furthermore, there are limited public health resources. In a number of endemic regions, public health resources may be limited, rendering it difficult to implement effective prevention and control strategies. Addressing these limitations requires a multi-pronged approach, including increased awareness among healthcare providers and the public, improved diagnostic tools, the development of new and effective treatments, and strengthened vector control measures. By working together, the burden of scrub typhus can be reduced, and improve patient recovery outcomes.

Emerging evidence from Africa, the Middle East and South America indicates that scrub typhus is more prevalent than previously recognized. These regions, undergoing significant demographic, economic and ecological shifts, are particularly vulnerable. Recent events, such as the earthquake in Nepal, underscore the potential for environmental disturbances to exacerbate the risk of scrub typhus (109). To accurately assess the global burden of this disease, comprehensive and up-to-date mapping of human cases and chigger mite distribution is essential. Given the lack of reliable data, increased attention and research are urgently needed to inform effective health policies.

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Authors' contributions

RY and AV were involved in the conceptualization of the study and in the preparation of the original draft of the manuscript. VK, GP and PS were involved in the reviewing and editing of the manuscript. GK, SB and RY prepared the figures and supervised the study. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Walker DH: Scrub Typhus-scientific neglect, Ever-widening impact. N Engl J Med 375: 913-915, 2016.
2. Dasgupta S, Asish PR, Rachel G, Bagepally BS and Chethrapilly Purushothaman GK: Global seroprevalence of scrub typhus: A systematic review and Meta-analysis. Sci Rep 14: 10895, 2024.
3. Huang J, Deng K, Chen J and Zhang M: Epidemiological and clinical characteristics of scrub typhus in northern Fujian, China, from 2015 to 2019. BMC Infect Dis 23: 479, 2023.

4. Coinfection with severe fever with thrombocytopenia syndrome and scrub typhus in Korea-PubMed.
5. Wang Q, Ma T, Ding F, Lim A, Takaya S, Saraswati K, Sartorius B, Day NPJ and Maude RJ: Global and regional seroprevalence, incidence, mortality of, and risk factors for scrub typhus: A systematic review and meta-analysis. Int J Infect Dis 146: 107151, 2024.
6. Jiang J and Richards AL: Scrub typhus: No longer restricted to the tsutsugamushi triangle. Trop Med Infect Dis 3: 11, 2018.
7. Watt G and Parola P: Scrub typhus and tropical rickettsioses. Curr Opin Infect Dis 16: 429-436, 2003.
8. Chakraborty S and Sarma N: Scrub typhus: An emerging threat. Indian J Dermatol 62: 478, 2017.
9. Singh OB and Panda PK: Scrub Typhus. In: StatPearls. StatPearls Publishing, Treasure Island, FL, 2024.
10. Paris DH, Shelite TR, Day NP and Walker DH: Unresolved problems related to scrub typhus: A seriously neglected Life-threatening disease. Am J Trop Med Hyg 89: 301, 2013.
11. Bal M, Kar CR, Behera HK, Kar PC, Biswas S, Dixit S, Khuntia HK, Pati S and Ranjit M: Scrub typhus associated acute kidney injury: An emerging health problem in Odisha, India. J Vector Borne Dis 58: 359-367, 2021.
12. Shivalli S: Diagnostic evaluation of rapid tests for scrub typhus in the Indian population is needed. Infect Dis Poverty 5: 40, 2016.
13. Khan SA, Bora T, Laskar B, Khan AM and Dutta P: Scrub typhus leading to acute encephalitis syndrome, assam, india. Emerg Infect Dis 23: 148, 2017.
14. Kundavaram AP, Jonathan AJ, Nathaniel SD and Varghese GM: Eschar in scrub typhus: A valuable clue to the diagnosis. J Postgrad Med 59: 177-178, 2013.
15. Singh P: Scrub typhus, a case report : Military and regional significance. Med J Armed Forces India 60: 89-90, 2004.
16. Xu G, Walker DH, Jupiter D, Melby PC and Arcari CM: A review of the global epidemiology of scrub typhus. PLoS Negl Trop Dis 11: e0006062, 2017.
