



Mortality due to scrub typhus – report of five cases

Rama Chaudhry¹, Chandan Kumar Thakur¹, Nitin Gupta^{1,2}, Tanu Sagar¹, Tej Bahadur¹, Naveet Wig², Rita Sood² & Mahesh Chandra Misra³

Departments of ¹Microbiology, ²Medicine & ³Surgery, All India Institute of Medical Sciences, New Delhi, India

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Scrub typhus is largely ignored in India particularly during outbreaks of viral fever. The disease course is often complicated leading to fatalities in the absence of treatment. However, if diagnosed early and a specific treatment is initiated, the cure rate is high. We report here five cases of scrub typhus to highlight the fact that high clinical suspicion for such a deadly disease is an absolute necessity.

Key words Acute febrile illness - chikungunya - dengue - mortality - scrub typhus

Scrub typhus is an acute undifferentiated febrile illness caused by the bacterium *Orientia tsutsugamushi*, which belongs to the family *Rickettsiaceae*. It is transmitted by the larval stage of trombiculid mite (chigger)¹. The clinical presentation often mimics other common febrile illnesses such as dengue virus infection and chikungunya. This disease is grossly under-reported and under-diagnosed owing to the misconception that scrub typhus is only a concern in heavily forested areas. Associated complications in severe cases include hepatitis, myocarditis, acute respiratory distress syndrome (ARDS), multiorgan dysfunction syndrome (MODS), disseminated intravascular clotting with shock and several neurological manifestations^{2,3}. Scrub typhus if not treated early may have a mortality rate of up to 30-35 per cent⁴⁻⁶. We report here five cases of scrub typhus with fatal outcome due to multiorgan dysfunction presented to the department of Medicine, All India Institute of Medical Sciences, New Delhi, India. The study was approved by the Institutional

Ethics Committee and written informed consent was taken from all patients.

In September 2016, a 21 yr old male patient, resident of Delhi (State in central India), with no prior co-morbidities presented with fever for 10 days and progressive shortness of breath and altered sensorium for two days. It was followed by the second case of a 16 yr old female patient, resident of Uttarakhand (State in north India), who presented with a history of fever, altered sensorium, shortness of breath, arthralgia and decreased urine output for 10 days. The third case was a 30 yr old female patient, resident of Bihar (State in east India), with a history of fever associated with vomiting and pain in the abdomen for 11 days. The series of fatalities continued with two more cases in November 2016. A 24 yr old male patient, resident of Delhi, with no prior co-morbidities presented with a history of fever associated with headache, myalgia and generalized swelling of the body for eight days. In the same month, a 62 yr old female patient, resident

of Haryana (State in north India), was referred from a private hospital with a history of fever for six days. She also had abdominal pain, dyspnoea and altered sensorium for one day. All these five patients succumbed to multiple organ dysfunctions. The clinical features and routine laboratory investigations of all these five patients are given in the Table.

Since these patients presented during the ongoing chikungunya outbreak in Delhi, serological tests were done first to rule out chikungunya and dengue followed by leptospirosis, malaria, enteric fever, hepatitis and scrub typhus to rule out other differentials. Scrub typhus IgM rapid flow assay (InBios International, Inc., Washington, USA) and ELISA (InBios) were positive for all five patients. This was confirmed by indirect immunofluorescent antibody assay (IFA) (Fuller Laboratories, Fullerton, USA) which is considered gold standard. Predetermined cut-offs for ELISA and IFA in New Delhi were used⁷. The whole blood sample was subjected to nested PCR targeting 56 kDa antigen and real-time PCR assay (qPCR) targeting 47 kDa antigen. DNA was extracted from 200 µl of whole blood using the QIAmp blood minikit (Qiagen GmbH, Germany). DNA amplification was performed by nested format and qPCR using conditions described by Furuya *et al*⁸ and Jiang *et al*⁹, respectively.

