Abstract
Scrub typhus is caused by Orientia tsutsugamushi (formerly Rickettsia) and is transmitted to humans by an arthropod vector of the Trombiculidae family (Leptotrombidium deliense and L. akamushi). It is the most common re-emerging Rickettsial infection in India and many other South East Asian countries. In fact, scrub typhus is confined geographically to the Asia Pacific region, a billion people are at risk and nearly a million cases are reported every year. Scrub typhus appears particularly to be distributed in the tsutsugamushi triangle which is distributed over a very wide area of 13 million km² bound by Japan in the east, through China, the Philippines, tropical Australia in the south, and west through India, Pakistan, possibly to Tibet to Afghanistan, and southern parts of the USSR in the north.

Eschar is the characteristic lesion that starts as a vesicular lesion at the site of mite feeding. Later, an ulcer forms with black necrotic center and an erythematous border along with regional lymphadenopathy. Other features are fever, maculopapular rash starting from the trunk, and spreading to the limbs. It may affect the central nervous system, cardiovascular system, renal, respiratory, and gastrointestinal systems. Serious complication in the form of myocarditis, pneumonia, meningoencephalitis, acute renal failure, gastrointestinal bleeding, and even acute respiratory distress syndrome may develop. Tetracycline or chloramphenicol remains the main stay of therapy.

Key Words: India, Orientia tsutsugamushi, Rickettsia, scrub typhus, South East Asia, Trombiculidae

Introduction
Scrub typhus, first described in Japan in 1899, caused by Orientia tsutsugamushi (formerly Rickettsia), is an acute infectious disease of variable severity that is transmitted to humans by an arthropod vector of the Trombiculidae family. It affects people of all ages including children. Humans are accidental hosts in this zoonotic disease. While scrub typhus is confined geographically to the Asia Pacific region, a billion people are at risk and nearly a million cases are reported every year.[1]

Mortality rates for scrub typhus range from <1% to 50% depending on proper antibiotic treatment, status of the individual infected, and the strain of O. tsutsugamushi encountered.[2]

During the Second World War, scrub typhus emerged out to be the most dreaded disease among the soldiers of the Far East. In India, scrub typhus broke out in an epidemic form in Assam and West Bengal during the Second World War. Gradually, the disease became prevalent in many parts of India.

Scrub typhus is endemic and re-emerging in eastern and southern parts of Asia.

History
The term “scrub” is used because of the type of vegetation (terrain between woods and clearings) that harbors the vector. The word “typhus” is derived from the Greek word “typhus,” which means “fever with stupor” or smoke.[1] “Tsutsuga” means small and dangerous and “mushi” means insect or mite.
Epidemiology and Distribution

Globally, over one billion people are at risk for scrub typhus and an estimated one million cases occur annually.[3]

Scrub typhus appears particularly to be distributed in the tsutsugamushi triangle, which is distributed over a very wide area of 13 million km² bound by Japan in the east, through China, the Philippines, tropical Australia in the south, and west through India, Pakistan, possibly to Tibet to Afghanistan, and southern parts of the USSR in the north. The disease is largely prevalent to southeastern and eastern parts of Asia; India, Pakistan, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, Thailand, and other islands in the region[4] [Figure 1].

In India, it is present in whole of the Shivalik ranges from Kashmir to Assam, Eastern and Western Ghats, and the Vindhyachal and Satpura ranges in the central part of India.

There were reports of scrub typhus outbreaks in Himachal Pradesh, Sikkim, and Darjeeling (West Bengal) during 2003–2004 and 2007.

This disease is also prevalent in areas such as sandy beaches, mountain deserts, and equatorial rain forests. Certain areas such as forest clearings, riverbanks, and grassy regions provide optimal conditions for the infected mites to thrive. These small geographic regions are high-risk areas for humans and have been called scrub typhus islands.

Season of Transmission

Transmission of scrub typhus disease occurs throughout the year in the tropical areas, whereas in the temperate zones, transmission is seasonal. Occurrence of *L. deliense* is influenced by rainfall, with more chiggers attached to the rodents in the wetter months of the year, which may be the reason for clustering of cases during the rainy season as shown by Gurung et al.[5] However, outbreaks have been reported during the cooler season in southern parts of India.[6]

Agent

*O. tsutsugamushi* is the agent of scrub typhus in India. It differs from other Rickettsiae in its antigenic structure. At least, eight serotypes are recognized.

