International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 28-11-2022, Accepted: 13-12-2022, Published: 30-12-2022
Volume 7, Issue 12, 2022, Page No. 181-186

Diversity, biology and pathogenesis of *Orientia tsutsugamushi* (Scrub typhus pathogen): A general review

Shweta Gupta
Department of Zoology, Mohanlal Sukhadia University (MLSU), Udaipur, Rajasthan, India

Abstract
Background: Scrub typhus is a neglected rickettsial vector-borne disease especially prominent in Asia Pacific region caused by pathogen *Orientia tsutsugamushi* which is a gram negative obligate intracellular parasitic bacterium transmitted to humans as well as other vertebrates by the biting of trombiculid mites (chigger mites). This review explores the strain diversity, biology and pathogenesity of scrub typhus pathogen *Orientia tsutsugamushi*.
Methods: Literature, related to scrub typhus disease and its causative agent *Orientia tsutsugamushi* was reviewed to extract all the information based on some immunological studies that have been done previously regarding the disease.
Result: *Orientia tsutsugamushi* is a pleiomorphic bacterium and shows very high variability in between its strains such as Karp, Kato, Gilliam, Boryong and Kawasaki. It completes its life cycle within the trombiculid mite (vector) and vertebrate hosts. It causes disease in human and affects the host's immune system and at present this disease spreads throughout the world and become a major health concern due to its severity and high mortality rate but neglected everywhere due to lack of awareness and there is no vaccine available currently.
Conclusion: This study provides the knowledge about genetic and evolutionary relationship of *Orientia spp.* strains. Understanding the strains diversity and life cycle are crucial for vaccine development as well as sero-diagnostics.

Keywords: *Orientia tsutsugamushi*, scrub typhus, trombiculid mites, strains, life cycle, pathogenesis

Introduction

Scrub typhus is a rickettsial vector-borne disease caused by pathogen *Orientia tsutsugamushi*, generally found in the cell cytoplasm and in some cases within the nucleus also. This pathogen is transmitted by the trombiculid mites (chigger mites) to human as well as other vertebrates especially in small mammals [6]. The disease scrub typhus has been neglected for a long time even if it was remained as a public health problem from the time of world war second. Previously it was found that this disease was confined to a fixed and limited area (Asia Pacific Region) in the world which is known as 'tsutsugamushi triangle' and because of this restricted area the disease was also named as 'tsutsugamushi disease' but at present it is spreading throughout the world population and become a major health concern. Headache, myalgia, fever, fatigue, cough, restlessness/insomnia, and eschar and maculopapular rashes are the common symptoms of this disease. The major difficulties of scrub typhus include hepatomegaly, pneumonia, meningoencephalitis, myocarditis, acute renal failure (ARF), gastrointestinal bleeding, splenomegaly, and seizures [20, 44]. Mortality rate ranges upto 30%. Due to the lack of specific manifestations it remains undiagnosed worldwide in a huge population.

Disease Scenario at Global and National Level

Scrub typhus is very common and widespread zoonotic disease amongst all the rickettsial diseases worldwide[28]. According to Watt and Parola, 2003, about one million global population has been suffered from scrub typhus disease and approximately one billion people are at risk per year.

Mortality rate depends on the pathogen strain, time of infection and different geographical area or environmental conditions. In untreated patients mortality ranges from 0-30%. Deaths occur due to the primary infection or due to secondary complications like ARDS, encephalitis, pneumonitis and failure of cardiovascular system [28].

Previously the disease scrub typhus was confined to Asia Pacific Region extending from Afghanistan and Pakistan in the west to China and Korea in the east and the islands of the south-western Pacific and northern Australia in the south which is known as 'tsutsugamushi triangle' and the disease was also named as 'tsutsugamushi disease' but at present it is spreading throughout the world population extends to UAE, South Chile, and South America and become a major health concern. In 1990, few case studies were reported and thus suggested the presence of scrub typhus in Africa also[37].

Distribution of vector is directly symbolize the area of scrub typhus disease worldwide and it is predominantly in certain specific geographical areas such as India, Nepal, Sri Lanka, Maldives, Myanmar, Indonesia and Thailand [28].

An outbreak was reported in Pondicherry, India and 50 patients were diagnosed as scrub typhus positive with common symptoms such as Lymphadenopathy, hepatosplenomegaly and in 46% patients had eschar formation. In this study, approximately one third patient population was suffered from multi organ dysfunction [50].