17. Mohapatra RK, Al-Haideri M, Mishra S, Mahal A, Sarangi AK, Khatib MN, Gaidhane S, Zahiruddin QS, Mohanty A and Sah R: Linking the increasing epidemiology of scrub typhus transmission in India and South Asia: Are the varying environment and the reservoir animals the factors behind? Front Trop Dis 5:1371905, 2024.
18. Sahu S, Misra SR, Padhan P and Sahu S: Scrub typhus in a tertiary care hospital in the eastern part of Odisha. Apollo Medicine 12: 2-6, 2015.
19. Agrawal A, Parida P, Rup AR, Patnaik S and Biswal S: Scrub typhus in paediatric age group at a tertiary care Centre of eastern India: Clinical, Biochemical Profile and Complications. J Family Med Prim Care 11: 2503-2506, 2022.
20. Seong SY, Choi MS and Kim IS: *Orientia tsutsugamushi* infection: Overview and immune responses. Microbes Infect 3: 11-21, 2001.
21. Devasagayam E, Dayanand D, Kundu D, Kamath MS, Kirubakaran R and Varghese GM: The burden of scrub typhus in India: A systematic review. PLoS Negl Trop Dis 15: e0009619, 2021.
22. Damodar T, Singh B, Prabhu N, Marate S, Gowda VK, Lalitha AV, Dsouza FS, Sajjan SV, Kariyappa M, Kinhal UV, *et al*: Association of scrub typhus in children with acute encephalitis syndrome and meningoencephalitis, southern India. Emerg Infect Dis 29: 711, 2023.
23. Garg D and Manesh A: Neurological facets of scrub typhus: A comprehensive narrative review. Ann Indian Acad Neurol 24: 849, 2021.
24. Varghese GM, Trowbridge P, Janardhanan J, Thomas K, Peter JV, Mathews P, Abraham OC and Kavitha ML: Clinical profile and improving mortality trend of scrub typhus in South India. Int J Infect Dis 23: 39-43, 2014.
25. Luo YY, Geater AF and Yin JX: The impact of meteorological parameters on the scrub typhus incidence in Baoshan City, western Yunnan, China. Front Public Health 12: 1384308, 2024.
26. Li Z, Xin H, Sun J, Lai S, Zeng L, Zheng C, Ray SE, Weaver ND, Wang L, Yu J, *et al*: Epidemiologic Changes of Scrub Typhus in China, 1952-2016. Emerg Infect Dis 26: 1091, 2020.
27. Peng PY, Duan HY, Xu L, Zhang LT, Sun JQ, Zu Y, Ma LJ, Sun Y, Yan TL and Guo XG: Epidemiologic changes of a longitudinal surveillance study spanning 51 years of scrub typhus in mainland China. Sci Rep 14: 3138, 2024.
28. Wangrangsimakul T, Elliott I, Nedsuwan S, Kumlert R, Hinjoy S, Chaisiri K, Day NPJ and Morand S: The estimated burden of scrub typhus in Thailand from national surveillance data (2003-2018). PLoS Negl Trop Dis 14: e0008233, 2020.
29. Yang S, Park G and Kim Y: Incidence of scrub typhus according to changes in geographic and demographic characteristic in the chungcheong region of Korea. Trop Med Infect Dis 9: 147, 2024.
30. Lu J, Liu Y, Ma X, Li M and Yang Z: Yang. Impact of meteorological factors and southern oscillation index on scrub typhus incidence in guangzhou, southern China, 2006-2018. Front Med (Lausanne) 8: 667549, 2021.
31. Pan K, Lin F, Xue H, Cai Q and Huang R: Exploring the influencing factors of scrub typhus in Gannan region, China, based on spatial regression modelling and geographical detector. Infect Dis Model 10: 28-39, 2025.
