# Molecular confirmation of scrub typhus infection and characterization of Orientia tsutsugamushi genotype from Karnataka, India

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**Case Reports**

# Molecular confirmation of scrub typhus infection and characterization of *Orientia tsutsugamushi* genotype from Karnataka, India

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Scrub typhus or rural typhus is a mite-borne acute febrile illness caused by the bacterium *Orientia tsutsugamushi*[1]. Morphologically the bacterium is a gram-negative bacillus, measuring approximately 0.5 µm wide and 1.2–3 µm in length[2]. The disease is maintained in nature by rodents, and in vectors by transovarial transmission[3]. Clinical diagnosis of scrub typhus is often difficult due to overlapping signs and symptoms with other tropical febrile illnesses. The disease commonly presents with acute fever, often associated with headache, vomiting, myalgia, cough, nausea and breathlessness. An eschar can be observed at the site of chigger larval mite bite but can be variably present across endemic countries. Diseases can range from self-limiting to mild to fatal illness. Complications in severe cases include multiorgan dysfunction syndrome (MODS), acute respiratory dysfunction syndrome (ARDS), meningoencephalitis, hepatitis and myocarditis with shock[4]. Molecular techniques aid in detecting the disease at an early stage of the illness, furthermore helping to understand the genetic relatedness of the causative agent to other genotypes. Antigenic heterogeneity is common in *Orientia* and is known to play a role in the disease's severity[5]. To date, >20 antigenically distinct strains have been identified, including the initially characterized prototypic strains of Karp, Gilliam, and Kato by Kelly *et al*[6]; and Shishido *et al*[7]. Scrub typhus has not been reported from Karnataka by direct evidence and the infecting genotype/serotype, has not been identified to the best of our knowledge. In addition, there is also a reemergence of scrub typhus in India, which interested us to look closer into the causative organism[8]. We present here, the first molecularly confirmed case of scrub typhus and the infecting genotype from a patient blood sample from endemic foci in Karnataka, southern India.

*Case report*

A 30-yr-old male patient was admitted to Kasturba Hospital, Manipal during the month of June 2012. The patient presented with high grade fever associated with chills, rigor, sweating, generalized myalgia, backache and headache that had persisted for a week. In order to determine the possible causes of acute febrile illness, clinical materials and general information was collected after obtaining informed consent. His appetite and sleep were both decreased and he had no history of recent travel. He had no comorbidities, except for a somatoform anxiety disorder which was diagnosed in 2008, but for which he had not been taking the prescribed amitriptyline treatment, causing a recurrence of symptoms. On examination, the patient was conscious and cooperative, with a pulse 90 beats per minute. His blood pressure was 110/70 mmHg, and the temperature was 38.4°C. His cardiovascular, abdominal, and central nervous system examinations were normal, and his lung fields were clear. A blanching rash was present over his chest. Laboratory investigations at the time of admission were: hemoglobin—12.2 g/dl, platelet count—$93\times10^3$/µl, white blood count (WBC) $2.5\times10^3$/µl (55% neutrophils), blood urea—38 mg/dl, serum creatinine—0.9 mg/dl, random blood sugar—165 mg/dl, serum sodium—136 mmol/l, and serum potassium—3.7 mmol/l. His liver functions were only mildly deranged with aspartate transaminase (AST)—70 IU/L, alanine transaminase (ALT)—49 IU/L, alkaline phosphatase (ALP)—75 U/L, total bilirubin—0.5 mg/dl, and direct bilirubin—0.2 mg/dl. His urine examination was normal. Suspecting fever due to common tropical febrile illness, investigations such as blood cultures, quantitative buffy coat for malaria, dengue IgM ELISA, leptospira IgM ELISA, Widal test, and tests for scrub typhus were performed according to the standard protocols. All the investigations performed were negative, except for scrub typhus specific investigations. A titer of 1:160 was observed for OX-K antigen, while a titer of 1:320 was observed for OX-2 antigen. Serological and molecular con-

firmation of the disease was done using immunofluorescence assay (IFA) and nested PCR for the 56-kDa type specific antigen TSA. Nested PCR was performed as previously described by Furuya *et al*[9]. An ultrasonography of the abdomen showed mild splenomegaly. He was started on a regimen of ceftriaxone and doxycycline, along with other supportive treatment that included antipyretics and nonsteroidal anti-inflammatory drugs (NSAIDs) for fever and myalgia. A psychiatric consultation was sought for his somatoform disorder and he was started on duloxetine in parallel to the antibiotic treatment. With his improved clinical status, he was discharged on Day 5 and was advised to continue doxycycline for three more days.

To exclude possible coinfection with other rickettsial infections, the patient sera was antibody screened for the spotted fever group species *Rickettsia australis*, *R. honei*, *R. conorii*, *R. africae*, *R. rickettsia*, and *R. akari*, and the typhus group species *R. typhi* and *R. prowazekii* using customized rickettsial IFA slides supplied by the Australian Rickettsial Reference Laboratory, Victoria, Australia. The patient's serum was negative for all of the above antigens indicating that the significant titer of OX-2 was false-positive. There was no sero-reactivity to Weil-Felix OX-19 antigen.