Dissimilarities of *Orientia tsutsugamushi* With Other Rickettsial Pathogens

Orientia is small obligate, intracellular [generally cytoplasmic but sometimes intra-nuclear], pleomorphic (0.5–0.8µm in width and 1.2–3.0µm in length), gram-negative bacteria of the family Rickettsiaceae. It has 2.0-2.1Mb genome size with highly repeated sequence. It may be either bacillus or coccus and grows promptly in the yolk sac of the embryonated egg [6]. Previously, the bacterium of

scrub typhus disease was placed in the genus Rickettsia under the family Rickettsiaceae but now it is classified under the genus *Orientia*, different from genus *Rickettsia* due to the different cell wall structure (absence of lipopolysaccharide (LPS) and peptidoglycan) and genetic makeup[45]. Within the genus Orientia, different species were found mainly *Orientia tsutsugamushi* and *Orientia chuto* (human pathogenic species). *O. tsutsugamushi* is prevalent in most of the regions globally and participation in disease transmission is also very much higher than others. It has vast genetic and antigenic variability also so that different serotype groups were isolated: majorly Karp, Kato, Gilliam, Boryong and Kawasaki, depend upon the different geographical areas and variations in environmental conditions [6].

Strains Diversity of *Orientia tsutsugamushi*

Orientia tsutsugamushi is the infectious agent of the disease scrub typhus and found globally at present. There are some genetic or structural variations occur in the pathogen that make differences within species and also lead to generate different strains or serotypes of *Orientia tsutsugamushi*. Antigenic variation in 56kDa type specific surface antigen leads to the origin of multiple strains. To identify such variations and establishment of the correlation amongst different strains, 56kDa protein antigen was amplified and sequenced followed by phylogenetic analysis. A relationship within different strains of *Orientia tsutsugamushi* was also observed that were isolated from different sources such as mites, rodents and humans [12, 21].

In context to India, the distribution of the *Orientia tsutsugamushi* strains was regional. The Karp-like strains were predominant in North India, Kato-like strains were predominant in South India, both Gilliam and Karp-like strains were predominant in Northeast India[5]. It is reported that Karp like strains are predominant in India followed by Gilliam like strains but Kato and Kawasaki like strains were not reported from India [27]. Dual infection of Karp and Kawasaki type strains was also reported in a few patients from Andhra Pradesh, India[48]. Less commonly found strains have also been reported from India, such as JG/Saitama type strains[31] Kuroki-like strains and Ikeda-like strains.

Life Cycle of *Orientia tsutsugamushi*

Orientia tsutsugamushi remains inside the cell cytoplasm of the salivary glands of larval trombiculid mites (chigger mites) and different type of cells in small mammals (mostly rodents) and humans. It causes scrub typhus disease in humans while the mites and small mammals act as reservoir hosts. Mites and small mammals play important role to complete the life cycle of *Orientia tsutsugamushi* while Humans are dead-end accidental hosts and don't play any role in its life cycle [1, 18].

Orientia tsutsugamushi multiplies in mites and transmits itself within mite population vertically by trans-ovarian and trans-stadial transmission process. The trombiculid mites lay their eggs (1-5 eggs per day) in the upper layers of the soil and they took about 6-12 weeks to hatch[46]. There are seven distinct stages occurs in the life cycle of trombiculid mites but the hexapod larval stage also known as chiggers (emerge after about 10-14 days) is considered as infective stage [46]. To transmit the pathogen it requires the suitable vertebrate host for attachment and feeding. They feed at once on tissue fluid through stylostome [13] and during this time *Orientia spp.* transfer to the host and start its life cycle inside the host tissue.

There is a variety of cell types in the host tissue and *Orientia* can also infect these different types of cells such as macrophages, endothelial cells, monocytes, dendritic cells, fibroblasts and the cultured polymorphonuclear leukocytes (PMNs) [11]. The ability to infect these cells depends upon the type of cells and the contact rate of pathogen with the tissue in the host. During the primary infection the dermal dendritic and macrophages act as target cells. These are the wandering cells which disperse the pathogen to the lymph nodes [39]. In lethal infection in humans, macrophages of liver and spleen and endothelial cells of the skin, lung, kidney, heart, pancreas and brain are the major target cells [35]. There is a process followed by bacterium to enter target cells from extracellular matrix.

