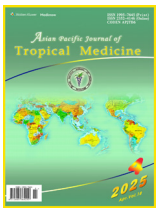

Review Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/apjtm.apjtm_95_24

# Immunogenic proteins of *Orientia tsutsugamushi* and implications for scrub typhus intervention: A narrative review

Shakshi Shah[1], Ankita Sharma[2], Alka Rana[1], Rakesh Kumar[1], Amit Kumar Sharma[1], Sunil Kumar[1]✉, Dixit Sharma[1]✉

[1]*Department of Animal Sciences, School of Life Sciences, Central University of Himachal Pradesh, District Kangra, Himachal Pradesh, 176206, India*

[2]*Dr. Ambedkar Centre of Excellence, Central University of Himachal Pradesh, District Kangra, Himachal Pradesh, 176215, India*

## ABSTRACT

Scrub typhus is an acute undifferentiated febrile infectious disease transmitted by a chigger (genus *Leptotrombidium*) bite carrying *Orientia (O.) tsutsugamushi*, affecting millions of people annually while more than one billion people are susceptible. Endemic areas are expanding to Africa, Europe, Middle East, and South America which is concerning, as despite best efforts, there is no vaccine to combat the bacteria. There are now three species of *Orientia* and over 20 strains of *O. tsutsugamushi*. The past attempts to develop a vaccine have been ineffective as they confer homologous strain-specific immunity. Various immunogenic proteins of *O. tsutsugamushi* have been identified that interact with the extracellular matrix (fibronectin) or vMLL5 receptor and modify the cytoskeleton of non-phagocytic host cells, which aids in host cell adhesion and invasion. These highly conserved proteins involve type specific antigen 56 (TSA56), 47 kDa, OmpA, and autotransporter proteins (ScaA, ScaB and ScaC). TSA56 is the most immunogenic and contains four types of hypervariable regions. Out of all autotransporter proteins, ScaA provides the homologous strains specific immunity and when coupled with TSA56 it shows better protective immunity against heterologous strains. The review provides detailed insight into the potential immunogenic proteins of *Orientia* which can be utilized to develop the vaccine. Furthermore, studies focused on highly antigenic proteins will provide more insight into their roles in developing therapeutics and easy-to-handle rapid diagnostic kits.

**KEYWORDS:** *Orientia tsutsugamushi*; Immunogenic genes; Microbial pathogenesis; Vaccine; Scrub typhus

## 1. Introduction

Scrub typhus is a zoonotic infectious disease caused by *Orientia (O.) tsutsugamushi* and infects millions of people annually around the globe[1−3]. The majority of cases occur along the Pacific coast of Asia, in a region known as the "tsutsugamushi triangle" which includes major regions of Japan, China, Korea, Russia, Thailand, Northern Australia and India. However, scrub typhus cases can be found beyond the endemic regions. Molecular and serological evidences have shown the presence of the pathogen in regions such as Africa, Europe, Middle East and South America[4,5]. The clinical picture is characterized by non-specific signs such as fever with or without eschar (inflammatory lesions), myalgia, headache, abdominal pain, vomiting, and capillary leak during first week of illness or after 6 to 21 days of incubation period[6]. Untreated infections leading to multiple organ failure may be observed in 7%-15% of confirmed cases[7]. Untreated scrub typhus can lead to organ specific infections and affects multiple organs including respiratory system (acute respiratory distress syndrome and interstitial pneumonia), renal system (acute renal failure), cardiovascular system (myocarditis, vasculitis, and rhythm abnormalities), central nervous system (encephalopathy, meningitis, cranial nerve palsies and deafness), and gastrointestinal system (diarrhoea, pancreatitis

✉To whom correspondence may be addressed. E-mail: sunilibes@gmail.com; sharmadixit17@gmail.com; sharmadixit17@hpcu.ac.in



**How to cite this article:** Shah S, Sharma A, Rana A, Kumar R, Sharma AK, Kumar S, et al. Immunogenic proteins of *Orientia tsutsugamushi* and implications for scrub typhus intervention: A narrative review. Asian Pac J Trop Med 2025; 18(4): 149-155.

**Article history:** Received 19 February 2024 Revision 21 March 2025
Accepted 3 April 2025 Available online 9 April 2025

and alteration in liver functions)[7,8]. Moreover, scrub typhus is now being associated with acute encephalitis syndrome among children in India with high case fatality rates[9,10]. Earlier, only one species of *Orientia* was known to cause scrub typhus but reports from Dubai and Chile identified *Candidatus O. chuto* and *Candidatus O. chiloensis* as causative agent of scrub typhus[11,12].