The 483 base pair amplified product by nested PCR corresponded to the partial coding sequences of the 56-kDa TSA gene of *O. tsutsugamushi*. Sequence analysis was performed with the DNA Baser software packages DNA Baser Sequence Assembler v4 (2013), Heracle BioSoft (*http://www.DnaBaser.com*) and designated as KMC001 in the study. Sequence identified in this study was deposited in GenBank under accession number KT345167. The 56-kDa sequences of the established genotypes were collected from the NCBI database. The clinical isolate sequence was aligned with the common genotype sequences of *O. tsutsugamushi* and evolutionary analyses was conducted in MEGA6[10] for 1000 bootstrap replicates; values above 75 were considered significant (Fig. 1).

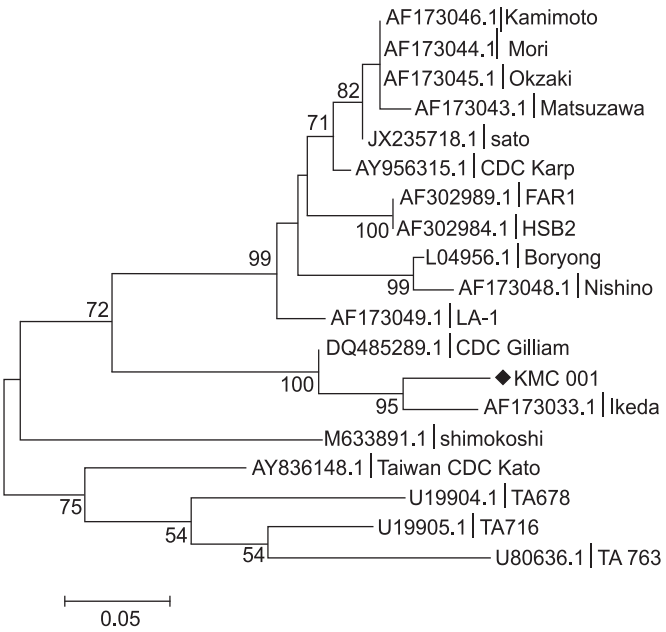


*Fig. 1:* Phylogenetic analysis of *Orientia tsutsugamushi* clinical isolate "KMC001" (indicated in black diamond shape) by maximum likelihood (ML) method based on Kimura 2-parameter model using the partial coding sequences of the 56-kDa TSA gene. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 19 nucleotide sequences. Codon positions included were I+II+III+ noncoding. All positions containing gaps and missing data were eliminated. There were a total of 417 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

## DISCUSSION

Case fatality due to scrub typhus can be as high as 30% in untreated cases[11]. Clinical and laboratory diagnosis of scrub typhus is often difficult due to overlapping clinical signs and symptoms and lack of sensitive and specific diagnostic assays respectively. Common clinical findings include fever with variable presence of rash, eschar and lymphadenopathy. Of late, scrub typhus cases are being reported in large numbers in India and probable reasons may be attributed to expanding mite islands and/or awareness about the disease among the medical fraternity.

In the present case, the patient's primary complaint was unresolved fever with a headache. As the patient was an agriculturist and had exposure to domestic animals, brucellosis was suspected, but due to the presence of lymphadenopathy and rash, the presumptive diagnosis shifted towards rickettsial diseases. The patient did not present with a pathognomonic eschar. All investigations for fever were negative except for that of scrub typhus. Based on the serological and molecular tests the patient was found to be infected with *O. tsutsugamushi*, and he responded dramatically to doxycycline therapy. False positive test results in Weil Felix has been observed in conditions such as leptospirosis, dengue and urinary tract infections due to *Proteus* species[12]. However, the present case had significant titer for OX-2 antigen without any of the probable reasons known for false positivity. Molecular methods provide direct evidence of the presence of the infective organism DNA in patients' blood on a single acute sample[13–14]. Among the genotypes of *O. tsutsugamushi* prevalent in India, there is very limited number of

studies and none from the state of Karnataka. Mahajan *et al*[15] have reported the presence of Kato, Karp, Kawasaki genotypes with two new genotypes, Karp and JP-1 types, and Saitama and JG types from the state of Himachal Pradesh. Varghese *et al*[16] reported that the predominant genotypes in their study were Karp and Kato, with a subset of Gilliam and Ikeda genotypes. In the phylogenetic analysis, our isolate clustered close to the Ikeda genotype, with significant bootstrap values, indicating the strain may be a unique genotype for which further characterization will define its lineage.

In conclusion, scrub typhus is one of the important cause of acute febrile illness in Karnataka, India. The present case was reported from the Davanagere district, in Karnataka state from where a large number of cases have been reported in recent years (unpublished data). Physicians in and around neighbouring districts of the Davanagere need to be aware of the disease prevalence, so that appropriate testing and treatment plans can be implemented. The infecting strain was phylogenetically closest to the Ikeda stain; however, there may be other genotypes in circulation that still need to be defined. A large-scale sero-survey and a hospital based cross sectional study on febrile illness would be imperative to determine the disease prevalence and confirm the common infective genotypes.

*Conflict of interest:* None to declare.

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