Entry of pathogen into host tissue cells

To enter into the host tissue cells pathogen first required to attach on the surface of the target cells. These target cells have some receptors such as fibronectin glycoprotein, towards the extra cellular matrix where *Orientia* attaches with the help of its 56kDa type specific antigen (TSA 56) [8] and ScaC (auto-transporter protein). There is one more transporter protein ScaA is also present at the surface of Orientia and involved in this process but its potent anti-bacterial component is yet unknown [14-15]. Though the involvement of heparin sulphate proteoglycans (HSPGs) present on the cell surfaces and ECM, and the trans-membrane molecules Integrin α5β1 and Syndecan 4 has been reported in *Orientia* attachment, their interactive components are not known [8, 17, 24]. There are some cytoplasmic enzymes named as Focal Adhesion Kinases (FAK), Src Kinases, Rho GTPases also involved in the attachment pathway. It was studied that Rho GTPases act as a target for treatment of inflammatory disorders as well as other diseases such as cancer, Alzheimer's and cardiovascular diseases [2, 24, 30]. Guanine nucleotide dissociation inhibitors (GDIs) are Rho GTPase inhibitors that bind to these GTPases in their inactive GDP bound form and limit their function in the attachment process.

After the attachment, to enter the host cells the bacterium follows clathrin mediated endocytosis pathway. Confirmation of the involvement of this pathway is by the *Orientia* Clathrin colocalization and inhibition studies in which known Clathrin-pathway inhibitors such as Chlorpromazine hydrochloride, Monodansyl cadaverine and Sucrose were used[9]. *Orientia* remains in the endosomes up to 2 h post infection, and after that they are released in the cytoplasm. The releasing process is also confirmed by using NH_4Cl and Bafilomycin A (increases the pH of endosome) which weaken this escape [9] that means the acidic environment is required for the escape. There are some pathogenic enzymes such as hemolysin and phopholipase D, involved in this type of escape process in other intracellular pathogens named as *Listeria* and *Mycobacterium*. According to these studies there could be an important role of such enzymes in *Orientia* also [36].

