

REVIEW ARTICLE



Orientia tsutsugamushi: Diversity and Distribution in India: A Review

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Scrub typhus is a disease caused by *Orientia tsutsugamushi* which is an obligate, intracellular gram-negative bacterium. It is endemic to a part of the world known as "tsutsugamushi triangle." This extends from Japan, China, Taiwan, South Korea, Nepal, Pakistan, Papua New Guinea, Australian states of Queensland and Northern New South Wales. In India, it has been recognized for several years and the disease widely spread all over the country. The severity of scrub typhus disease has been reported to be strain dependent in humans. *Orientia tsutsugamushi* strains have been examined by various research tools. PCR amplification and sequencing of 56-kDa antigen gene is one of the most advanced tool in identifying *O. tsutsugamushi* strains and genotypes. In the present review, English-language literature for reports of *Orientia tsutsugamushi* genotype diversity and distribution in India was searched using PubMed and Google Scholar databases. The search terms scrub typhus or mite typhus or tropical typhus or tsutsugamushi disease or *Orientia tsutsugamushi* or *Rickettsia tsutsugamushi* were used in combination with the term genotypes or strains or serotypes. The result of present review revealed limited information related to genotype diversity and distribution in the country. Understanding a more detailed regional genetic diversity is required in region-specific vaccine development strategies and sero-diagnostics tools.

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Introduction

Scrub typhus also called tsutsugamushi disease is an acute, febrile illness among humans caused by the infection of a gram-negative bacterium *Orientia tsutsugamushi* following the bite of infected mites.^{1,2} *Orientia tsutsugamushi* is an obligate, intracellular bacterium and the genus *Orientia* belongs to the order Rickettsiales within the family Rickettsiaceae. In addition to scrub typhus caused by *O. tsutsugamushi*, the rickettsial (spotted fever and typhus group), ehrlichial, and anaplasma diseases

are also included within the order Rickettsiales.³ Scrub typhus is endemic to an area of the Asia-Pacific rim, extending from Afghanistan to China, Korea, the islands of the south-western Pacific, and northern Australia.⁴ It threatens one billion people globally and causes in about one million people each year.⁵ Transmission of the agent to the human incidental host occurs during feeding of the parasitic larval or "chigger" of mites belonging to the genus *Leptotrombidium*. Vertical or transovarial

transmissions are essential in maintaining the infection in nature; thus, the mite serves as vector as well as reservoir. Clinical presentation in humans vary from mild to fatal disease, with varying mortality rates ranging between 35%–50% in the pre-antibiotic era.^{6,7} Within 5 to 14 days after being bitten by a vector, *Leptotrombidium* mite, patients begin to exhibit non-specific flu-like symptoms, fever, rash, eschar at the bite site, headache, myalgia, cough, generalized lymphadenopathy, nausea, vomiting, and abdominal pain.^{8,9} Fever and headache are the most common symptoms among the patients. Several studies showed 95% to 100% of confirmed cases were noted to have fever.^{10,11} Currently, there is no vaccine available against scrub typhus, therefore effective management highly relies upon rapid diagnosis and antibiotic treatment with doxycycline, tetracycline, or chloramphenicol.¹² The disease has been recorded in writings from as early as 313 a.d. in China and in later descriptions from the early 1800s in Japan.^{4,13}

In India, scrub typhus was recognized as a typhus-like fever since 1917.^{5,14} During World War II and the 1965 Indo-Pak war, scrub typhus was a major cause of fever among military personnel along the country border. There was a resurgence of the disease at the Pakistan border of India in 1990. It is still an under-diagnosed disease in India.¹⁵ It occurs all over India, from South to Northeast and Northwest India. Cases have been reported from the state of Karnataka, Kerala, Maharashtra, Tamil Nadu, Himachal Pradesh, West Bengal, Bihar, Jammu and Kashmir, Uttaranchal, Rajasthan, Meghalaya, and Nagaland.¹⁶⁻

¹⁹ *Leptotrombidium deliense* is the primary vector of *O. tsutsugamushi*.²⁰ Some studies revealed that majority of scrub typhus patients in India are uneducated living in rural areas.^{15,16}

The purpose of this review is to provide information currently associated with the diversity and distribution of *Orientia tsutsugamushi* strains and genotypes in India. It is our belief that understanding of the present status of *Orientia tsutsugamushi* diversity at different geographical areas in the country must be considered when implementing future research strategies.

