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Original Research Article

Clinical, Laboratory, and Molecular Profiling of Scrub Typhus in Southern Rajasthan: A Study of Clinical Spectrum, Strain Variability, and Disease Outcomes

Rambabu Sharma[1], Jyoti Tomar[2], Sanjay Kumar Datta[3]

[1]Ph.D. Scholar, Department of Microbiology, PIMS, Udaipur

[2]Associate Professor, Department of Microbiology, PIMS, Udaipur

[3]Professor, Department of Microbiology, PIMS, Udaipur

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Corresponding Author: Dr. Rambabu Sharma

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Abstract:

Background: Acute Undifferentiated Febrile Illness (AUFI) is a significant health concern, especially in tropical regions like India, where it poses diagnostic challenges due to its nonspecific symptoms. Scrub typhus, caused by Orientia tsutsugamushi, is a common but underdiagnosed infectious disease in these regions, leading to severe complications and high mortality if untreated.

Objective: To characterize the clinical, demographic, and molecular features of scrub typhus in southern Rajasthan, India, and assess the correlation between laboratory parameters, strain identification and patient outcomes.

Material & Methods: A prospective, analytical study was conducted at the Pacific Institute of Medical Sciences, Udaipur, from November 2020 to November 2024. Blood samples from patients with acute febrile illness were tested for scrub typhus using serological (ELISA) and molecular (PCR) diagnostic methods. Clinical features, demographic data, laboratory parameters, strain variability and disease outcome were analyzed.

Results: A total of 350 scrub typhus cases were confirmed. The majority of patients (55.71%) were female, with the highest prevalence observed in the 21-50 years age group (68.28%). Rural and tribal backgrounds predominated (86% combined). The most common clinical manifestations were fever (100%), myalgia (98%), headache (88.57%), and rash (75.14%). Eschar was present in 23.71% of cases. Hepatomegaly and splenomegaly were observed in 41.14% and 41.42% of cases, respectively. Laboratory findings included raised liver enzymes (33.14% for SGPT and 32% for SGOT), thrombocytopenia (36.85%), and raised ESR (48%). Complications: Major complications included ARDS (38.28%), Multi-organ Dysfunction Syndrome (MODS) (40.28%), jaundice (31.14%), and acute kidney injury (17.42%). The mortality rate was 14%, with male patients (18.06%) showing higher mortality than females (10.76%). Agricultural workers had the highest mortality (15.83%). Laboratory parameters correlated significantly with mortality, with elevated ESR, urea, creatinine, SGOT, SGPT, and bilirubin levels in deceased patients. Molecular Findings: PCR sequencing revealed the presence of Kato-like genes in 70% of deceased patients and Karp-like genes in 80%. The Gilliam strain was not detected in any sample.

Conclusion: Scrub typhus is a significant cause of febrile illness in southern Rajasthan, with high mortality, particularly among males and agricultural workers. Early diagnosis, prompt treatment, and management of complications are crucial for improving patient outcomes. Molecular studies reveal strain-specific variations that may influence disease severity and outcomes.

Keywords: Scrub Typhus, Acute Febrile Illness, Orientia tsutsugamushi, Mortality, Molecular Diagnosis, ELISA, PCR, Southern Rajasthan.

Introduction

Acute undifferentiated febrile illness (AUFI) is a serious public health problem particularly in the developing countries where it affects both urban and rural communities having limited healthcare infrastructure and resources. [1] AUFI is characterized by sudden onset of fever with associated symptoms which are unable to establish immediate cause as per initial clinical assessment. This is common in many tropical and subtropical areas. In India Acute Febrile Illness epidemics have been a major source of concern for Indian health officials, because most of these illnesses pose considerable public health challenges. [2] Therefore, such severe diseases as malaria, Japanese encephalitis, leptospirosis, rickettsiosis, enteric fever, dengue fever and other infectious diseases should be considered under differential diagnosis for AUFI in these areas. [3] Epidemiological data show that viral infections contribute much to acute febrile diseases that can be prevented through vaccination and controlled by vector management strategies. [4]

Acute Febrile Illness (AFI) is characterized by a sudden onset of fever lasting less than 14 days and may result from various infectious or non-infectious causes. [5-9] Among the infectious etiologies is scrub typhus, caused by Orientia tsutsugamushi, an obligate intracellular gram-negative bacterium from the family Rickettsiaceae. [10] This pathogen, known for over 200 years, was first described as "Japanese flood fever" by Hashimoto in 1810 and later by Baelz & Kawakami in 1879. [11]