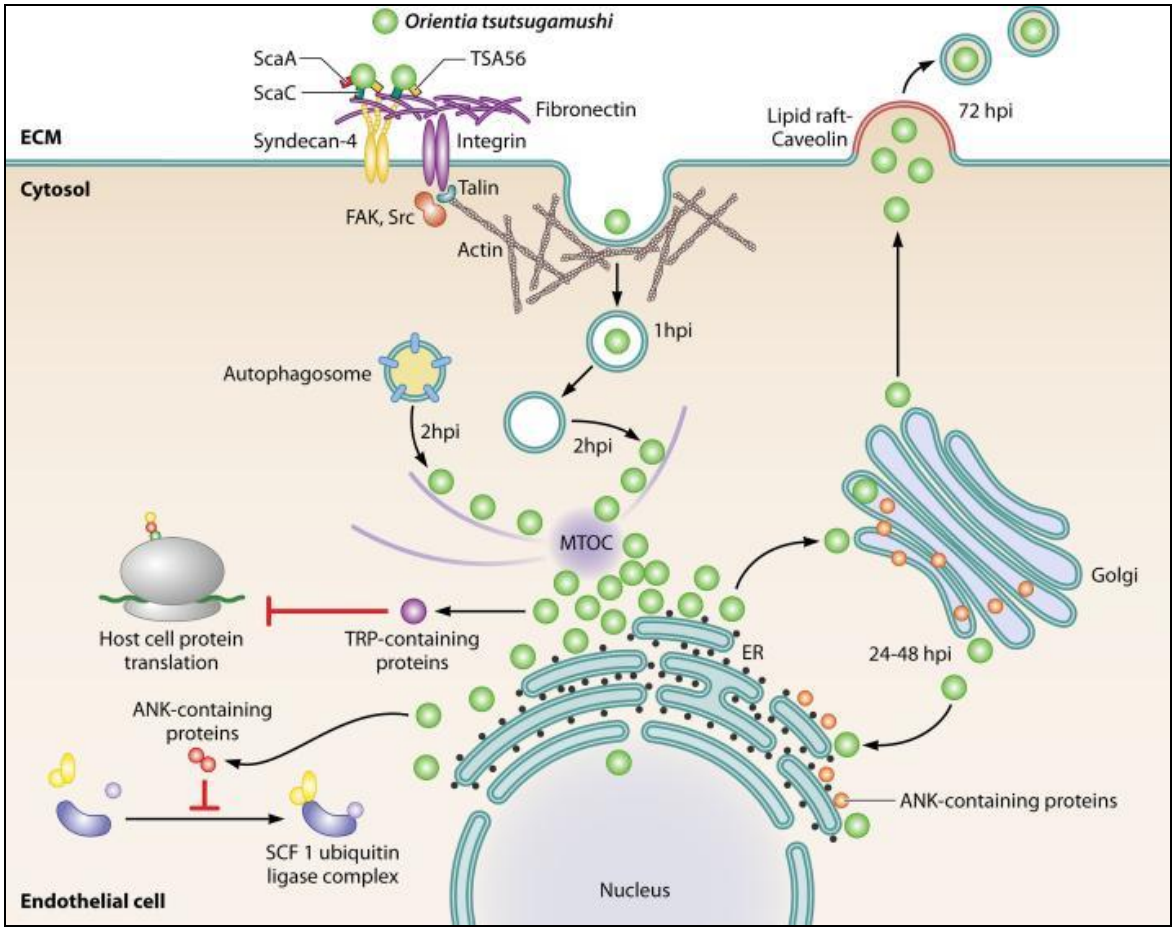

Fig 1: Shows life cycle of *Orientia tsutsugamushi* in non-phagocytic human cell [11]

Multiplication of pathogen into host cells

From the cytoplasm, *Orientia* move into the perinuclear space with the help of microtubules and dynein/dynectin motor proteins complex. In perinuclear space, they start binary fission and increase their number [24]. Intranuclear localization of *Orientia tsutsugamushi* has also been reported in mouse fibroblast cell line [49]. Cellular nitric oxide (NO) was reported as an enhancer molecule for the *Orientia* replication process while in other intracellular bacteria it has no effect on multiplication [36].

Infection of the pathogen in surrounding cells

To infect the surrounding cells, *Orientia* undergoes in the budding process and these buds like structures are released outside to the surrounding cells. In this process lipid rafts provide the needed support. The host tissue cell membrane must be present around the infected cell in order for the immune cells to internalize it during the phagocytosis process [10].

Pathogenesity of *Orientia tsutsugamushi*

To cause the infection in human tissues *Orientia* requires attachment to the host tissue. There are some important protein molecules; Fibronectin is one of them, present in the extra cellular matrix which facilitates the attachment and entry of pathogen inside the host [29]. Integrins and syndecans are the receptor families that mediate the interaction and these interactions initiate synergistic signaling pathway for cell adhesion [34]. *Orientia* multiplies within the host cells and target the endothelial cells of different organs [35]. Walsh *et al*., 2001 showed the pathogen within mononuclear WBCs in patients with acute infection and suggest direct blood-borne spread of the pathogen within the host.

Orientia tsutsugamushi induced both the humoral and cell mediated immune response within the host. In response to that level of some cytokines macrophage colony-stimulating factor (M-CSF), interferon-gamma (IFN-γ) and granulocyte colony-stimulating factor (G-CSF) increase during acute infection. Whereas the tumor necrosis factor (TNF-α) level rises during the recovery phase.

Orientia tsutsugamushi down regulates the expression of the glycoprotein 96 (gp96) in endoplasmic reticulum of infected macrophages and endothelial cells of the host which hinder multiple pathways of immune system [8]. Another study supports the increasing level of IFN-γ, interleukin-15 (IL-15) and IL-18 in scrub typhus patients [7].

It was demonstrated that cytotoxic T- lymphocytes (CTLs) cells (cytotoxic T cells and natural killer (NK) cells) activate during acute infection which destroy infected host cells. As a result, the severity of illness is determined by both host and pathogen factors [41].

Strategies Adopted by the Pathogen to Survive within The Host

When a pathogen comes inside the host tissue, the immune system of host gets activated and gives protection against the pathogen. To reside in the host tissue, pathogen has to do some modifications either inside the host body or in the outer surface of itself to become unrecognizable by the host's immune system. *Orientia tsutsugamushi* is also adopted some modification strategies within the host so that

it can reside inside the host tissue alive. These modifications have to be such type that intact the properties of the pathogen so that without elimination and being unrecognizable it can survive, multiply and infect its host.

Modification in apoptosis pathway inside the host

As *Orientia tsutsugamushi* is an intracellular bacterium and replicate within the cell's perinuclear space, if the host tissue cells die via apoptotic pathway it indirectly affects the replication of *Orientia*. This pathway has to be inhibited or modified by the *Orientia* to survive. Some studies have been done regarding these strategies. Based on some studies, apoptosis pathway is inhibited by decreasing the intracellular Ca^{+2} level in human monocytes cell line THP-1 when infected with the Boryong strain of *Orientia tsutsugamushi* [31]. Modulations in the apoptotic pathway depend on the different stages of infection, different strains of pathogen different cell types. There is a Ca^{+2} dependent signaling molecule involved in this pathway, known as Protein Kinase C (PKC). So if there is decrease in Ca^{+2}, it might affect the PKC function and finally the apoptosis pathway.