According to recent studies, chiggers can play dual roles, serving as both host and reservoir for the disease. *Orientia* is sustained in the environment by means of transstadial and transovarial transmission in trombiculid mites. The process by which *O. tsutsugamushi* is transmitted through eggs is termed transovarial transmission which is found to be 100% effective in the laboratory environment. The transfer of *O. tsutsugamushi* across various life cycle stages is termed as transstadial transmission and the transmission percentage is modest[13]. Chigger bite is responsible for the transmission of *O. tsutsugamushi* which invades dermal cells forming eschar. It infects a wide range of cells; however, initial target cells appear to be activated monocytes/macrophages and dermal dendritic cells. Since these cells are the component of the lymphatic system, this appears to be a mechanism of dissemination for the pathogen[14]. The characteristics of lethal infection in humans encompass the endothelial cells infection entailing several organs such as pancreas, lung, skin, heart, kidney, and brain. This is further associated with infection of macrophages in liver and spleen[15].

Although, the exact comprehension of how host cells are invaded remains unclear at the time, some molecular interactions between bacteria and host cells have been described. In phagocytic cells like activated monocytes or dendritic cells, *O. tsutsugamushi* is internalized through phagocytosis; whereas in non-phagocytotic cells, it takes advantage of integrin-mediated signalling and the actin cytoskeleton for invasion, particularly clathrin-mediated endocytic pathway[7,16]. After successful invasion, *O. tsutsugamushi* escapes phagocytotic vacuole and can multiply *via* binary fission within the host cell, particularly in the perinuclear region. The interaction of *O. tsutsugamushi* with the host cell microtubules in a distinctive manner might contribute to its intracellular movement and replication. Following an ample number of replication, *O. tsutsugamushi* exits from the host cell in a manner similar to the budding process seen in enveloped viruses[17]. Despite fighting more than a decade long battle against *O. tsutsugamushi*, there is still a great deal about *O. tsutsugamushi* that is unknown and requires more research. With the addition of new areas to endemicity and a rise in number of scrub typhus cases to already endemic areas, it is now critical to produce effective vaccines against a wide range of *O. tsutsugamushi* strains[4,18,19]. Thus, we need to investigate different proteins in bacteria having the ability to elicit the needed immunogenic response, using combination of computational biology and experimental techniques[20]. Furthermore, omics and genome-wide approaches can be explored which can be useful for the identification of gene-specific function at the cellular, biological, and molecular level[21,22].

## 2. Immunogenic genes in *O. tsutsugamushi* genome

*O. tsutsugamushi* contains a circular genome ranging from 1.93 Mb to 2.47 Mb, with no plasmid or prophages. Study of the set of 8 full single-contig genomes suggests that a total of 657 genes forms alleged core genome, accounting for 28%-35% of the genome of all strains. The analysis of genome structure, specifically the order and clustering of conserved core genes indicates significant rearrangements in the genome over the time. These conserved core genes are found in 'core gene islands' and repeat regions are distributed between these islands. Genome of *O. tsutsugamushi* are highly repetitive among the bacterial genera and repeat density is 200 fold higher than other bacteria[23,24]. These repetitive sequences are classified into three types: (1) *O. tsutsugamushi* amplified genetic element (OtAGE) which is large genetic element encoding 33-38 genes and approximately 33 kb in size; (2) transposable elements, which are mobile DNA sequences that can replicate themselves within genomes irrespective of host cell DNA[25]; and (3) other repetitive sequences, which are short repeated sequences present in *O. tsutsugamushi* genome[24]. Percentage of repetitive genome varies remarkably from 33% in UT176 strain to 51% in Gilliam strain of *O. tsutsugamushi*. The existing literature has elucidated a set of genes highly expressed in *O. tsutsugamushi* genome and may be crucial in electing immune responses against bacteria. The 22, 47, 56, 58, and 110 kDa proteins of *O. tsutsugamushi* are the product of these genes identified during western blotting[23].

## 3. 56 kDa protein immunogenic responses

56 kDa type specific antigen (TSA) is an immunodominant, major integral outer membrane protein of *O. tsutsugamushi*, accounting for 10%-15% of the total bacterial cellular protein composition[26]. It consists of an open reading frame approximately 1 600 bp, encoding 516-541 amino acids[27]. This protein contains both conserved as well as variable regions varying in different strains of *O. tsutsugamushi*. There are four types of hypervariable regions (variable domains Ⅰ, Ⅱ, Ⅲ & Ⅳ) accountable for antigenic variation in the *TSA56* gene[26]. The maximum fibronectin binding activity is seen by TSA56 peptides that fall between amino acid 312 and amino acid 341 and from amino acid 292 to amino acid 321. TSA56 kDa protein interacts with fibronectin, a glycoprotein of extracellular matrix, which facilitated phagocytic and non-phagocytic host cell invasion[28,29]. Further interaction of fibronectin with integrin, such as $\alpha5\beta1$, stimulates downstream signaling molecules and endocytosis in non-phagocytic host cells. Utilizing host cell integrin signaling pathways, *O. tsutsugamushi* promotes actin cytoskeleton rearrangement to enter non-phagocytic host cells. Many invasive pathogens, such as *Staphylococcus aureus*, *Yersinia*,