Scrub Typhus was identified by the World Health Organization (WHO) as one of the most unrecognised, ignored and serious public health problems in Asia-Pacific region. [12] Seroprevalence studies indicate that between 9-31% of population are affected in different Asian countries. [13-17] It is estimated that one billion people are at risk of the disease in endemic regions, with almost a million cases every year. Traditionally, scrub typhus has been endemic to the “tsutsugamushi triangle” which includes parts of East and Southeast Asia. [18] The disease covers approximately 13 million square kilometers of the Asia–Pacific rim extending from Afghanistan to China, Korea, south western pacific islands, and north Australia as it is endemic. [19]

Scrub typhus is transmitted through bites from infected mites (chiggers), which act as both vectors and reservoirs [20]. These mites are more common in densely vegetated areas during the rainy season. The disease is region-specific and presents symptoms such as high fever, headache, muscle ache, rash, swollen lymph nodes, coughing, and digestive issues, particularly in tropical regions like India.[21] Scrub typhus has been recognized in India since ancient times. Now, India has seen a significant rise in scrub typhus cases, making it a leading cause of severe febrile illness with high mortality rates. Notable outbreaks include southern India (2001-2002) and Himachal Pradesh during autumn. Epidemics have also been reported in Tamil Nadu, Pondicherry, Goa, Assam, Nagaland, and other regions. [22-26]

Scrub typhus is challenging to diagnose due to its nonspecific symptoms, including fever, headache, joint pain, rash, lymphadenopathy, respiratory and gastrointestinal issues, and fatigue. A key feature is the eschar—a dark scab at the bite site—though not always present. Severe complications, if untreated, include ARDS, myocarditis, meningoencephalitis, septic shock, and multi-organ dysfunction syndrome (MODS). The variability in disease severity depends on the patient's immune response, bacterial strain, and timely intervention. [26] Diagnosis often requires serological tests (e.g., Weil-Felix, ELISA) or molecular tests (PCR), as symptoms can resemble other febrile illnesses like dengue or malaria. Prompt treatment with antibiotics like doxycycline or azithromycin is crucial to prevent severe outcomes.

The antigenic diversity of Orientia tsutsugamushi, due to its strain-specific outer membrane proteins (e.g., TSA56), complicates vaccine development and diagnostic tests. Common strains include Karp, Gilliam, and Kato, and the shift from “serotypes” to “genotypes” reflects advanced understanding of its genetic variability. Comprehensive research on strain diversity and regional distribution, alongside diagnostic and molecular studies, is essential for combating the disease. This study focuses on characterizing circulating serotypes in southern Rajasthan, India, using ELISA and PCR-based investigations.

Materials and Methods

Study Design: Prospective and Analytical study.

source of Sample: All age groups having acute under diagnosed febrile illness patients.

Study Place and Duration: Study was done at Department of Microbiology, Central Research Laboratory, Pacific Institute of Medical Sciences, Udaipur, Rajasthan, from November 2020 to November 2024.

Inclusion Criteria: All age group patients with acute under-diagnosed febrile illness presenting with or without eschar in our hospital were included in the study.

Exclusion Criteria: All other patients with co-tropical illness, such as dengue, typhoid fever, and malaria were excluded from the study. All duplicate patients were excluded from the study. Scrub typhus patients with chronic liver and CKD. Samples that were lysed, icteric or turbid were not included.

Statistical Analysis: The statistical analysis was carried out by using MedCalc software for window version 16. The continuous data was expressed as mean±SD. Descriptive statistics for the categorical variables were performed by computing the frequencies (percentage) in each category. The association between two categorical variables was analyzed by Chi-square test or student t-test as appropriate. P-value <0.05 considered statistically significant.

Ethical Approval: The study was approved by Institutional ethics committee of Sai Tirupati University, Udaipur, (Rajasthan), India by Approval number STU/IEC/2022/95.

Blood Sample Collection: Blood samples are collected for Serology, hematology, biochemistry, and molecular testing by appropriate aseptic collection techniques into three different sample collection tubes.

Sero-Diagnosis of Scrub Typhus by Elisa: The Scrub Typhus Detect ELISA system for IgM test is a diagnostic tool designed to detect IgM antibodies specific to O. tsutsugamushi in serum samples. The assay utilizes an enzyme-linked immunosorbent assay (ELISA) format, a common technique for detecting antibodies in biological samples. In this assay, the wells of each ELISA plate are coated with a mix of recombinant antigens derived from O. tsutsugamushi. These antigens serve as the target molecules for binding with IgM antibodies present in the serum samples. During testing, serum samples are first diluted in a sample diluent provided by J-Mitra, the manufacturer of the kit. These diluted samples are then applied to the wells of the ELISA plate.