32. Kweon SS, Choi JS, Lim HS, Kim JR, Kim KY, Ryu SY, Yoo HS and Park O: Rapid increase of scrub typhus, south Korea, 2001-2006. Emerg Infect Dis 15: 1127, 2009.
33. Lee IY, Lim JW, Seo JH, Kim HC, Lee KJ, Yong TS, Lee WJ, Yu JR and Sim S: Geographical distribution and epidemiologic factors of chigger mites on apodemus agrarius during autumn in Korea. Korean J Parasitol 59: 473, 2021.
34. Dhimal M, Dumre SP, Sharma GN, Khanal P, Ranabhat K, Shah LP, Lal BK, Jha R, Upadhyaya BP and Acharya B: An outbreak investigation of scrub typhus in Nepal: Confirmation of local transmission. BMC Infect Dis 21: 193, 2021.
35. Yao H, Wang Y, Mi X, Sun Y, Liu K, Li X, Ren X, Geng M, Yang Y, Wang L, *et al*: The scrub typhus in mainland China: Spatiotemporal expansion and risk prediction underpinned by complex factors. Emerg Microbes Infect 8: 909-919, 2019.
36. Ding F, Wang Q, Hao M, Maude RJ, John Day NP, Lai S, Chen S, Fang L, Ma T, Zheng C and Jiang D: Climate drives the spatiotemporal dynamics of scrub typhus in China. Glob Chang Biol 28: 6618, 2022.
37. Banerjee A and Kulkarni S: *Orientia tsutsugamushi*: The dangerous yet neglected foe from the East. Int J Med Microbiol 311: 151467, 2021.
38. Phetsouvanh R, Sonthayanon P, Pukrittayakamee S, Paris DH, Newton PN, Feil EJ and Day NPJ: The diversity and geographical structure of *Orientia tsutsugamushi* strains from scrub typhus patients in Laos. PLoS Negl Trop Dis 9: e0004024, 2015.
39. Kumar A, Biswal M, Zaman K, Sharma N, Suri V and Bhalla A: Genetic diversity of *Orientia tsutsugamushi* strains from patients in north India. Int J Infect Dis 84: 131-135, 2019.
40. Kumar D and Jakhar SD: Emerging trends of scrub typhus disease in southern Rajasthan, India: A neglected public health problem. J Vector Borne Dis 59: 303-311, 2022.
41. Sadanandane C, Jambulingam P, Paily KP, Kumar NP, Elango A, Mary KA, Agatheswaran S, Sankari T and Mishra BB: Occurrence of *Orientia tsutsugamushi*, the etiological agent of scrub typhus in animal hosts and mite vectors in areas reporting human cases of acute encephalitis syndrome in the Gorakhpur Region of Uttar Pradesh, India. Vector Borne Zoonotic Dis 18: 539-547, 2018.
42. Gurung S, Pradhan J and Bhutia PY: Outbreak of scrub typhus in the North East Himalayan region-Sikkim: An emerging threat. Indian J Med Microbiol 31: 72-74, 2013.
43. Viswanathan S, Muthu V, Iqbal N, Remalayam B and George T: Scrub typhus meningitis in South India-a retrospective study. PLoS One 8: e66595, 2013.
44. Varghese GM, Janardhanan J, Mahajan SK, Tariang D, Trowbridge P, Prakash JA, David T, Sathendra S and Abraham OC: Molecular epidemiology and genetic diversity of *Orientia tsutsugamushi* from patients with scrub typhus in 3 regions of India. Emerg Infect Dis 21: 64-69, 2015.
45. Jain HK, Das A, Dixit S, Kaur H, Pati S, Ranjit M, Dutta A and Bal M: Development and implementation of a strategy for early diagnosis and management of scrub typhus: An emerging public health threat. Front. Public Health threat. Front Public Health 12: 1347183, 2024.
46. Sharma P, Kakkar R, Kaore S, Yadav V and Sharma RK: Geographical distribution, effect of season & life cycle of scrub typhus, 2010.