*Streptococcus pyogenes*, and *Neisseria* use this method for host cell invasion[16]. Integrin engagement causes conformational changes across the membrane to the extracellular domains of integrin, and multiprotein complexes compile at cytoplasmic domain of integrin, which connects integrin to the actin cytoskeleton and relay signals to the cell. Focal adhesion kinase (FAK) is one such integrin signalling molecule that links stimuli to the action cytoskeleton. Autophosphorylation of FAK generates a docking site for binding of Src tyrosine kinase, which results in the tyrosine phosphorylation of several substrates, causing activation of Rho GTPases that facilitates the attachment of the pathogen to the cell and ultimately leads to significant cytoskeleton rearrangements[1,16,30]. Proteins like talin, which has a role in activation of integrin and paxillin, is a FAK-binding protein performing several functions like regulating protein-protein interaction, mediate binding of kinases and participate in the process of *O. tsutsugamushi* cell invasion (Figure 1)[7]. Due to abundance of TSA56 in bacterial outer membrane, it is also utilized in commonly used molecular method such as nested PCR for diagnosis and genotyping of *O. tsutsugamushi*[31]. TSA56 exhibits high immunogenicity and hence, making it one of the best candidates for vaccine development against *O. tsutsugamushi* as evident from previous studies[32–35]. Nevertheless, even though this major outer membrane protein is an immunodominant antigen, the genotypic diversity makes it difficult to create a vaccine against scrub typhus. The variability of *O. tsutsugamushi* genotypes among different strains restricts the ability of cross-protective immunity against heterologous strains[36]. It has been demonstrated that in earlier investigations, fusion of 56 kDa and 47 kDa, as well as plasmid vaccine encoding 56 kDa, offers homologous protective immunity against the bacteria in mice and non-human primate model[26,32,37]. Subsequent research demonstrates immunization mediated by conserved blocks of 56 kDa (cTSA56) exhibits superior heterologous protective immunity compared to the whole 56 kDa[36]. These results indicate approaches towards a multisubunit or multivalent immunization incorporating 56 kDa proteins together with additional immunogenic components could be a viable strategy.

## 4. 47 kDa protein immunogenic responses

47 kDa outer membrane protein (OMP) is one of the major outer membrane proteins and an immunodominant antigen of *O. tsutsugamushi*, a member of high-temperature requirement A (HtrA) family of serine proteases. The 47 kDa OMP makes a good candidate for the development of the vaccine as it is a highly conserved protein in 25 different strains of *O. tsutsugamushi*. Previously, it was found that a recombinant 47 kDa protein could promote the growth of polyclonal helper T cell line derived from *O. tsutsugamushi*-immune mice, suggesting the protein might have a potential T cell epitopes[38]. The 47 kDa protein has a considerable sequence homology to human HtrA1 and like other proteases, contains trypsin domain. Despite being a strong candidate for vaccine development