Of these five patients, three were found to be positive by both nested PCR targeting 56 kDa antigen and real-time PCR targeting 47 kDa antigen (Table). PCR product was sequenced and deposited in NCBI GenBank under accession numbers -

KY319228, KY319229 and KY319231. Basic Local Alignment Search Tool analysis (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) revealed homology with *O. tsutsugamushi*. Phylogenetic tree was drawn, with branch lengths in the same units as those of the evolutionary distances used to infer the tree. The evolutionary history was inferred using the UPGMA method¹⁰. The optimal tree was constructed with the sum of branch length=0.29878659. The evolutionary distances were computed using the maximum composite likelihood method¹¹. Phylogenetic analyses involved 13 nucleotide sequences and were conducted in Molecular Evolutionary Genetics Analysis software, version 4.0 (MEGA4, USA) (<https://www.megasoftware.net/mega4/>). Of the three sequences, two were clustered close (100%) to Gilliam and one isolate (89%) with Karp genotype (Figure).

There have been concerns in the recent times regarding the specificity of IFA and the current gold standard for diagnosis of scrub typhus. The molecular assays like PCR have good specificity, but these are usually positive only in the first week of disease during the period of rickettsemia. The sensitivity of PCR beyond the first week of illness is low. Most of the patients presented late to the hospital explaining the negativity of PCR in two patients. Although Lim *et al*¹² described scrub typhus infection criteria (STIC) using a combination of culture, PCR assays and IFA IgM for diagnosis of scrub typhus, yet its applicability and feasibility in resource-limited setting remained questionable. Three of our five patients met

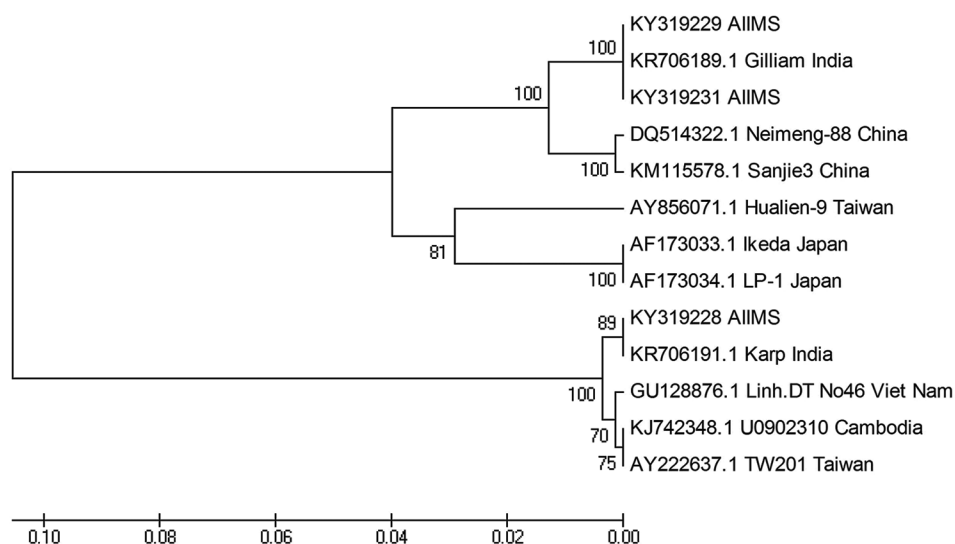


Figure. Phylogenetic analysis of *Orientia tsutsugamushi* clinical isolates. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Table. Clinical, haematological, biochemical and microbiological details of the five cases of scrub typhus