After incubation to allow antigen-IgM binding, wells are washed to remove unbound components. Polyclonal goat anti-human IgM antibodies conjugated with HRP are added, binding to IgM attached to *O. tsutsugamushi* antigens. After further incubation and washing, TMB substrate is added, producing a blue color via HRP catalysis. An acidic solution stops the reaction, and absorbance is measured at 450nm, proportional to IgM concentration. Hematological tests (WBC, platelets, ESR) were performed using autoanalyzers (ERBA Elite 580, ALI FAX ROLLER 20), while biochemical tests (renal and liver function) were conducted using an ERBA XL 640 autoanalyzer.

Molecular Diagnosis of Scrub Typhus by Pcr:

Following sero-diagnosis of scrub typhus, the samples underwent molecular analysis. DNA was extracted from blood clots, and the presence of O. tsutsugamushi DNA in the blood was determined using a polymerase chain reaction (PCR) targeting the type-specific antigen gene. The expected amplicon size was 410bp. The oligonucleotide primers used were as follows: forward OtsuF: 5` - AATTGCTAGTGCAATGTCTG-3`; reverse OtsuR: 5`-GGCATTATAGTAGGCTGAC-3`. The PCR amplification mixture (final volume 25ul) contained dATP, dGTP, dTTP, 0.2 u M of primers and 1 U of Taq DNA polymerase. The reaction was carried out in a DNA thermal cycler (Bio Rad) machine.

Dna Sequencing

By the primer sequence of (5'-GAT CAA GCTTCCTCAGCCTACTATAATGCC-3'), and Primers (5'-CTAGGGATCCCGAC AGATGCACTATTAGGC-3'), which overlap, were utilized to amplify a 483-bp fragment of the 56-kDa TSA gene. The amplified DNA was purified with a QIAquick gel extraction kit (Qiagen) and sent to Studio Diagnostics (Genetix Asia Pvt Ltd, India) for sequencing. The sequencing was performed using an ABI Prism 3730xl DNA analyzer (Applied Biosystems, Foster, CA). The resulting sequence data were analyzed using bioinformatics tools. The sequence data were assembled and refined using the DNA Baser software package (DNA Baser Sequence Assembler v4, 2013, HeracleBioSoft, (www.DnaBaser.com). The organism was identified by analyzing the sequencing results through the BLAST tool available on the NCBI platform.

Results:

This study focused on the clinical features, diagnostic methods, and strain identification of scrub typhus in Southern Rajasthan, India, with 350 confirmed cases. A subset of 175 randomly selected samples was tested using PCR, and 158 samples were positive for scrub typhus. Further analysis of 59/158 PCR-positive samples identified *O. tsutsugamushi strains*, with the Kato-like gene found in 24 samples and Karp-like in 11 samples.

In the present study out of 350 confirmed cases of scrub typhus, female patients were predominance over males. Male patients constituted in number of 155 (44.29%) and females were 195 (55.71%). Male patients tended to be 41.4 years old, while female patients were 41.44 years old on average (**Table 1**).

The highest percentage of patients (68.28%) was observed in age group of 21-50 years. The percentage was highest in this age group due adult patients worked in filed had more chances of mite exposure. In the age group 51 and above had percentage of 22.86%. lowest percentage 8.86% was observed in age groups of 11 – 20 years.

Table 1: Sociodemographic characteristics of scrub typhus patients

Gender distribution (n=350)	Total (%)	Mean age
Male	155 (44.29%)	41.40 years
Female	195 (55.71%)	41.44 years
Distribution of patients according to residence		
Residence	**Total**	**%**
Urban	49	14%

Rural (non-tribal)	154	44%
Tribal (non-tribal)	147	42%
Distribution of patients according to occupation		
Housewife	24	6.86%
Farmers	240	68.57%
Mine worker/drivers	10	2.85%
Others	76	21.72%
Clinical features in scrub typhus patients		
Fever with chills	350	100%
Rashes	263	75.14%
Vomiting	60	17.14%
Lymphadenopathy	104	29.71%
Headache	310	88.57%
Myalgia	343	98%
Shortness of breath	221	63.14%
Diarrhoea	53	15.14%
Conjunctival suffusion	37	10.57%
Presence of eschar	83	23.71%
Hepatomegaly	144	41.14%
Splenomegaly	145	41.42%

Rural (non-tribal) patients were highest in number 154 (44%) followed by Trial (non-rural) patients 147 (42%). Lowest number of patients were observed in urban area 49 (14%).