Vector

The vectors of scrub typhus are *L. deliense* and *Leptotrombidium akamushi* which are present in most countries of the South-East Asian region and they are endemic in certain geographical regions of India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka, and Thailand. The vector mite is mostly present in diverse ecological niches such as equatorial rain forests, semi deserts, and subarctic terrains in the Himalayan regions. These ecological patches are called mite islands and within this there may be a limited area of intense transmission called typhus islands.

The infection is transmitted through the larval mites or “chiggers.” Only the larval stage takes a blood meal.[7]

Host

A number of small rodents, particularly wild rats of subgenus Rattus, are the natural hosts for scrub typhus as the rodents and acarine hosts do not succumb to the disease.[3] Thus, the field rodents and the vector mites act as a reservoir and between the two the infection perpetuates in nature.

Transmission and Life Cycle

The infection is transmitted to humans and rodents by some species of infective trombiculid mites (“chiggers,” *L. deliense*, and others), feeds on lymph and tissue fluid rather than blood. Once they are infected in nature by feeding on the body fluid of small mammals, including the rodents, they maintain the infection throughout their life stages and as adults, pass the infection on to their eggs in a process called transovarial transmission.

Similarly, the infection passes from the egg to the larva or adult in a process called transstadial transmission.

Rather than biting or piercing the skin, mite larvae prefer to insert their mouthparts down the hair follicles or pores. A large number of *O. tsutsugamushi* are present in the salivary glands of the larvae and these are injected into their host when they feed.[8]
Mode of Human Infection
Infection takes place when humans accidentally pick up an infective larval mite while walking, sitting, or lying on the infested ground. The adult mites have a four-stage lifecycle: egg, larva, nymph, and adult. The larva is the only stage (chigger) that can transmit the disease to humans and other vertebrates.

Agent
Rickettsiae are small pleomorphic organisms (0.3–0.5 mm × 0.8–1.5 mm), Gram-negative bacterium of family Rickettsiaceae adapted to obligate intracellular parasitism. They are either short rods or as cocci and occur singly, in pairs, in short chains, or filaments. They grow readily in the yolk sac of the embryonated egg. There are several serologically distinct strains.

They include heterogeneous strains classified into five major serotypes: (1) boryong, (2) Gilliam, (3) Karp, (4) Kato, and (5) Kawasaki.

In each geographic area, there are several genetic variations and these differ from the genetic variants in other regions. Differentiation of serotypes is important for laboratory diagnosis.

Pathogenesis
The severity of the disease depends on the strain of organism involved and also on the host.

*O. tsutsugamushi* invades endothelial cells to produce disseminated vasculitic and perivascular inflammatory lesions, which results in significant vascular leakage and ensuing end-organ injury of various organs such as lungs, heart, and kidney.[9]

It induces the formation of several cytokines such as granulocyte-colony-stimulating factor (CSF), macrophage-CSF, interferon γ, and tumor necrosis factor-α. The cytotoxic T-lymphocytes and the NK T-cells play an important role in destroying the infected host cells.

The organism downregulates the host defense mechanism by downregulating the GP-96 on the macrophages and the endothelial cells, which plays a prime role in antigen presentation, functioning of the dendritic cells, antibody production, and cross-priming of the immune system.

The immune response against *O. tsutsugamushi* is both humoral and cellular. Humoral immunity involves the production of strain-specific antibodies against the organism which might reduce the organisms’ capacity to enter cells by altering nonspecific attractions between infectious agents and target cells.[10]

T-lymphocytes are involved in cell-mediated immunity against *Orientia*, producing interferon γ by mononuclear cells in the peripheral blood.

Clinical Features
The average incubation period of *O. tsutsugamushi* in humans is 10–12 days, the onset of disease is characterized by fever, headache, myalgia, cough, and gastrointestinal symptoms. Patients often present with pyrexia of unknown origin (PUO).[11] The severity of the symptoms varies widely, depending on the susceptibility of the host, the virulence of the bacterial strain, or both.