Modifications in autophagic pathway inside the host

During infection of any pathogen to the host, pathogen comes inside the host in very less number. To survive and multiply themselves, they have to remain in hidden condition until they make their population sufficient. But for infection, host's system is also prepared already to suppress the growth of pathogen. To this, host can use the process of autophagy to destroy its own cells in which pathogen gets multiply. When endosomal membrane-based organelles called autophagosomes join forces with other endosomal compartments to absorb the cytosolic components intended for breakdown, this is a controlled catabolic process. These autophagosomes appear in the *Orientia* infected polymorpho-nuclear cells [42] and within 1 h of *Orientia* infection. Autophagy marker LC3B recruitment is a sign of it [25]. This autophagic pathway takes some time to initiate due to the process of antigen presentation so the pathogen can evade from this pathway as early as 2 h post infection even in the presence of autophagy inducing factors like rapamycin [25].

Modification in ER associated degradation (ERAD) pathway inside the host cells

Misfolded proteins are known to be degraded by the ERAD pathway. The unfolded protein response (UPR) is triggered when misfolded proteins build up in the ER lumen. The cytoplasmic misfolded proteins in this route attach to ubiquitin molecules and are then degraded by the proteasome [33]. OT controls the UPR pathway by preventing misfolded protein ERAD. Till 2 days of infection, *Orientia* inhibits the ERAD pathway and multiply slowly in the leg phase and after 3 days of infection ERAD pathway is activated and generation of amino acids occur due to the protein degradation, it enhances the growth of *Orientia* and it undergoes in log phase of rapid growth. Ank4 is the molecule released by *Orientia* which plays important role in this pathway [44].

Detection Methods of Scrub Typhus Pathogen

Scrub typhus disease was not identified as a mite borne disease previously due to the lack of specific signs and symptoms. It was known as the rickettsial disease having the common symptoms like headache, fever, fatigue, myalgia, restlessness/insomnia, cough, eschar and maculopapular rash. Eschar formation is the specific sign to detect the disease but it is not appeared in 100% scrub typhus positive population. Gastrointestinal symptoms, encephalitis, respiratory and kidney failure, and very hardly ever disseminated intravascular coagulation (DIC) are the symptoms, arise in complicated condition. In untreated condition multi-organ failure occurs with high mortality rates depending on the strains of *Orientia* and immune competence of the patient.

There are three strains of *Proteus spp.* OX2, OX19, and OXK that have been used as antigens in the Weil-Felix test (agglutination based) for the diagnosis of rickettsial diseases. OXK is used for the detection of scrub typhus disease. Previously it was identified as a rickettsial disease and thought that the structure of outer membrane is similar to the membrane of *Proteus* bacteria in terms of lipopolysaccharide (LPS) and showing the antigenic similarity with rickettsia but based on chemical composition analysis, Amano *et al.,* 1987 has reported that *R. tsutsugamushi* has little or no peptidoglycan or lipopolysaccharide (LPS) in its outer membrane [3].

The rapid immunochromatographic test (ICT) is the method to detect the IgM and IgG antibodies against the outer membrane protein antigen of scrub typhus disease pathogen. The sensitivity, specificity of this rapid ICT was 66.7% and 98.4% respectively according to studies conducted in Thailand.

The Immunofluorescent assay (IFA) is the gold standard test for the diagnosis of scrub typhus, but it is expensive and requires trained individuals to perform the test. It cannot be used in early diagnosis and treatment of this disease[26]. Further, IgM capture ELISA with IFA can be used as a reliable method of diagnosing early infections due to high specificity [19].

PCR is another method of diagnosis the scrub typhus disease based on 56kDa, 47kDa, 16SrRNA and GroEL genes. The alternative highly sensitive method of PCR is LAMP (Loop mediated isothermal amplification). Different PCR methods have been used previously. For proper diagnosis both serological and DNA based methods have been used.