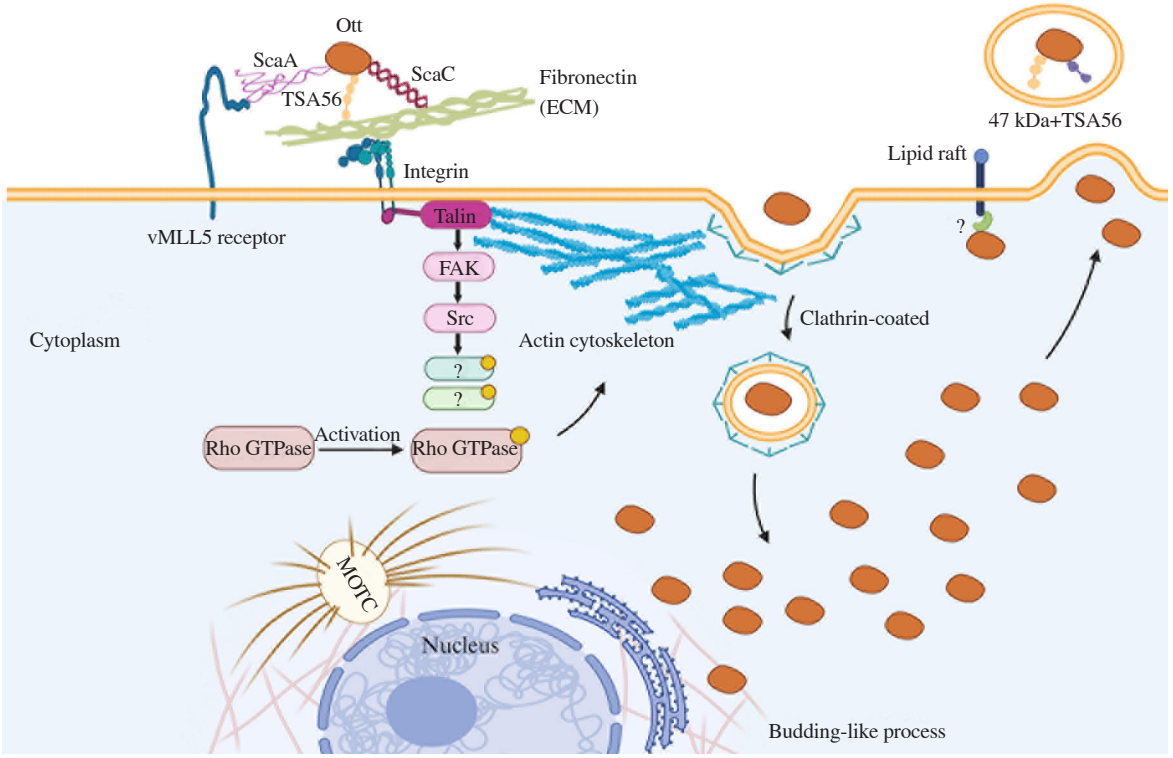


**Figure 1.** Interactions of outer membrane proteins of *Orientia tsutsugamushi* with the non-phagocytotic host cells of infected individuals. "?" stands for unidentified substrates.

since it promotes humoral and cell-mediated immunity, it was found that it can also trigger autoimmune reactions as it shares similarities to human HtrA proteins[39]. Although the molecular function and cellular location of the 47 kDa in *O. tsutsugamushi* are not yet understood, recent research suggests that during the budding-like process, HtrA is secreted within the host cell. It could be transported to the surface as complexes with 56 kDa protein, where it is secreted into the host cell. The HtrA might interact with specific lipid raft proteins, which could initiate the budding-like process and facilitate the exit of the bacterium[40]. Further studies are required regarding finding of specific T or B-cell epitopes for the development of vaccine using 47 kDa.

## 5. Outer membrane protein A (OmpA) immunogenic responses

Outer membrane protein A, a peptidoglycan-associated lipoprotein, is a major heat-modifiable outer membrane protein conserved in the majority of Gram-negative bacteria[41,42]. The molecular mass of OmpA ranges from 28 kDa to 36 kDa, depending on the temperature and conditions to which it is exposed before SDS-PAGE. The molecular structure of OmpA is well described in *Escherichia coli*, revealing that the *N*-terminal domain makes up an eight-stranded, anti-parallel β barrel embedded in the outer membrane; while the globular *C*-terminal domain is found in periplasmic space[41]. Order Rickettsiales contains various arthropod vector-transmitted pathogens including genera like *Anaplasma*, *Ehrlicia*, *Orientia* and *Rickettsia*. According to several studies, OmpA participates in a variety of functions like adherence and invasion of the host cell and virulence in obligate intracellular bacteria belonging to the genus *Anaplasma*, *Ehrlicia*, and *Rickettsia*, where targeting this protein prevents cell invasion of host cells in *Anaplasma* spp, *Ehrlicia chaffeensis* and *Rickettsia conorii*[43-45]. In addition, OmpA shows high immunogenicity by inducing specific humoral and cytotoxic responses, and has therefore been proposed in the design of various anti-infectious vaccines[46]. Keeping the role of the protein in the survival of intracellular bacteria belonging to Order Rickettsiales in mind, a study was conducted, in which 51 geographically diverse *O. tsutsugamushi* isolates were examined and it was discovered that these isolates show 93.6%-100% conservation at the nucleotide level and 90.6%-100% conservation at protein level. It was also revealed that the tertiary structure of OmpA protein from *O. tsutsugamushi* is remarkably similar to OmpA of *Anaplasma phagocytophilum*, when the two were superimposed on each other. Therefore, OmpA of *O. tsutsugamushi* protein might be a good contender for vaccine development that would offer heterologous protection[42].

## 6. Autotransporter proteins of *O. tsutsugamushi*

Autotransporter proteins comprise the largest protein family with over 1 000 distinct Family members in pathogenic Gram-negative bacteria. The typical sequence organization of autotransporter proteins consists of *C*-terminal autotransporter domain and *N*-terminal passenger domain. Passenger domain ranging from less than 20 kDa to more than 400 kDa, can be quite diverse and is linked with virulence-related phenotypes of specific bacterium including adhesion, invasion, cytotoxicity, and biofilm formation. The autotransporter domain about 30 kDa is vital for the transfer of the passenger domain to bacterial surface, and as a result, it shares common characteristics with other autotransporter proteins[47-49]. Examination of the whole genome of two strains of *O. tsutsugamushi* unveiled genes encoding for autotransporter proteins, which were then divided into five different groups of orthologs (ScaA, ScaB, ScaC, ScaD, and ScaE) based on their sequence similarity[48]. Another study found additional ScaF proteins in a few *O. tsutsugamushi* strains[50]. Additionally, these may be helpful for serodiagnostic tests since the *O. tsutsugamushi* autotransporter genes, *ScaA* is the largest and most widely distributed and *ScaC* is the smallest and most conserved gene[50].