The first sign of scrub typhus disease in patients is a vesicular lesion at the site of mite feeding, which later on becomes an eschar or an ulcer with regional lymphadenopathy. An eschar typically presenting with black necrotic center and an erythematous border is seen at the site of the chigger bite and is often found in the groin, axilla, genitalia, and neck. The prevalence of eschar in patients infected with scrub typhus ranges from 7% to 80%. It is the single most important clue for diagnosis and is a pathognomonic sign.

Eschar-inducing capacity of different strains of *O. tsutsugamushi* is variable. The detection rate of eschar depends on the skin color of the individual (difficult in dark-skinned people) and the anatomical location of the eschar, and also the type of clothing. Rarely, multiple eschars may be found, for example, under a trouser belt. Typically, eschar is formed at the time when symptoms are manifested.

Occurrence of eschar is rare in South-East Asian patients. Moreover, indigenous people of endemic areas commonly have a less severe illness, often without any rash or eschar.[12]

Cases from Korea have reported a high number of eschar,[12] but those from Thailand and Taiwan have reported very low incidence. This may be due to variation in serotypes. Chance of underdetection cannot be overruled.

Immunohistochemical staining of skin biopsy specimens, particularly that of eschars, is sensitive and specific, and this technique can be reliable for confirming the diagnosis of scrub typhus.[13,14]

Fever is the most common complaint starting abruptly and has the usual typhus accompaniments of suffused conjunctiva, severe headache, drowsiness, apathy, pain in the shins and other muscles, and more characteristically lymphadenopathy and hepatosplenomegaly.

At the end of the 1st week, there appears a maculopapular rash starting from the trunk and spreading to the limbs. At the end of the 2nd week, systemic symptoms ensue mostly involving the central nervous system, cardiovascular system, renal, respiratory, and gastrointestinal systems. Serious complication in the
form of myocarditis, pneumonia, meningoencephalitis, acute renal failure, and gastrointestinal bleeding may occur. The chances of developing acute respiratory distress syndrome are more in patients of scrub typhus who have higher white blood cell (WBC) counts, lower hematocrit, higher bilirubin levels, and delayed treatment with antibiotics.

An unusual presentation with acute abdomen is also known to occur, especially in patients coming from hyperendemic areas.[15]

According to Kim et al., the potential markers for developing complications are age (≥60 years), scrub typhus patients who present to the hospital without an eschar, and laboratory findings such as WBC counts >10,000/mm and serum albumin level ≤3.0 g/dL.[15]

Deafness, dysarthria, and dysphagia may occur, but are usually transient, although deafness can last for several months.[16] Patients with untreated disease remain febrile for about 2 weeks and have a long convalescence of 4–6 weeks thereafter.

Diagnosis

The presence of fever and eschar supports the diagnosis. Serology remains the mainstay of diagnosis. In primary infection with O. tsutsugamushi, a significant antibody titer is observed at the end of the 1st week, which are mainly IgM antibodies, whereas IgG antibodies appear at the end of the 2nd week.[17,18] In the case of re-infection with O. tsutsugamushi, IgG antibodies are detectable by day 6, with IgM antibody titers being variable.

The cheapest and most easily available serological test is the Weil-Felix (WF) test. The WF test has a high specificity but a low sensitivity and is based on the detection of antibodies to various Proteus species which contain cross-reacting antigenic epitopes to antigens from members of the genus Rickettsia with the exception of Rickettsia akari. The test is said to be positive when there is a titer of 1:320 or greater or a 4-fold rise in titer starting from 1:50.

The gold standard is indirect immunofluorescence antibody (IFA).[19] This detects the presence of scrub typhus-specific antibody bound to smears of scrub typhus antigen. This can confirm infection before their seroconversion.[20]

IFA is expensive, requires specialized laboratories and considerable training. The immunochromatographic test (ICT) to detect antibodies against O. tsutsugamushi also serves as a rapid diagnostic test which is available in some of the commercial laboratories in India.[21]

Indirect immunoperoxidase eliminates the expense of a fluorescent microscope by substituting peroxidase for fluorescein. Results are interpreted by an ordinary microscope. Hence, it can serve as a useful tool in an even resource-poor setup.[22]

Western immunoblot assay with sodium dodecyl sulfate-gel electrophoresed and electroblotted antigens, useful for large-scale screening, is a powerful and specific serodiagnostic tool for sero-epidemiology and confirmation of serologic diagnoses. It also helps in exploring the cross-reactive strain.[23]

A recombinant protein-based enzyme-linked immunosorbent assay using the most abundant and immunodominant protein for the detection of Orientia-specific antibodies in serum has been developed.