Clinical/laboratory parameters	Reference range	Case 1	Case 2	Case 3	Case 4	Case 5
Complications	-	ARDS, shock, myocarditis	AKI, ARDS	Transaminitis, AKI	AKI, ARDS	ARDS, AKI
Treatment	-	Ceftriaxone, artesunate, doxycycline	Ceftriaxone, azithromycin, doxycycline	Ceftriaxone, piperacillin-tazobactam, doxycycline	Levofloxacin, cefoperazone-sulbactam, doxycycline	Ceftriaxone, amikacin, doxycycline
Death (day post-admission)	-	Second day	Third day	Fifth day	Third day	Second day
Haemoglobin (g/dl)	12.0-16.0	11.2	4.9	7.9	9.9	9.8
TLC (per µl)	4500-11,000	9800	3500	14,700	11,600	9800
Platelet count (per µl)	150,000-400,000	26,000	20,000	30,000	40,000	8000
Total bilirubin (mg/dl)	0.3-1.0	0.4	2.4	5.3	4.4	2.5
Direct bilirubin (mg/dl)	0.0-0.4	-	-	-	3.7	3.5
ALP (IU/l)	30-100	296	403	396	449	226
ALT (IU/l)	0-50	70	95	108	277	966
AST (IU/l)	0-50	149	315	290	483	2160
Total protein (g/dl)	6.0-8	-	4.6	5.1	5.6	-
Urea (mg/dl)	20-50	147	93	256	169	165
Creatinine (mg/dl)	<1.2	5.7	3.9	7.7	4.7	4.1
Sodium (mmol/l)	135-145	145	113	135	143	137.1
Potassium (mmol/l)	3.4-5	5.7	3.4	3.95	5.5	4.81
CRP (mg/l)	<8.0	-	-	-	129.22	100
ESR (mm/h)	0-25	-	-	-	40	55
IFA titre [‡]						
Kato	≥64	512	512	512	512	256
Karp	≥64	512	256	256	256	256
Boryong	≥64	512	512	512	512	256
Gilliam	≥64	512	256	512	512	256
IgM ELISA [§] (OD)	≥0.89	2.89	2.85	1.28	3.12	1.512
RFA IgM	-	+	+	+	+	+
Nested PCR (56 kDa)*	483 bp	+	-	+	+	-
qPCR (47 kDa)*	118 bp	+	-	+	+	-

ARDS, acute respiratory distress syndrome; AKI, acute kidney injury; TLC, total leucocyte count; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IFA, immunofluorescent antibody assay; OD, optical density; RFA, rapid flow assay

the STIC criteria. The other two patients could not meet the criteria because we could not perform all the recommended tests or dilutions due to feasibility issues. Besides, all five patients met the Indian criteria for diagnosis of scrub typhus¹³.

There have been reports of emergence/re-emergence of scrub typhus in locations where it was previously unknown¹⁴. Although scrub typhus is known to be endemic in India, the suspicion and identification of this disease have been neglected for long. Similar seasonal prevalence (post-monsoon period) of other febrile diseases and overlapping clinical manifestations also contribute to misdiagnosis. During 2016, there was an upsurge in the total number of cases where scrub typhus was suspected. A total of 317 samples were tested between August and November 2016 at the time of ongoing chikungunya outbreak¹⁵, of which 34 were found to be positive by IFA and ELISA (13 PCR positive) including these five fatal cases.

The mortality due to scrub typhus depends on the geographical location, pathogenic strain involved and various host factors such as age, immune status and the co-morbidities. In a systemic review by Taylor *et al*¹⁶, higher mortality was seen in patients over 30 yr of age and was highest among patients over 60 yr. The presence of specific clinical symptoms, namely myocarditis, haemorrhagic, neurological or pulmonary symptoms may also contribute to the higher mortality among patients. Among the cases we report here, it was worth noting that four patients were under 30 yr of age and none of them had the presence of eschar, but all had multiple complications (cardiological, pulmonary or neurological), which might have contributed to their unfortunate outcome. Doxycycline is universally the drug of choice for treatment of scrub typhus irrespective of the day of presentation. However, it might be ineffective if the treatment is delayed. In our case, all patients were referred late and were already in MODS which could not be reversed inspite of initiation of effective treatment.

In conclusion, scrub typhus need to be included in the differential diagnosis of patients presenting with acute febrile illness even in the absence of characteristic eschar. High index of clinical suspicion should be kept, particularly during viral outbreaks where the diagnosis can be missed. Timely diagnosis and appropriate management can prevent fatal outcomes associated with scrub typhus.

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For correspondence: Dr Rama Chaudhry, Department of Microbiology, All India Institute of Medical Sciences, 2059, II Floor, Teaching Block, New Delhi 110 029, India
e-mail: drramach@gmail.com