Scrub typhus is an acute rickettsial infectious disease caused by *Orientia tsutsugamushi*, a gram-negative intracellular coccobacillus. It is transmitted to humans and other vertebrates through the bite of the larval trombiculid mites (known as Chiggers) harbouring the etiological agent. The mites normally feed upon single vertebrate hosts, usually rodents. Febrile illness typically begins after the bite of an infected chigger and lasts for 7–10 days. Headache, lymphadenopathy, hepatosplenomegaly, rash, and an eschar usually develop in the infected person. Scrub typhus have been reported from Southeast Asia, Japan, Malaysia, Kampuchea, Thailand, Vietnam, Southeastern Siberia, Sri Lanka, Indonesia, Philippines, Korea, Western Pacific Islands, Pakistan, Astrakhan, India, and northern Australia forming the so called “tsutsugamushi triangle.” In India, epidemics of scrub typhus have been reported from states of Andhra Pradesh and neighbouring states, as well as from the sub-Himalayan states. The aim of the present study was to diagnose the clinically suspected scrub typhus patients serologically by Weil-Felix (WF) test, IgM ELISA and nested-PCR targeting 56-kDa type-specific antigen gene.

From January 2013 to July 2014, 113 blood samples of patients clinically suspected to have scrub typhus, attending Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, India were included in the study. Patients presented with intermittent fever and having five out of the following eight clinical features, namely headache, myalgia, malaise, cough, nausea, abdominal pain, lymphadenopathy, hepatosplenomegaly, rash and eschar were included in the study. Two ml of blood samples were collected without anticoagulant before the administration of empirical treatment with antibiotics. The Institutional Ethical Clearance was taken from Institute Ethics Committee, SVIMS, Tirupati, India. Serum and blood clot were separated and preserved at −80°C until processing. Demographic details of patients, the clinical course of illness and complications of infection were reviewed from the medical records.

The samples were tested serologically by two techniques. The WF test was performed with each sample by using in-house prepared *Proteus mirabilis* OX-K antigens. Detection of IgM antibodies against *O. tsutsugamushi* was performed by commercial ELISA kit (InBiOS International Inc., USA) as per manufacturer’s instructions. The IgM ELISA test was initially standardized using serum samples from healthy blood donors and the OD cut-off of 0.5 was taken 3±SD from the mean. Further, validation was done using known scrub typhus sera (confirmed by PCR) and sera from patients with other diseases like malaria and enteric fever and also healthy controls. A positive and negative control provided in the kit as well as in-house positive control for every run were used. Molecular analysis of the serologically positive samples were carried at Department of Virology, Sri Venkateswara University (SVU), Tirupati, India. DNA was extracted from blood clot of each sample by using the method as described by Furuya *et al.* The 56-kDa type-specific antigen (TSA) gene was amplified by nested-PCR (N-PCR) using the following primers:

**Primer set 1:**

m35-5′ TCA AGC TTA TTG CTA GTG CAA TGT CTG C 3′
m55-5′ AGG GAT CCC TGC TGC TGT GCT TGC TGC G 3′

**Primer set 2:**

m10-5′ GAT CAA GCT TCC TCA GCC TAC TAT AAT GCC 3′
m11-5′ CTA GGG ATC CCG ACA GAT GCA CTA TTA GGC 3′

Primer set 1 amplified a 1003 bp fragment and primer set 2 amplified 483 bp fragment. PCR products were electrophoresed in 1% agarose gel containing ethidium bromide (0.5 μg/ml) and visualized using a gel documentation system (BioRad, USA). The expected 483-bp products were purified by using QIAquick PCR purification kit (Qiagen, USA) according to the manufacturer’s instructions. The purified product was sequenced by Sanger’s dideoxy method on ABI 3730 × 1 automated sequencer. Samples were selected for sequencing, based on the expected fragment sizes and consistent cycle graph profiles.
Weil-Felix test showed more positives when compared with ELISA and N-PCR tests. It was also found that there was good agreement between WF test and ELISA when compared with N-PCR. So, WF and ELISA tests can be used in laboratories where PCR is not available. The scrub typhus ELISA is a flexible alternative to the IFA technique and have specificities and sensitivities of >90% for detecting specific-antibodies. The ELISA format is very convenient for large-scale testing in laboratory and takes about 50 min to perform. Since, serological tests have low sensitivities in the early stage of scrub typhus due to insufficient production of antibodies, frequent follow-up tests are needed. PCR methods when used independently or in conjugation with WF test can be employed as a specific diagnostic tool for diagnosis of scrub typhus in developing countries, aiding in the surveillance and effective treatment of this emerging infectious disease.

All the WF positive samples which were tested positive by N-PCR had titer of 1:160 or above whereas those which tested negative had a titer of 1:80. The strains obtained in our study, namely SVU/SVIMS CTR, SVU/SVIMS ANTPR, and SVU/SVIMS 28 formed a cluster close to Japanese Gilliam (JG) type comprising of Hualien-7, Taitung-2 and UT329 (Fig. 1).

During the study period it was found that most of the positive scrub typhus cases occurred during the months of October to December. Clinically suspected cases were tested by WF and IgM ELISA tests. Serologically positive samples were further confirmed by N-PCR assay. Despite of growing knowledge on epidemiology and phylogeny of Orientia strains in endemic regions, there is a true paucity of information from India. For genotyping we have targeted the 56-kDa protein gene as it is a major outer membrane protein containing both group-specific and type-specific epitopes, which are useful for diagnosis of scrub typhus. The genotyping method also has the advantage of detecting unknown serotypes that are not identified by assays based on reactions with monoclonal antibodies against known strains.

In the present investigation, we found that our study strains were similar to JG type that refers to “Gilliam type” in Japan on the basis of serological cross-reactions. But by the analysis of sequences by sequence similarity, JG was found closely related to Kawasaki, but not to Gilliam and forms significant group within Orientia species. Kawasaki type sequences have been found in Japan and China, whereas JG and JG-v sequences have been reported from Japan, China, and Southeast Asia. A study from Himachal Pradesh, India reported that their study strain sequences (ISS-1 and 2) closely matched the Japanese...
Gilliam cluster. In another study from India, among the two new genotypes that were causing scrub typhus in Himalayan regions, the phylogenetic position of one was between Karp and JP-1 while the other was between Saitama and JG type. Karp and Kato like strains have been reported in Vellore, Tamil Nadu by Vargheese et
In conclusion, it can be said that there exists genotypic variation among the strains prevalent in India which needs further evaluation.

REFERENCES