Three recombinant protein antigens were derived from four prototype strains of Karp and TA763 (r56C1), Kato (r56Kt), and Gilliam (r56Gm) Orientia are used.[24] It can be considered an improved, easy-to-operate, and cost-effective alternative to the gold standard IFA for acute diagnosis and seroprevalence.[25]

Culture

The samples which can be collected for isolation are buffy coat of heparinized blood, defibrinated whole blood, triturated clot, plasma, necropsy tissue, skin biopsy, and arthropod samples. The various methods adopted to identify the rickettsial strains are embryonated chicken yolk sacs, cell culture in Vero cells, MRC 5 cells, BHK21, L929 mouse fibroblast cell monolayer in tube culture, shell-vial assay, etc. Vero or L929 cells have been shown to allow better and faster isolation of Rickettsia while HEL or MRC5 cells prevent contact inhibition.[24]

Cell culture is time taking (an average of 4 weeks).

Polymerase chain reaction

Molecular detection using polymerase chain reaction (PCR) is possible from skin rash biopsies, lymph node biopsies, or ethylenediaminetetraacetic acid blood. GroEL-based real-time PCR assays are more sensitive and give a more quantitative assay.[29]

Loop isothermal amplification is a technique for amplifying DNA that makes use of three specially designed primer pairs and the Bst DNA polymerase. It is inexpensive and simple to perform. However, these methods need to be validated.[28] Nested PCR method is known to be 100 times more sensitive than performing single PCR. This is a fast method and takes only 24 h.[29]

Nested PCR technique can detect it as early as day 3 of the fever phase which is even before the appearance of antibody. Some studies suggest that nested PCR in conjunction with IFA may serve as a rapid and reliable method for diagnosing scrub typhus.

Eschar samples can be used for conducting PCR.
Leukocytosis and thrombocytopenia may be found. Elevated transaminases may be seen in 75%–95% of cases. Hypoalbuminemia and hyperbilirubinemia are not uncommon. In severe cases, there may be creatinine elevation. Ultrasonography may reveal liver and spleen enlargement. There may be pleural effusion and bilateral infiltrates in chest X-ray.

**Differential Diagnosis**

The differential diagnosis includes fever of unknown origin, typhoid fever, dengue hemorrhagic fever, malaria and other rickettsioses, tularemia, anthrax, dengue, leptospirosis, hemorrhagic fevers, and infectious mononucleosis. It may be considered as a possibility of PUO in endemic areas under strong background.

**Management**

Tetracycline or chloramphenicol remains the main stay of therapy in patients in whom scrub typhus is suspected. The recommended treatment regimen for scrub typhus is doxycycline (2.2 mg/kg/dose bid PO or intravenous [IV], maximum 200 mg/day for 7–15 days) and tetracycline (25–50 mg/kg/day divided every 6 h PO, maximum 2 g/day per oral, duration 7-15 days). For prophylaxis, 200 mg may be taken as a single dose. However, reports of natural resistance make choosing appropriate antibiotic difficult.[30]

Alternative regimens include chloramphenicol (50–100 mg/kg/day divided every 6 h IV, maximum 3 g/24 h, or 500 mg qid orally for 7–15 days for adults). If used, chloramphenicol should be monitored to maintain serum concentrations of 10-30 μg/mL. Therapy should be continued for a minimum of 5 days and until the patient has been afebrile for at least 3–4 days to avoid relapse. Chloramphenicol is best avoided during pregnancy and reduced doses should be given in hepatic impairment.