Treatment of Scrub Typhus Disease

Scrub typhus is caused by the bacteria so different antibiotics are used as a treatment of the disease. Due to its affordability, broad spectrum of activity, and absorption, doxycycline is the most widely used and most effective antibiotic for rickettsial infections [10]. For the treatment of scrub typhus, the dosage of Doxycycline is 100 mg twice day for seven days. Intravenous doxycycline can be used in serious patients [40]. Early antibiotic intervention shortens the duration of the illness, averts major consequences, and lowers mortality. Putting off commencing antibiotic therapy for 24 hours increases the chance of death by 20% [29]. However, due to the dangers of teratogenicity, hepatotoxicity, and child teeth discolouration, doxycycline is not advised for usage in pregnant women or children [10].

Another antibiotic used to treat scrub typhus is chloramphenicol, however it works less well than doxycycline. Additionally, Azithromycin is advised for the treatment of scrub typhus in pregnant women instead of

Doxycycline [24]. Macrolides are also used for the treatment of scrub typhus disease in pregnant females and children. Ciprofloxacin was also tried on pregnant females but it didn't work effectively [32].

Precautions to Prevent From the in fection of The Scrub Typhus Disease

Scrub typhus disease is a vector borne zoonotic disease even if it is caused by the pathogen *Orientia tsutsugamushi* but transmitted to humans by the biting of chigger mites so to control this transmission to human population it has to be required to avoid the contact with trombiculid mites.

To avoid human-vector contact in mite-infested areas, the following measures are necessary:

- Wear full and protective cloths or impregnating clothes with miticidal chemicals such as permethrin and benzyl benzoate and apply mites' repellent diethyltoluamide to exposed skin surfaces. Lathering with soap in a hot bath or shower will remove both attached and unattached chiggers [7].
- Lindane, dieldrin, and chlordane are the chlorinated hydrocarbons that should be used to the ground and vegetation in endemic areas to eliminate the mites from site [5].

Conclusion

Scrub typhus is highly fetal and neglected vector borne disease worldwide and symptoms are resembled with other vector borne diseases, leads to the misdiagnosis of this disease. The area of spreading scrub typhus disease depends on different geographical and environmental conditions and presence of suitable host in that particular area and the rate of transmission of the disease. To overcome the disease burden all these factors should be included for proper treatment.