### 6.1. ScaA autotransporter proteins immunogenic responses

A study on the prevalence of Sca was performed with 178 *O. tsutsugamushi* DNA samples collected from 12 endemic countries, where ScaA, ScaD, ScaE and ScaC were widely detected. ScaA is one of the largest autotransporter protein of *O. tsutsugamushi* universally distributed. ScaA has a predicted mass of 156 kDa[51]. It is expressed on the periphery of bacterial cell and serves as an adhesion factor during infection. Recently, a study discovered ScaA interacts with a host receptor which is a novel splicing variant of MLL5 (vMLL5). The *MLL5* (lysine *N*-methyltransferase 2) gene consists of 25 coding exons translated into a protein of 1 858 amino acid residue. MLL5 has a single plant homeodomain, an enhancer of zeste, Su(var)3-9, and a SET domain near *N*-terminal region, and participates in several biological functions, including genomic maintenance, cell cycle progression, and spermatogenesis. The novel *vMLL5* gene is expected to encode the transmembrane protein at the *C*-terminus of the intron region and is found to be expressed in both subcellular regions and plasma membrane. ScaA particularly interacts with vMLL5 *via* the SET domain[52]. ScaA showed sequence similarity of 83.5%-87.5% at nucleotide level and 78.8%-90.2% at amino acid level among different strains which can help in providing protective immunity against *O. tsutsugamushi* as effectively as TSA56. When coupled with TSA56, ScaA provides enhanced protective immunity against heterologous strains of *O.*

*tsutsugamushi*[51]. Another study revealed zinc oxide nanoparticle (ZNP) used as an adjuvant complexes with zinc oxide binding peptide (ZBP)-ScaA antigen results in a strong antibody response resistant to the lethal challenges posed by *O. tsutsugamushi*[53].

### 6.2. ScaC autotransporter proteins

Another autotransporter protein, ScaC shows a high level of conservation among different strains of *O. tsutsugamushi*[54]. The predicted mass of ScaC was found to be approximately 60 kDa. It is found to be localized on the periphery of bacterial cells, suggesting that it is expressed on the outer membrane and can induce an immune response. It simply enhances the affinity of bacteria to host cells and has no connection with cell invasion. ScaC also serves as a ligand binding with fibronectin, which shows the possibility of ScaC participating in the fibronectin-integrin pathway. Targeting this protein as a multiepitope vaccine candidate could show promising results as it induces specific antibody responses in host cell[48].

### 6.3. ScaB autotransporter proteins

Out of six identified autotransporter proteins in *O. tsutsugamushi*, ScaB and ScaF occur less frequently in different samples, which is about 33.7%-43.35%[54]. ScaB is absent in Ikeda strain of *O. tsutsugamushi*, and present in some strains where it is amplified differently[51]. However, a recent study confirms the presence of *ScaB* genes in Boryang, Sido, TA716, Kuroki and TA686 strains of *O. tsutsugamushi* and shows high level of amino acid sequence variation at the passenger domain. The molecular mass of the protein encoded by *ScaB* gene is predicted approximately 73 kDa in *O. tsutsugamushi*. *ScaB* gene is actively transcribed and translated and may be seen on the bacterial outer membrane. ScaB seems to facilitate bacteria in the initial stages of adherence and promotes host cell invasion in cultured epithelial and endothelial cells. Hence, ScaB is found to be related to bacterial pathogenesis[54]. The utilization of *O. tsutsugamushi* ScaB protein as an antigen to produce vaccine could be an approach but may be limited to specific strains due to its non-existence in some strains.

## 7. Future prospect and conclusion

Scrub typhus disease has been present among the human population for a very long period. At first, it was prevalent in a specific region, *i.e.*, "tsutsugamushi triangle" but now it has spread globally with the emergence of two new *Orientia* species. Furthermore, the identification of two new species (*Candidatus Orientia chuto* and *Candidatus Orientia chiloensis*) provides even more justification for research and studies aimed at creating vaccine against this bacteria. Past studies suggest that a lack of prolonged protective immunity and poor cross-reactive immunity cause recurrent reinfection episodes. Despite the development of advanced molecular techniques, scrub typhus is highly misdiagnosed and the endemic areas are typically rural, where necessary infrastructure is generally not available. The review majorly focuses on the immunogenic proteins but there are some other auspicious vaccine approaches, *i.e.*, vector based vaccines, live attenuated vaccines or DNA vaccines which can be utilized for vaccine development. The antigenic proteins can also aid in diagnostics but the paper does not discuss about the challenges in development of sensitive and field friendly diagnostic tool for scrub typhus. Several studies have been conducted relating to immunogenic antigens present in *O. tsutsugamushi*; nevertheless, the majority of vaccines developed from immunogenic antigens do not produce desirable protective immunity against the bacteria in the animal model. The TSA56, 47 kDa, OmpA, and autotransporter proteins (ScaA and ScaB) are the most potential immunogenic antigens in *O. tsutsugamushi*. The profound research examining the role of these proteins and the interaction of host cells with bacteria will be useful in the development of vaccines which is in pressing demand. This can be possible by integrating computational biology with experimental techniques for better results. Furthermore, the adoption of omics technologies will help to speed up the process for the development of vaccines and cheap or easy-to-handle molecular diagnostic tool kits.