The other antibiotics which are found to be effective are azithromycin (500 mg orally for 3 days), rifampicin, and roxithromycin. Rifampicin has been shown to be superior to doxycycline in several studies. Roxithromycin (150 mg twice a day) was reported to be as effective as either doxycycline or chloramphenicol, suggesting a role as an alternative therapy for children or pregnant women.[9]

For short exposure, chemoprophylaxis with doxycycline (200 mg weekly) can prevent the disease but permits infection. Prophylaxis for scrub typhus with doxycycline has shown promising results when started before exposure to infection.

**Disease in High-risk Groups**

**Scrub typhus in pregnancy**

There have been reports of vertical transmission from transplacental infection and transmission in perinatal blood-borne infection during labor, causing neonatal scrub typhus in mothers with acute febrile illness during pregnancy.[31] It may be associated with increased fetal loss, preterm delivery, and small-for-gestational-age infants.[32] Chloramphenicol is a category C drug and may be used with caution during late trimester of pregnancy to prevent fetal transmission.[33,34] Doxycycline, a category D drug, is contraindicated in pregnant women. A retrospective case series was reported by Poomalar et al. who assessed eight cases of scrub typhus in pregnancy for the clinical features, complications, and maternal and neonatal outcomes.[35]

**Scrub typhus in children**

In children, scrub typhus may be mild or severe. Most patients present with fever and regional/generalized lymphadenopathy.[36] A single painless eschar, maculopapular rash hepatomegaly, splenomegaly, and gastrointestinal symptoms (abdominal pain, vomiting, and diarrhea) may be present. Case fatality rate in untreated patients may be as high as 30%, although deaths in children are infrequent.

**Indian scenario**

Scrub typhus was an endemic disease in many parts of India in the 1960s and 1970s. However, due to widespread use of insecticidal in the later years, it seemed to have virtually disappeared from our country. However, in the recent years, there had been resurgence and re-emergence of the disease in our country [Figure 2].[34,37-46]
Resurgence may be due to changes in the human behavior, unplanned urbanization, deforestation, and rapid transport leading to displacement of vectors as well as rodents from one place to another. Earlier, the habitat of the mite was restricted to the shrubs in hilly and forest terrains. However, recent studies have shown that rodents carrying the mite are transmitting the disease in the urban locales as well. Human host in urban areas may get bitten by the disease-causing mite while jogging in parks, doing yoga, or during any other recreational activities such as camping in the jungles.[45]

There have been outbreaks in areas located in the sub-Himalayan belt, from Jammu to Nagaland.[43]

The notable outbreaks reported from India in the last 10 years were Tamil Nadu (28 cases in 2001–2002), Himachal Pradesh (200 cases, 13 fatal in 2011), Nagaland (9 cases, 3 fatal in 2011), Meghalaya (80 cases, 5 fatal in 2010), and Puducherry in 2008.

In West Bengal, scrub typhus is mostly prevalent in the hilly forest belt. The subdivision Kurseong is full of thick vegetation, predominantly tea plantation. This presents a suitable habitat for the mite to survive in the grassy fields, shrubby areas, forests, tea plantation, and cleared forests.[67] Regular outbreaks of scrub typhus have been reported in the Integrated Disease Control Programme, West Bengal State Surveillance unit in Kurseong, Mirik (Darjeeling district of West Bengal), in 2010, 2011, and 2012. However, no incidence of fatality was reported, and all the cases were treated with doxycycline to which response was adequate.

The district of Darjeeling has also been historically considered as one of the scrub typhus-endemic areas in the country with scrub typhus outbreaks reported until the 1960s. Thereafter, for a long time, no outbreaks were reported which continued till 2005. The outbreaks of scrub typhus were reported in Darjeeling district of West Bengal in 2005 and subsequently were thoroughly investigated epidemiologically.[67]

The earlier outbreaks had been associated with the predominance of the vector Leptotrombidium deliense. There has been an important observation that, in the recent outbreaks, the L. deliense was missing and an interesting finding came up, that is, emergence of Schoengastiella ligula, as the primary vector in the outbreak of Kurseong district.[48]

### Preventive Measures

**Avoidance of Mite - Human Contact**

- To avoid mite-infested areas, the following measures should be followed:
  - Wearing protective clothing
  - Following personal prophylaxis against the mite vector by impregnating clothes with miticidal chemicals (permethrin and benzyl benzoate) and the application of mite repellants (diethyltoluamide) to exposed skin surfaces
  - Mites from sites should be eliminated by application of chlorinated hydrocarbons (lindane, dieldrin, and chlordane) to the ground and vegetation in camps and other populated zones in endemic areas.