47. Lai CH, Huang CK, Chen YH, Chang LL, Weng HC, Lin JN, Chung HC, Liang SH and Lin HH: Epidemiology of acute q Fever, scrub typhus, and murine typhus, and identification of their clinical characteristics compared to patients with acute febrile illness in southern taiwan. J Formos Med Assoc 108: 367-376, 2009.
48. Acosta-Jamett G, Martínez-Valdebenito C, Beltrami E, Silva-de La Fuente MC, Jiang J, Richards AL, Weitzel T and Abarca K: Identification of trombiculid mites (Acari: Trombiculidae) on rodents from Chiloé Island and molecular evidence of infection with Orientia species. PLoS Negl Trop Dis 14: e0007619, 2020.

49. Chaisiri K, Linsuwanon P and Makepeace BL: The chigger microbiome: Big questions in a tiny world. Trends Parasitol 39: 696-707, 2023.
50. Shatrov AB: Stylostome formation in trombiculid mites (Acariformes: Trombiculidae). Exp Appl Acarol 49: 261-280, 2009.
51. Luce-Fedrow A, Lehman ML, Kelly DJ, Mullins K, Maina AN, Stewart RL, Ge H, John HS, Jiang J and Richards AL: A Review of Scrub Typhus (*Orientia tsutsugamushi* and Related Organisms): Then, Now, and Tomorrow. Trop Med Infect Dis 3: 8, 2018.
52. Lipovsky PJ: Studies of the food habits of postlarval stages of chiggers (Acarina, Trombiculidae). University of Kansas, Lawrence, Kan, 1954.
53. Li W, Huang L and Zhang W: Scrub typhus with multi-organ dysfunction syndrome and immune thrombocytopenia: A case report and review of the literature. J Med Case Rep 13: 358, 2019.
54. Sharma N, Biswal M, Kumar A, Zaman K, Jain S and Bhalla A: Scrub Typhus in a Tertiary Care Hospital in North India. Am J Trop Med Hyg 95: 447-451, 2016.
55. Mahajan SK and Mahajan SK: Neuropsychiatric manifestations of scrub typhus. J Neurosci Rural Pract 8: 421-426, 2017.
56. Berman SJ and Kundin WD: Scrub typhus in South Vietnam. A study of 87 cases. Ann Intern Med 79: 26-30, 1973.
57. Basu S and Chakravarty A: Neurological manifestations of scrub typhus. Curr Neurol Neurosci Rep 22: 491-498, 2022.
58. Chen PH, Hung KH, Cheng SJ and Hsu KN: Scrub typhus-associated acute disseminated encephalomyelitis. Acta Neurol Taiwan 15: 251-254, 2006.
59. Kim J, Kim JS, Shin DI, Lee SH, Lee SS and Choi SY: Ophthalmoplegia due to scrub typhus. J Neuroophthalmol 35: 284-286, 2015.
60. Mahajan SK and Bakshi D: Acute reversible hearing loss in scrub typhus. J Assoc Physicians India 55: 512-514, 2007.
61. Rana A, Mahajan SK, Sharma A, Sharma S, Verma BS and Sharma A: Neurological manifestations of scrub typhus in adults. Trop Doct 47: 22-25, 2017.
62. Ray A, Nangia V, Chatterji RS and Dalal N: Scrub typhus infection presenting as acute heart failure: A case report and systematic review of literature of cardiopulmonary involvement in scrub typhus infection. Lung India 33: 439, 2016.
63. Zhang M, Zhao ZT, Wang XJ, Li Z, Ding L and Ding SJ: Scrub typhus: Surveillance, clinical profile and diagnostic issues in Shandong, China. Am J Trop Med Hyg 87: 1099-1104, 2012.
64. Pannu AK, Debnath MK, Sharma N, Biswal M, Vijayvergia R, Bhalla A, Kaur J and Kumar S: Circulating cardiac biomarkers and echocardiographic abnormalities in patients with scrub typhus: A prospective cohort study from a tertiary care center in North India. J Vector Borne Dis 58: 193-198, 2021.