## Conflict of interest statement

The authors declare no conflict of interest.

## Acknowledgments

D.S. acknowledges Department of Health Research, Government of India for Young Scientist Fellowship. Authors duly acknowledge Central University of Himachal Pradesh for providing computational facilities.

## Funding

This research was funded by Department of Health Research, Government of India, New Delhi, India (grant number: YSS/2020/000116/PRCYSS).

## Authors' contributions

Conceptualization, D.S. and S.K.; methodology, D.S. and A.S.; validation, S.S., A.R. and A.K.S.; formal analysis, A.S., R.K. and A.R.; investigation, S.S. and A.S.; data curation, D.S.; writing-original draft preparation, S.S.; writing-review and editing, D.S.,

A.S., S.K.; funding acquisition, D.S., S.K. All authors have read and agreed to the published version of the manuscript.

## References


[1] Devamani C, Alexander N, Chandramohan D, Stenos J, Cameron M, Abhilash KPP, et al. Incidence of scrub typhus in rural South India. *N Engl J Med* 2025; **392**(11): 1089-1099.

[2] Kim S, Kim AR, Lim S, Lee SJ, Bae M. Real-world utility of serological tests in patients with suspected scrub typhus in the Republic of Korea: A single-center, retrospective, observational study. *Asian Pac J Trop Med* 2024; **17**(6): 273-280.

[3] Long JL, Zeng ZY, Chen HY, Tao X, Wu XW, Chen SY, et al. Correlation between genotypes of *Orientia tsutsugamushi* and clinical characteristics of patients with scrub typhus in Guangzhou, China. *Asian Pac J Trop Med* 2024; **17**(7): 299-309.

[4] Jiang J, Richards AL. Scrub typhus: No longer restricted to the tsutsugamushi triangle. *Trop Med Infect Dis* 2018; **3**(1): 11.

[5] Sharma D, Kumar S, Sharma A, Kumar R, Kumar R, Kulharia M, et al. Functional assignment to hypothetical proteins in *Orientia tsutsugamushi* strain Ikeda. *Bioinformation* 2022; **18**(3): 188.

[6] Kispotta R, Kasinathan A, Kommu PPK, Mani M. Analysis of 262 children with scrub typhus infection: A single-center experience. *Am J Trop Med Hyg* 2021; **104**(2): 622.

[7] Díaz FE, Abarca K, Kalergis AM. An update on host-pathogen interplay and modulation of immune responses during *Orientia tsutsugamushi* infection. *Clin Microbiol Rev* 2018; **31**(2): e00076-17.

[8] Sharma D, Sharma A, Verma SK, Singh B. Targeting metabolic pathways proteins of *Orientia tsutsugamushi* using combined hierarchical approach to combat scrub typhus. *J Mol Recognit* 2019; **32**(4): e2766.

[9] Mittal M, Thangaraj JWV, Rose W, Verghese VP, Kumar CG, Mittal M, et al. Scrub typhus as a cause of acute encephalitis syndrome, Gorakhpur, Uttar Pradesh, India. *Emerg Infect Dis* 2017; **23**(8): 1414.

[10] Damodar T, Singh B, Prabhu N, Marate S, Gowda VK, Lalitha A, et al. Association of scrub typhus in children with acute encephalitis syndrome and meningoencephalitis, southern India. *Emerg Infect Dis* 2023; **29**(4): 711.

[11] 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. *J Clin Microbiol* 2010; **48**(12): 4404-4409.

[12] Abarca K, Martínez-Valdebenito C, Angulo J, Jiang J, Farris CM, Richards AL, et al. Molecular description of a novel *Orientia* species causing scrub typhus in Chile. *Emerg Infect Dis* 2020; **26**(9): 2148.

[13] Elliott I, Pearson I, Dahal P, Thomas NV, Roberts T, Newton PN. Scrub typhus ecology: A systematic review of *Orientia* in vectors and hosts. *Parasit Vectors* 2019; **12**(1): 513.

[14] 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 Negl Trop Dis* 2012; **6**(1): e1466.

[15] Moron CG, Popov VL, Feng HM, Wear D, Walker DH. Identification of the target cells of *Orientia tsutsugamushi* in human cases of scrub typhus. *Mod Pathol* 2001; **14**(8): 752-759.

[16] Cho BA, Cho NH, Seong SY, Choi MS, Kim IS. Intracellular invasion by *Orientia tsutsugamushi* is mediated by integrin signaling and actin cytoskeleton rearrangements. *Infect Immun* 2010; **78**(5): 1915-1923.