Those people working in infested areas should consider impregnating clothing with permethrin. When sitting around or camping, groundcovers and tents with closed floors should be used. Lathering with soap in a hot bath or shower will remove both attached and unattached chiggers.

### Chemoprophylaxis

Weekly once dose of 200 mg doxycycline is effective. It should be considered for nonimmune people sent to work in endemic areas and in high-risk travelers.

**Vaccine**

Initial efforts of preparation of vaccines with killed O. tsutsugamushi were disappointing, because results of animal studies did not prove to be equally successful in human studies. This has been also been attributed to the diversity of the strains, lack of tolerability of live vaccines in volunteers due to absence of natural attenuated strains of the organism, and lack of achieving long-term heterologous protection even with irradiated strains.

The different types of vaccines which were attempted to develop immunoprophylaxis were killed vaccines, live vaccines, live vaccines with antibiotics, attenuated vaccines, and subunit vaccines. The killed vaccines were developed as formalin-treated strains such as Karp and Gilliam strains.

Smadel et al. demonstrated that immunization with a live vaccine followed by antibiotic treatment produced protective immunity to the homologous strain that lasted at least 1 year in most volunteers.[69] Gamma irradiation produced a successfully attenuated Karp strain of O. tsutsugamushi.[68] This agent was capable of infecting cells, but did not produce disease. Irradiated strains were also shown to provide homologous immunity for 1 year and heterologous immunity for <6 months. However, due to difficulties in producing, storing, and standardizing the vaccine candidates, development of the irradiated vaccines for scrub typhus was stopped and a shift in emphasis toward the development of subunit vaccines began.

Subunit vaccines that focused mainly on the 22, 47, 56, 58, and 110-kDa protein antigens have been developed.[51, 52] There were also attempts to develop recombinant fusion proteins to provide a more efficient cellular and humoral immunity against scrub typhus.[51, 52] The role of the 47-kDa antigen as an important vaccine candidate against scrub typhus needs to be further explored. The 110-kDa O. tsutsugamushi
antigen is a less abundant protein that contains both group and strain specific epitopes. Since this antigen is recognized by humans following natural infection, it is a potential candidate for a scrub typhus vaccine.[53,54]

Immunization with a combination of conserved Orientia antigens recognized, as a whole, by CD4+ T-cells, CD8+ T-cells, and antibodies will lead to cross-reactive and sustained anti-orientational immunity.

An autotransporter protein (ScmA) of O. tsutsugamushi plays an important role in bacterial pathogenesis.[95]

Immunization with ScmA not only provides protective immunity against lethal challenges with the homologous strain, but also confers significant protection against heterologous strains when combined with TSA56, a major outer membrane protein of O. tsutsugamushi.

**Conclusion**

Scrub typhus is showing a recent resurgence in our country as evidenced by reports from different parts of India in the last two decades. Important outbreaks have been noticed in India in Tamil Nadu (28 cases in 2001-2002), Himachal Pradesh (200 cases, 13 fatal in 2011), Nagaland (9 cases, 3 fatal in 2011), Meghalaya (80 cases, 5 fatal in 2010), and in Puducherry in 2008.[56] It is a serious acute febrile illness associated with significant morbidity and mortality. A high index of suspicion is needed in patients presenting with fever during the monsoon months.

Diagnosis is often missed, and tools for confirming diagnosis are often not available in resource-poor setups. Delay in initiating treatment owing to this may lead to untoward fatality. More widespread access to medical care, close suspicion of the disease along with the increased use of affordable and accurate rapid tests, is mandatory to improve diagnosis and treatment of this condition which can be easily treated with antibiotics.

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**Conflicts of interest**

There are no conflicts of interest.

**What is new?**

There has been a resurgence of scrub typhus from several parts of our country. Its being reported from both hilly, forest areas as well as plain land. There has been reports of disease from urban areas also.

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