65. Karthik G, Sudarsan TI, Peter JV, Sudarsanam T, Varghese GM, Kundavaram P, Sathyendra S, Iyyadurai R and Pichamuthu K: Spectrum of cardiac manifestations and its relationship to outcomes in patients admitted with scrub typhus infection. World J Crit Care Med 7: 16-23, 2018.
66. Jang SY, Kang KW, Kim JH, Kim B, Chin JY, Park SH, Choi YJ, Jung KT and Lee SK: New-onset atrial fibrillation predicting for complicating cardiac adverse outcome in scrub typhus infection. Clin Cardiol 42: 1210-1221, 2019.
67. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, Tracy RP, Van Wagoner DR, Psaty BM, Lauer MS and Chung MK: Inflammation as a risk factor for atrial fibrillation. Circulation 108: 3006-3010, 2003.
68. Kumar V, Kumar V, Yadav AK, Iyengar S, Bhalla A, Sharma N, Aggarwal R, Jain S and Jha V: Scrub typhus is an under-recognized cause of acute febrile illness with acute kidney injury in India. PLoS Negl Trop Dis 8: e2605, 2014.
69. Yen TH, Chang CT, Lin JL, Jiang JR and Lee KF: Scrub typhus: A frequently overlooked cause of acute renal failure. Ren Fail 25: 397-410, 2003.
70. Thap LC, Supanaranond W, Treeprasertsuk S, Kitvatanachai S, Chinprasatsak S and Phonrat B: Septic shock secondary to scrub typhus: Characteristics and complications. Southeast Asian J Trop Med Public Health 33: 780-786, 2002.
71. Upadhyaya A, Alam MR, Raeen AA, Upadhyaya S, Pathania M, Upadhyaya S and Sivanu K: Scrub Typhus Meningoencephalitis: An Overlooked Entity. Cureus 14: e28989, 2022.
72. Young PC, Hae CC, Lee KH and Hoon CJ: Tsutsugamushi infection-associated acute rhabdomyolysis and acute renal failure. Korean J Intern Med 18: 248-250, 2003.
73. Sun IO, Shin SH, Cho AY, Yoon HJ, Chang MY and Lee KY: Clinical significance of NGAL and KIM-1 for acute kidney injury in patients with scrub typhus. PLoS One 12: e0175890, 2017.
74. Sankuratri S, Kalagara P, Samala KB, Veledandi PK and Atiketi SB: Scrub typhus with acute respiratory distress syndrome (ARDS) and its management in intensive care unit: A case report. J Clin Diagn Res 9: OD10-OD11, 2015.
75. Lee CH, Lee JH, Yoon KJ, Hwang JH and Lee CS: Peritonitis in patients with scrub typhus. Am J Trop Med Hyg 86: 1046-1048, 2012.
76. Bozeman FM and Elisberg BL: Serological diagnosis of scrub typhus by indirect immunofluorescence. Proc Soc Exp Biol Med 112: 568-573, 1963.
77. Oberhansli G: Contribution to the serological diagnosis of classic and murine stain fever. Schweiz Z Pathol Bakteriol 10: 545-564, 1947 (In German).
78. Fletcher W: Typhus-like Fevers of Unknown Ætiology, with special reference to the malay states. Proc R Soc Med 23: 1021-1030, 1930.
79. Brown GW, Shirai A, Rogers C and Groves MG: Diagnostic criteria for scrub typhus: Probability values for immunofluorescent antibody and Proteus OXK agglutinin titers. Am J Trop Med Hyg 32: 1101-1107, 1983.
80. Robinson DM, Brown G, Gan E and Huxsoll DL: Adaptation of a microimmunofluorescence test to the study of human Rickettsia tsutsugamuskh antibody. Am J Trop Med Hyg 25: 900-905, 1976.