[17] 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. *Infect Immun* 2001; **69**(1): 494-500.

[18] Sharma D, Sharma A, Singh B, Verma SK. Bioinformatic exploration of metal-binding proteome of zoonotic pathogen *Orientia tsutsugamushi*. *Front Genet* 2019; **10**: 797.

[19] Valbuena G, Walker D. Approaches to vaccines against *Orientia tsutsugamushi*. *Front Cell Infect Microbiol* 2013; **2**: 170.

[20] Sharma D, Sharma A, Singh B, Verma SK. Pan-proteome profiling of emerging and re-emerging zoonotic pathogen *Orientia tsutsugamushi* for getting insight into microbial pathogenesis. *Microb Pathog* 2021; **158**: 105103.

[21] Sharma A, Sharma D, Verma SK. A systematic *in silico* report on iron and zinc proteome of Zea mays. *Front Plant Sci* 2023; **14**: 1166720.

[22] Sharma D, Chauhan D, Kumar S, Sharma A, Thakur K, Choudhary K, et al. Omics in livestock animals: Improving health, well-being and production. *Syst Biol Bioinforma Livest Sci* 2023; **1**: 129.

[23] Batty EM, Chaemchuen S, Blacksell S, Richards AL, Paris D, Bowden R, et al. Long-read whole genome sequencing and comparative analysis of six strains of the human pathogen *Orientia tsutsugamushi*. *PLoS Negl Trop Dis* 2018; **12**(6): e0006566.

[24] Nakayama K, Yamashita A, Kurokawa K, Morimoto T, Ogawa M, Fukuhara M, et al. The Whole-genome sequencing of the obligate intracellular bacterium *Orientia tsutsugamushi* revealed massive gene amplification during reductive genome evolution. *DNA Res Int J Rapid Publ Rep Genes Genomes* 2008; **15**(4): 185-199.

[25] Wells JN, Feschotte C. A field guide to eukaryotic transposable elements. *Annu Rev Genet* 2020; **54**: 539-561.

[26] Chattopadhyay S, Jiang J, Chan TC, Manetz TS, Chao CC, Ching WM, et al. Scrub typhus vaccine candidate Kp r56 induces humoral and cellular immune responses in cynomolgus monkeys. *Infect Immun* 2005; **73**(8): 5039-5047.

[27] Varghese GM, Janardhanan J, Mahajan SK, Tariang D, Trowbridge P, Prakash JAJ, et al. Molecular epidemiology and genetic diversity of *Orientia tsutsugamushi* from patients with scrub typhus in 3 regions of India. *Emerg Infect Dis* 2015; **21**(1): 64-69.

[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. *J Infect Dis* 2008; **198**(2):

250-257.

[29]Luce-Fedrow A, Lehman ML, Kelly DJ, Mullins K, Maina AN, Stewart RL, et al. A review of scrub typhus (*Orientia tsutsugamushi* and related organisms): Then, now, and tomorrow. *Trop Med Infect Dis* 2018; **3**(1): 8.

[30]Harburger DS, Calderwood DA. Integrin signalling at a glance. *J Cell Sci* 2009; **122**(2): 159-163.

[31]Janardhanan J, Prakash JAJ, Abraham OC, Varghese GM. Comparison of a conventional and nested PCR for diagnostic confirmation and genotyping of *Orientia tsutsugamushi*. *Diagn Microbiol Infect Dis* 2014; **79**(1): 7-9.

[32]Chan TC, Dasch GA, Chao CC, Richards AL, Ching WM, Ni YS. Protection against scrub typhus by a plasmid vaccine encoding the 56-KD outer membrane protein antigen gene. *Am J Trop Med Hyg* 2005; **73**(5): 936-941.

[33]Cho H, Lee WH, Kim YK, Kim K. Extracellular vesicle-associated antigens as a new vaccine platform against scrub typhus. *Biochem Biophys Res Commun* 2020; **523**(3): 602-607.

[34]Choi S, Jeong HJ, Ju YR, Gill B, Hwang KJ, Lee J. Protective immunity of 56-kDa type-specific antigen of *Orientia tsutsugamushi* causing scrub typhus. *J Microbiol Biotechnol* 2014; **24**(12): 1728-1735.

[35]Dolley A, Goswami HB, Dowerah D, Dey U, Kumar A, Hmuaka V, et al. Reverse vaccinology and immunoinformatics approach to design a chimeric epitope vaccine against *Orientia tsutsugamushi*. *Heliyon* 2024; **10**(1): e23616.

[36]Kim HI, Ha NY, Kim G, Min CK, Kim Y, Yen NTH, et al. Immunization with a recombinant antigen composed of conserved blocks from TSA56 provides broad genotype protection against scrub typhus. *Emerg Microbes Infect* 2019; **8**(1): 946-958.