Methods

In the present studies, the electronic databases related to scrub typhus and its causative agent, *Orientia tsutsugamushi* in India were searched in the PubMed and Google Scholar databases. The following search terms were used: scrub typhus or mite typhus or tropical typhus or tsutsugamushi disease or *Orientia tsutsugamushi* or *Rickettsia tsutsugamushi*. These terms were combined with the terms: genotypes or strains or serotypes. No unpublished literature or conference abstracts were included. Only English language peer-reviewed articles were included in the study.

Strain variation in *O. tsutsugamushi*

The severity and clinical presentation of scrub typhus have been reported to be strain dependent in humans.^{21,22} The nature of antigens of *Orientia tsutsugamushi* strains was examined by various assays and research tools. Methods of serological characterization include toxin and serum neutralization, complement fixation (CF), direct fluorescent antibody assay (DFA) and indirect fluorescent antibody assay (IFA). Strain identification by use of these serological techniques requires comparison with established "prototypes" available at the time of the study. Early CF characterization compared only three distinct strains, Karp, Gilliam, and Kato with new isolates,^{23,24} whereas IFA and DFA analyses included selected Thai strains that were also shown by CF, IFA, and DFA to be antigenically distinct.²⁵⁻²⁷ More recently, genetic analysis of the 56 kDa cell-surface antigen gene of *Orientia* species has resulted to differentiate "genotypes".²⁸ These molecular techniques include PCR in conjunction with gel electrophoresis, restriction fragment-length polymorphism (RFLP) mapping, and sequencing for comparison of products from the same genes of multiple *Orientia* strains. Complete DNA sequencing of Boryong and Ikeda strains have been done.^{29,30} As whole-genome sequences of *Orientia* strains are available, the enormous scope of strain variation is becoming more evident, and also resulting in increased interest in the species status of strains within the genus.^{29,31}

O. tsutsugamushi in India

Scrub typhus epidemics have been reported from various parts of North, South, and Eastern India.³² It account for approximately 50% of cases with undifferentiated febrile illness in many parts of the country especially during cooler season.³³ However, due to the limited access to laboratory tests in the country, diagnosis of scrub typhus is difficult.^{33,34} Introducing advanced diagnostic methods would highly improve detection of the disease.³⁵

Prototypes and serotypes of *O. tsutsugamushi* have been identified based on the variation in 56 kDa type-specific surface antigen. Gilliam, Karp and Kato are considered as prototypes, while Kuroki, Shimokoshi, Irie, Hirano, Kawasaki, Boryong etc. are considered genotypes.³⁶⁻³⁸ There are few reports indicating variation in clinical presentation and severity of scrub typhus based on the infecting strain of *O. tsutsugamushi*.²¹ Studies in mice model demonstrated differences in the virulence of *O. tsutsugamushi* strains, and the strains were classified as a high virulence group (Karp, Kato and KN-3 genotypes), intermediate virulence group (Gilliam genotype) and a low virulence group (Kuroki, Kawasaki and KN-2).³⁹ Considering the differences in clinical manifestations, it is important to investigate

genotypic distribution across endemic areas and their association with clinical manifestations. A number of scrub typhus cases have been reported from several geographic areas of India. However, there is limited information on the genotypes of *O. tsutsugamushi* and the associated clinical presentations.

Patricia et al., (2017)⁴⁰ has conducted studies on the occurrence of *O. tsutsugamushi* genotypes in Puducherry and surrounding areas of Tamil Nadu state. In their studies, genotyping was done based on the variation of 56 kDa antigen gene using nested PCR. They have reported Gilliam and Karp prototypes along with other genotypes such as Kuroki, Boryong, Chuto and Kato. Their findings also indicated that Karp is a dominant prototype in the study areas. It was also revealed that each genotype is associated with a different set of clinical manifestations with some of them showing a wider spectrum of manifestations involving multiple organs and more severe manifestations like hypertension.

During the year 2009 and 2010, Varghese et al.⁴¹ conducted studies on the genetic variability of *O. tsutsugamushi* in Christian Medical College, Vellore, Tamil Nadu. The 410 bp region of the 56 kDa TSA was amplified and sequenced for genotyping the causative bacteria. Based on the phylogenetic and sequence analysis, they have identified Kato (65%), Karp-like isolates (35%) and one Gilliam strain. It was also observed that majority of the isolates have 95-98% sequence similarity with *O. tsutsugamushi* isolates from Cambodia and Vietnam while some of the Kato-like isolates showed 90-95% sequence similarity to the Hualein 3 from Taiwan. Their findings clearly indicate that Keto-like and Karp-like strains were the predominant circulating ones in the study area. During the year 2010 to 2012, evaluation on genetic diversity of *Orientia tsutsugamushi* was carried out by Varghese and his colleagues in three different parts of India such as Christian Medical College in Vellore, Indira Gandhi Medical College in Shimla and Dr. H. Gordon Roberts Hospital in Shillong.⁴² Their evaluation includes 263 scrub typhus patients from the three study sites out of which 56 kDa PCR products from 130 patients were used for phylogenetic analysis. The result of their studies revealed that Kato-like strains predominated (61.5%) followed by Karp-like strains with 27.7% and Gilliam and Ikeda strains with 2.3% each. Four samples (3.1%) from Shillong resemble to Neimeng-65 and also four samples (3.1%) from Shimla were found similar to HIS-II. Kato-like and Karp-like strains were commonly distributed in the three study sites. Gilliam strain was detected in Vellore and Shillong, Ikeda in Vellore and Shimla whereas HIS-II was found only in Shimla. Sequence comparison of samples from the three study sites in their studies revealed separate clustering on the neighbor-joining tree. The Vellore strains forming a distant clade,