81. Blacksell SD, Bryant NJ, Paris DH, Doust JA, Sakoda Y and Day NPJ: Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: A lack of consensus leads to a lot of confusion. Clin Infect Dis 44: 391-401, 2007.
82. Blacksell SD, Tanganuchitcharnchai A, Nawtaisong P, Kantipong P, Laongnualpanich A, Day NPJ and Paris DH: Diagnostic accuracy of the InBios scrub typhus detect enzyme-linked immunoassay for the detection of IgM antibodies in northern Thailand. Clin Vaccine Immunol 23: 148-154, 2016.
83. Blacksell SD, Paris DH, Chierakul W, Wuthiekanun V, Teeratakul A, Kantipong P and Day NPJ: Prospective evaluation of commercial antibody-based rapid tests in combination with a loop-mediated isothermal amplification PCR assay for detection of *Orientia tsutsugamushi* during the acute phase of scrub typhus infection. Clin Vaccine Immunol 19: 391-395, 2012.
84. Paris DH, Blacksell SD, Nawtaisong P, Jenjaroen K, Teeraratkul A, Chierakul W, Wuthiekanun V, Kantipong P and Day NP: Diagnostic accuracy of a loop-mediated isothermal PCR assay for detection of *Orientia tsutsugamushi* during acute Scrub Typhus infection. PLoS Negl Trop Dis 5: e1307, 2011.
85. Paris DH and Dumler JS: State of the art of diagnosis of rickettsial diseases: The use of blood specimens for diagnosis of scrub typhus, spotted fever group rickettsiosis, and murine typhus. Curr Opin Infect Dis 29: 433-439, 2016.
86. Paris DH, Blacksell SD, Newton PN and Day NPJ: Simple, rapid and sensitive detection of *Orientia tsutsugamushi* by loop-isothermal DNA amplification. Trans R Soc Trop Med Hyg 102: 1239-1246, 2008.
87. Nakayama K, Kurokawa K, Fukuhara M, Urakami H, Yamamoto S, Yamazaki K, Ogura Y, Ooka T and Hayashi T: Genome comparison and phylogenetic analysis of *Orientia tsutsugamushi* strains. DNA Res 17: 281-291, 2010.
88. Wongprompitak P, Duong V, Anukool W, Sreyrath L, Mai TT, Gavotte L, Moulia C, Cornillot E, Ekpo P, Suputtamongkol Y, *et al*: *Orientia tsutsugamushi*, agent of scrub typhus, displays a single metapopulation with maintenance of ancestral haplotypes throughout continental South East Asia. Infect Genet Evol 31: 1-8, 2015.
89. Paris DH, Phetsouvanh R, Tanganuchitcharnchai A, Jones M, Jenjaroen K, Vongsouvath M, Ferguson DP, Blacksell SD, Newton PN, Day NP and Turner GD: *Orientia tsutsugamushi* in human scrub typhus eschars shows tropism for dendritic cells and monocytes rather than endothelium. PLoS Negl Trop Dis 6: e1466, 2012.
90. Valbuena G and Walker DH: Approaches to vaccines against *Orientia tsutsugamushi*. Front Cell Infect Microbiol 2: 170, 2012.
91. Mathai E, Rolain JM, Verghese GM, Abraham OC, Mathai D, Mathai M and Raoult D: Outbreak of scrub typhus in southern India during the cooler months. Ann N Y Acad Sci 990: 359-364, 2003.

92. Watt G, Chouriyagune C, Ruangweerayud R, Watcharapichat P, Phulsuksombati D, Jongsakul K, Teja-Isavadharm P, Bhodhidatta D, Corcoran KD, Dasch GA and Strickman D: Scrub typhus infections poorly responsive to antibiotics in northern Thailand. Lancet 348: 86-89, 1996.
93. Longley RL, Woods A, Fleetwood A, Cowling GJ, Gallagher JT and Couchman JR: Control of morphology, cytoskeleton and migration by syndecan-4. J Cell Sci 112: 3421-3431, 1999.