[37]Yu Y, Wen B, Wen B, Niu D, Chen M, Qiu L. Induction of protective immunity against scrub typhus with a 56-kilodalton recombinant antigen fused with a 47-kilodalton antigen of *Orientia tsutsugamushi* Karp. *Am J Trop Med Hyg* 2005; **72**: 458-464.

[38]Niu D, Chen W, Zhang X, Chen M, Cui H, Wei W, et al. Immunogenicity of a 40 kDa fragment of the 47 kDa recombinant protein and DNA vaccine from Karp strain of *Orientia tsutsugamushi*. *Ann N Y Acad Sci* 2003; **990**(1): 527-534.

[39]Chen HW, Zhang Z, Huber E, Chao CC, Wang H, Dasch GA, et al. Identification of cross-reactive epitopes on the conserved 47-kilodalton antigen of *Orientia tsutsugamushi* and human serine protease. *Infect Immun* 2009; **77**(6): 2311-2319.

[40]Kim MJ, Kim MK, Kang JS. Involvement of lipid rafts in the budding-like exit of *Orientia tsutsugamushi*. *Microb Pathog* 2013; **63**: 37-43.

[41]Confer AW, Ayalew S. The OmpA family of proteins: Roles in bacterial pathogenesis and immunity. *Vet Microbiol* 2013; **163**(3-4): 207-222.

[42]Evans SM, Adcox HE, VieBrock L, Green RS, Luce-Fedrow A, Chattopadhyay S, et al. Outer membrane protein A conservation among *Orientia tsutsugamushi* isolates suggests its potential as a protective antigen and diagnostic target. *Trop Med Infect Dis* 2018; **3**(2): 63.

[43]Hebert KS, Seidman D, Oki AT, Izac J, Emani S, Oliver LD, et al. *Anaplasma marginale* outer membrane protein A is an adhesin that recognizes sialylated and fucosylated glycans and functionally depends on an essential binding domain. *Infect Immun* 2017; **85**(3): e00968-16.

[44]Hillman RD, Baktash YM, Martinez JJ. OmpA-mediated rickettsial adherence to and invasion of human endothelial cells is dependent upon interaction with 2β1 integrin. *Cell Microbiol* 2013; **15**(5): 727-741.

[45]Ojogun N, Kahlon A, Ragland SA, Troese MJ, Mastronunzio JE, Walker NJ, et al. *Anaplasma phagocytophilum* outer membrane protein A interacts with sialylated glycoproteins to promote infection of mammalian host cells. *Infect Immun* 2012; **80**(11): 3748-3760.

[46]Jeannin P, Magistrelli G, Goetsch L, Haeuw JF, Thieblemont N, Bonnefoy JY, et al. Outer membrane protein A (OmpA): A new pathogen-associated molecular pattern that interacts with antigen presenting cells-impact on vaccine strategies. *Vaccine* 2002; **20**: A23-A27.

[47]Benz I, Schmidt MA. Structures and functions of autotransporter proteins in microbial pathogens. *Int J Med Microbiol IJMM* 2011; **301**(6): 461-468.

[48]Ha NY, Cho NH, Kim YS, Choi MS, Kim IS. An autotransporter protein from *Orientia tsutsugamushi* mediates adherence to nonphagocytic host cells. *Infect Immun* 2011; **79**(4): 1718-1727.

[49]Hor L, Pilapitiya A, McKenna JA, Panjikar S, Anderson MA, Desvaux M, et al. Crystal structure of a subtilisin-like autotransporter passenger domain reveals insights into its cytotoxic function. *Nat Commun* 2023; **14**(1): 1163.

[50]Koralur MC, Ramaiah A, Dasch GA. Detection and distribution of Sca autotransporter protein antigens in diverse isolates of *Orientia tsutsugamushi*. *PLoS Negl Trop Dis* 2018; **12**(9): e0006784.

[51]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 Negl Trop Dis* 2015; **9**(3): e0003585.

[52]Nguyen YTH, Kim C, Kim HI, Kim Y, Lee SE, Chang S, et al. An alternative splicing variant of the mixed-lineage leukemia 5 protein is a cellular adhesion receptor for ScaA of *Orientia tsutsugamushi*. *mBio* 2022; **14**(1): e01543-22.

[53]Ha NY, Shin HM, Sharma P, Cho HA, Min CK, Kim H, et al. Generation of protective immunity against *Orientia tsutsugamushi* infection by immunization with a zinc oxide nanoparticle combined with ScaA antigen. *J Nanobiotechnol* 2016; **14**(1): 76.

[54]Nguyen YTH, Kim C, Kim Y, Jeon K, Kim H, Ha NY, et al. The *Orientia tsutsugamushi* ScaB autotransporter protein is required for adhesion and invasion of mammalian cells. *Front Microbiol* 2021; **12**: 626298.

## Publisher's note



Edited by Zhang Q, Lei Y, Pan Y