whereas Shimla and Shillong strains clustered more closely together. Mahajan et al. (2006)⁴³ also identified two genotypes of *O. tsutsugamushi* in Himachal Pradesh using 56-kDa antigen gene. The phylogenetic position of one genotype is between Karp and JP-1, and the other genotype is between JG type and Saitama.

Studies conducted to characterize the circulating strains of *O. tsutsugamushi* prevailing in North-eastern region of India revealed the circulation of different strains with a pre-dominance of Karp-like strains.⁴⁴ Molecular characterization of *Orientia* species was done using PCR amplification of partial 56-kDa, 47-kDa and 16SrRNA genes. Kumar et al. (2019)⁴⁵ reported genetic diversity of *Orientia tsutsugamushi* strains in north India covering different states such as Punjab, Haryana, Himachal Pradesh, Uttar Pradesh and Jammu and Kashmir. Molecular characterization of scrub typhus causative agent was done by nested PCR amplification of 483-bp region of the 56-kDa antigen gene. The PCR product sequences and others obtained from the GenBank database were used to construct a phylogenetic tree using neighbor-joining algorithms. The result of their studies revealed the presence of Karp-like strains predominating in all states studied comprising 64.7% which is followed by Gilliam-like (26.47%) and 8.82% similar to Hualien 1 or S072. However, Kato or Kawasaki-like strains were not detected in the areas at the time of study.

Usha et al. (2016)⁴⁶ conducted studies for the diagnosis of scrub typhus using the method of IgM ELISA and detection of *O. tsutsugamushi* serotypes in southern part of Andhra Pradesh. Nested PCR was also performed targeting 56-kDa antigen gene. Out of 230 samples detected, only Karp and Kawasaki serotypes were identified with Karp strain being predominant (70.43%). Dual infection with the two serotypes was also detected among seven patients. Studies from Himachal Pradesh also reported the presence of Kuroki serotype by serotype-specific PCR amplification.⁴⁷

Conclusion

Descriptive studies in the epidemiology and genetic diversity of *O. tsutsugamushi* strains is important in developing rapid diagnostics and vaccines in endemic areas. It will also be helpful in early recognition and treatment of the disease. Currently, the most commonly used method for strain identification of *O. tsutsugamushi* is sequence analysis of the 56-kDa type-specific antigen (TSA). With an open reading frame of 1,600 bp, the 56-kDa antigen contains 516-541 amino acids which are involved in host cell invasion. There are a number of studies conducted in India which employed 56-kDa antigen-based PCR method for strain and genotype identification of *O. tsutsugamushi*. However, a limited number of serotypes/genotypes have been

reported which include Karp, Gilliam, Kuroki, Boryong, Chuto, Kato, Ikeda, Hualien and Kawasaki. Based on the information available, Karp-like *O. tsutsugamushi* strain seemed to be the most commonly detected and widely distributed strain in the country followed by Kato-like strain.

Scrub typhus cases has been reported from different states of India including Tamil Nadu, Bihar, Maharashtra, Kerala, Karnataka, Andhra Pradesh, Jammu Kashmir, Rajasthan, Meghalaya, Sikkim, Himachal Pradesh, Uttara-ranchal, Mizoram and West Bengal.^{43,48-57} Although there are a large number of reports on the occurrence of scrub typhus throughout India, information related to genotype diversity and geographical distribution in the country is limited. Scrub typhus cases have also been reported from neighbouring countries. However, limited information in genotype data from these neighbouring countries is still creating difficulties in comparative studies. The prevalence of antigenic diversity must have implications in the development of vaccine. Thus, understanding regional genetic diversity is crucial in region-specific vaccine development strategies as well as sero-diagnostics.

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Conflict of interest

The authors have no conflicts of interest concerning this work.

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