Ethical statement: Not Applicable

Conflict of interest: None

References

1. Aggarwal P, Mahesh DM, Ravi Kumar V, Himral P, Kaushal SS, Verma BS. Atypical eschar sites in scrub typhus in sub-Himalayas. J Assoc Physicians India,2009:57:154.
2. Aguilar BJ, Zhu Y, Lu Q. Rho GTPases as therapeutic targets in Alzheimer's disease. Alzheimer's research & therapy,2017:9(1):1-10.
3. Amano K, Tamura A, Ohashi N, Urakami H, Kaya S, Fukushi K. Deficiency of peptidoglycan and lipopolysaccharide components in Rickettsia tsutsugamushi. *Infection and immunity*,1987:*55*(9):2290-2292.
4. Bora T, Khan SA, Jampa L, Laskar B. Genetic diversity of Orientia tsutsugamushi strains circulating in Northeast India. Transactions of The Royal Society of Tropical Medicine and Hygiene,2018:112(1):22-30.
5. Chakraborty S, Sarma N. Scrub typhus: an emerging threat. Indian Journal of Dermatology,2017:62(5):478.
6. Chierakul W, de Fost M, Suputtamongkol Y, Limpaiboon R, Dondorp A, White NJ *et al*. Differential expression of interferon-γ and interferon-γ-inducing cytokines in Thai patients with scrub typhus or leptospirosis. Clinical immunology,2004:113(2):140-144.
7. Cho BA, Cho NH, Min CK, Kim SY, Yang JS, Lee JR *et al*. Global gene expression profile of Orientia tsutsugamushi. Proteomics,2010:10(8):1699-1715.
8. Chu H, Lee JH, Han SH, Kim SY, Cho NH, Kim IS *et al*. Exploitation of the endocytic pathway by Orientia tsutsugamushi in nonprofessional phagocytes. Infection and immunity,2006:74(7):4246-4253.
9. Cross R, Ling C, Day NP, McGready R, Paris DH. Revisiting doxycycline in pregnancy and early childhood–time to rebuild its reputation?. Expert opinion on drug safety,2016:15(3):367-382.
10. Díaz FE, Abarca K, Kalergis AM. An update on host-pathogen interplay and modulation of immune responses during Orientia tsutsugamushi infection. Clinical microbiology reviews,2018:31(2):e00076-17.
11. Enatsu T, Urakami H, Tamura A. Phylogenetic analysis of Orientia tsutsugamushi strains based on the sequence homologies of 56-kDa type-specific antigen genes. FEMS microbiology letters,1999:180(2):163-169.
12. Ewing Jr EP, Takeuchi A, Shirai A, Osterman JV. Experimental infection of mouse peritoneal mesothelium with scrub typhus rickettsiae: an ultrastructural study. Infection and immunity,1978:19(3):1068-1075.
13. Fukuhara M, Fukazawa M, Tamura A, Nakamura T, Urakami H. Survival of two Orientia tsutsugamushi bacterial strains that infect mouse macrophages with varying degrees of virulence. Microbial pathogenesis,2005:39(5-6):177-187.
14. Ha NY, Sharma P, Kim G, Kim Y, Min CK, Choi MS *et al*. Immunization with an autotransporter protein of Orientia tsutsugamushi provides protective immunity against scrub typhus. PLoS neglected tropical diseases,2015:9(3);e0003585.
15. Ihn KS, Han SH, Kim HR, Huh MS, Seong SY, Kang JS *et al*. Cellular invasion of Orientia tsutsugamushi requires initial interaction with cell surface heparan sulfate. *Microbial pathogenesis*,2000:*28*(4):227-233.
16. Iwasaki H, Takada N, Nakamura T, Ueda T. Increased levels of macrophage colony-stimulating factor, gamma interferon, and tumor necrosis factor alpha in sera of patients with Orientia tsutsugamushi infection. Journal of clinical microbiology,1997:35(12):3320-3322.
17. Izzard L, Fuller A, Blacksell SD, Paris DH, Richards AL, Aukkanit N *et al*. Isolation of a novel Orientia species (O. chuto sp. nov.) from a patient infected in Dubai. Journal of clinical microbiology,2010:48(12):4404-4409.
18. Jiang J, Richards AL. Scrub typhus: no longer restricted to the Tsutsugamushi Triangle. Tropical medicine and infectious disease,2018:3(1):11.
19. Kala D, Gupta S, Nagraik R, Verma V, Thakur A, Kaushal A. Diagnosis of scrub typhus: recent advancements and challenges. *3 Biotech*,2020:*10*(9):1-21.
20. Kim HR, Choi MS, Kim IS. Role of Syndecan-4 in the cellular invasion of Orientia tsutsugamushi. Microbial pathogenesis,2004:36(4):219-225.
21. Kim MK, Seong SY, Seoh JY, Han TH, Song HJ, Lee JE *et al*. Orientia tsutsugamushi inhibits apoptosis of macrophages by retarding intracellular calcium release. Infection and immunity,2002:70(8):4692-4696.