94. Song JH, Kang HB, Park SH, Jeong JH, Park J, You Y, Lee YH, Lee J, Kim E, Choi KC and Jun W: Extracts of Porphyra tenera (Nori Seaweed) activate the immune response in mouse RAW264.7 macrophages via NF-κB signaling. J Med Food 20: 1152-1159, 2017.
95. Netea MG, Simon A, van de Veerdonk F, Kullberg BJ, Van der Meer JWM and Joosten LAB: IL-1beta processing in host defense: Beyond the inflammasomes. PLoS Pathog 6: e1000661, 2010.
96. Page AV and Liles WC: Biomarkers of endothelial activation/dysfunction in infectious diseases. Virulence 4: 507-516, 2013.
97. Chousterman BG, Swirski FK and Weber GF: Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol 39: 517-528, 2017.
98. Shelite TR, Liang Y, Wang H, Mendell NL, Trent BJ, Sun J, Gong B, Xu G, Hu H, Bouyer DH and Soong L: IL-33-Dependent endothelial activation contributes to apoptosis and renal injury in *Orientia tsutsugamushi*-Infected mice. PLoS Negl Trop Dis 10: e0004467, 2016.
99. El Sayed I, Liu Q, Wee I and Hine P: Antibiotics for treating scrub typhus. Cochrane Database Syst Rev 9: CD002150, 2018.
100. Watt G, Kantipong P, Jongsakul K, Watcharapichat P and Phulsuksombati D: Azithromycin activities against *Orientia tsutsugamushi* strains isolated in cases of scrub typhus in Northern Thailand. Antimicrob Agents Chemother 43: 2817-2818, 1999.
101. Song JH, Lee C, Chang WH, Choi SW, Choi JE, Kim YS, Cho SR, Ryu J and Pai CH: Short-course doxycycline treatment versus conventional tetracycline therapy for scrub typhus: A multicenter randomized trial. Clin Infect Dis 21: 506-510, 1995.
102. Rajapakse S, Rodrigo C and Fernando SD: Drug treatment of scrub typhus. Trop Doct 41: 1-4, 2011.
103. Ives TJ, Manzewitsch P, Regnery RL, Butts JD and Kebede M: In vitro susceptibilities of Bartonella henselae, B. quintana, B. elizabethae, Rickettsia rickettsii, R. conorii, R. akari, and R. prowazekii to macrolide antibiotics as determined by immunofluorescent-antibody analysis of infected Vero cell monolayers. Antimicrob Agents Chemother 41: 578-582, 1997.
104. Strickman D, Sheer T, Salata K, Hershey J, Dasch G, Kelly D, Kuschner R: In vitro effectiveness of azithromycin against doxycycline-resistant and-susceptible strains of Rickettsia tsutsugamushi, etiologic agent of scrub typhus. Antimicrob Agents Chemother 39, 2406-2410, 1995.
105. Watt G, Kantipong P, Jongsakul K, Watcharapichat P, Phulsuksombati D and Strickman D: Doxycycline and rifampicin for mild scrub-typhus infections in northern Thailand: A randomised trial. Lancet 356, 1057-1061, 2000.
106. Jensenius M, Han PV, Schlagenhauf P, Schwartz E, Parola P, Castelli F, von Sonnenburg F, Loutan L, Leder K and Freedman DO; GeoSentinel Surveillance Network: Acute and potentially life-threatening tropical diseases in western travelers-a GeoSentinel multicenter study, 1996-2011. Am J Trop Med Hyg 88: 397-404, 2013.
107. Nachega JB, Bottieau E, Zech F and Van Gompel A: Travel-acquired scrub typhus: Emphasis on the differential diagnosis, treatment, and prevention strategies. J Travel Med 14: 352-355, 2007.
108. Jensenius M, Fournier PE and Raoult D: Rickettsioses and the international traveler. Clin Infect Dis 39: 1493-1499, 2004.
109. Basnyat B: Typhoid versus typhus fever in post-earthquake Nepal. Lancet Glob Health 4: e516-517, 2016.