22. Kim SW, Ihn KS, Han SH, Seong SY, Kim IS, Choi MS. Microtubule-and dynein-mediated movement of Orientia tsutsugamushi to the microtubule organizing center. Infection and immunity,2001:69(1):494-500.
23. Ko Y, Choi JH, Ha NY, Kim IS, Cho NH, Choi MS. Active escape of Orientia tsutsugamushi from cellular autophagy. Infection and immunity,2013:81(2):552-559.
24. Koh GC, Maude RJ, Paris DH, Newton PN, Blacksell SD. Diagnosis of scrub typhus. The American journal of tropical medicine and hygiene,2010:82(3):368.
25. Koraluru M, Bairy I, Singh R, Varma M, Stenos J. Molecular confirmation of scrub typhus infection and characterization of Orientia tsutsugamushi genotype from Karnataka, India. Journal of Vector Borne Diseases,2016:53(2):185.
26. Kumar A, Biswal M, Zaman K, Sharma N, Suri V, Bhalla A. Genetic diversity of Orientia tsutsugamushi strains from patients in north India. International Journal of Infectious Diseases,2019:84:131-135.
27. Larsson P. 6 Serology of Proteus mirabilis and Proteus vulgaris. In *Methods in Microbiology*. Academic Press,1984:14:187-214.
28. Lee JH, Cho NH, Kim SY, Bang SY, Chu H, Choi MS *et al*. Fibronectin facilitates the invasion of Orientia tsutsugamushi into host cells through interaction with a 56-kDa type-specific antigen. The Journal of infectious diseases,2008:198(2):250-257.
29. Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Prasher BS *et al*. Scrub typhus in Himalayas. Emerging infectious diseases,2006:12(10):1590.
30. Mathai E, Rolain JM, Verghese GM, Abraham OC, Mathai D, Mathai M *et al*. Outbreak of scrub typhus in southern India during the cooler months. Annals of the New York Academy of Sciences,2003:990(1):359-364.
31. Meusser B, Hirsch C, Jarosch E, Sommer T. ERAD: the long road to destruction. Nature cell biology,2005:7(8):766-772.
32. Morgan MR, Humphries MJ, Bass MD. Synergistic control of cell adhesion by integrins and syndecans. Nature reviews Molecular cell biology,2007:8(12):957-969.
33. Moron CG, Popov VL, Feng HM, Wear D, Walker DH. Identification of the target cells of Orientia tsutsugamushi in human cases of scrub typhus. Modern Pathology,2001:14(8):752-759.
34. Ogawa M, Satoh M, Kataoka M, Ando S, Saijo M. Nitric oxide enhanced the growth of an obligated intracellular bacterium Orientia tsutsugamushi in murine macrophages. Microbial Pathogenesis,2017:107:335-340.
35. Osuga K, Kimura M, Goto H, Shimada K, Suto T. A case of tsutsugamushi disease probably contracted in Africa. European Journal of Clinical Microbiology and Infectious Diseases,1991:10(2):95-96.
36. Paris DH, Chattopadhyay S, Jiang J, Nawtaisong P, Lee JS, Tan E *et al*. A nonhuman primate scrub typhus model: protective immune responses induced by pKarp47 DNA vaccination in cynomolgus macaques. The Journal of Immunology,2015:194(4):1702-1716.
37. Paris DH, Phetsouvanh R, Tanganuchitcharnchai A, Jones M, Jenjaroen K, Vongsouvath M *et al*. Orientia tsutsugamushi in human scrub typhus eschars shows tropism for dendritic cells and monocytes rather than endothelium. PLoS neglected tropical diseases,2012:6(1):e1466.
38. Peter JV, Sudarsan TI, Prakash JAJ, Varghese GM. Severe scrub typhus infection: Clinical features, diagnostic challenges and management. World journal of critical care medicine,2015:4(3):244.
39. Rikihisa Y. Glycogen autophagosomes in polymorphonuclear leukocytes induced by rickettsiae. The Anatomical Record,1984:208(3):319-327.
40. Rikihisa Y, Ito S. Intracellular localization of Rickettsia tsutsugamushi in polymorphonuclear leukocytes. The Journal of experimental medicine,1979:150(3):703-708.
41. Rodino KG, VieBrock L, Evans SM, Ge H, Richards AL, Carlyon JA. Orientia tsutsugamushi modulates endoplasmic reticulum-associated degradation to benefit its growth. Infection and immunity,2018:86(1):e00596-17.
42. Shimokawa H, Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. Arteriosclerosis, thrombosis, and vascular biology,2005:25(9):1767-1775.
43. Tamura A, Ohashi N, Urakami H, Miyamura S. Classification of Rickettsia tsutsugamushi in a new genus, Orientia gen. nov., as Orientia tsutsugamushi comb. nov. International Journal of Systematic and Evolutionary Microbiology,1995:45(3):589-591.
44. Traub R, Wisseman Jr CL. Ecological considerations in scrub typhus: 2. Vector species. Bulletin of the World Health Organization,1968:39(2):219.
45. Traub R, Wisseman Jr CL. The ecology of chigger-borne rickettsiosis (scrub typhus). Journal of medical entomology,1974:11(3):237-303.
46. Usha K, Kumar E, Kalawat U, Kumar BS, Chaudhury A, Gopal DS. Molecular characterization of Orientia tsutsugamushi serotypes causing scrub typhus outbreak in southern region of Andhra Pradesh, India. The Indian Journal of Medical Research,2016:144(4):597.
47. Varghese GM, Janardhanan J, Mahajan SK, Tariang D, Trowbridge P, Prakash JA *et al*. Molecular epidemiology and genetic diversity of Orientia tsutsugamushi from patients with scrub typhus in 3 regions of India. Emerging infectious diseases,2015:21(1):64.
48. Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak of scrub typhus in Pondicherry. J Assoc Physicians India,2010:58(1):24-8.
49. Walsh DS, Myint KS, Kantipong PACHAREE, Jongsakul KRISADA, Watt GEORGE. Orientia tsutsugamushi in peripheral white blood cells of patients with acute scrub typhus. The American journal of tropical medicine and hygiene,2001:65(6):899-901.
50. Xu G, Walker DH, Jupiter D, Melby PC, Arcari CM. A review of the global epidemiology of scrub typhus. PLoS neglected tropical diseases,2017:11(11):e0006062.