Majority of the 240 (68.57%) scrub typhus cases were agriculturist by profession, followed by non-agricultural profession i.e. students 31 (8.85%). House workers 24 (6.85%), labour 23 (6.57%) and other job 22 (6.28%).

Scrub typhus is more frequent in autumn (57.14 percent) than summer (24.57 percent). Prevalence was highest in these months when the rains set in. Furthermore, there were 10.28% of cases recorded during November and December which are colder months. The incidence of cases reduces slowly from January to June, with a percentage decrease of about 7.99% (**Table 1**).

Among the varied clinical manifestations in scrub typhus, the common clinical findings were fever with chills, myalgia, headache, conjunctival chemoses. Breathlessness and rashes was commonly associated in female group than in male group

Fever was the predominant symptom present in 100% of the patients followed by Myalgia 98%, headache 88.57%, rashes 75.14%, shortness of breath 63.14%, lymphadenopathy 29.71%, vomiting 17.14%, diarrhoea 15.14% and conjunctival suffusion in 10.57% of the patients (**Table 1**).

Eschar was observed in 83 patients (23.71%) out of 350. Eschar was present mainly in female patients 47 (24.01%) over males 36 (23.22%).

Hepatomegaly was present in 41.14% among Scrub Typhus patients. Splenomegaly was present in 41.42% of the patients. No statistically significant difference was seen among parameters and gender (**Table 1**).

Table 2: Laboratory parameters in scrub typhus patients

Parameters	**No. of patients (n=350)**	**%**
Leucocytosis (WBCs >11,000)	104	29.71%
Thrombocytopenia (PLT <1,00000)	129	36.85%
SGOT (>45 IU/l)	112	32%
SGPT (>45 IU/l)	116	33.14%
Bilirubin Total (>1.2mg/dl)	51	14.57%
Bilirubin Direct (>0.4 mg/dl)	68	19.42%
Urea (> 45 mg/dl)	87	24.85%
Serum creatinine (>1.2 mg/dl)	89	25.42%
ESR (>20 mm/hr)	168	36.85%

Leucocytosis was observed in 29.71% of the patients, thrombocytopenia in 36.85% patients. ESR was raised in 48% patients. Serum glutamic pyruvic transaminase (SGPT), and serum glutamic oxaloacetic transaminase (SGOT) levels were found to be higher than the normal range in 33.14%, and 32% patients, raised level of urea in

24.85%, and creatinine 25.42% patients respectively (**Table 2**).

Complication in scrub typhus was observed i.e. ARDS, Jaundice, AKI (Creatinine>2.5mg/dl) and MODS. ARDS was present in 38.28%, MODS 40.28%, Jaundice 31.14%, and AKI 17.42%. Complications were present in 94 recovered patients (26.85%) and 40 (11.42%) in dead patients (**Table 3**).

Table 3: Complications in scrub typhus patients

Complications	No. of cases, (%)	Recovered No of cases, (%)	Death No of cases, (%)	p-Value
ARDS	134, (38.28%)	94, (26.85%)	40, (11.43%)	*<0.0001
Jaundice	109, (31.14%)	63, (18%)	46, (13.14%)	*<0.0001
AKI (Creatinine >2.5mg/dl	61, (17.42%)	16, (4.57%)	45, (12.85%)	*<0.0001
MODS	141, (40.28%)	92, (26.28%)	49, (14%)	*<0.0001

All the patients confirmed to have scrub typhus recovered except for 49 cases with a mortality of 14%. Twenty-eight (18.06%) cases were males and twenty-one (10.76%) of cases were female. Mortality was present in male patients 18.06% and females 10.76% (**Table 4**).

Table 4: Mortality according to gender wise among scrub typhus patients

Mortality	Gender distribution		No of patients (n=350)	Percentage
	Male (n=155)	Female (n=195)		
Present	28 (18.06%)	21 (10.76%)	49	14%
Absent	127 (81.94%)	174 (89.24%)	301	86%
Total	100%	100%	350	100%

Mortality was further distributed according to occupation of the patients. Highest percentage of mortality was observed among agricultural workers 15.83%, followed non-agricultural workers i.e. mine workers or drivers 40%, labour 17.38%, and other job employees 4.54%, and housewives 9%. No mortality was observed among paediatric patients (**Table 5**).

Table 5: Distribution of patients according to mortality and Occupation.

Occupation	Mortality Present		No of patients, %
	Male No of patients, %	Female No of patients, %	
Housewife (n=22)	NA	02, (9%)	02, 9%
Farmers (n=240)	20, (8.33%)	18, (7.50%)	38, 15.83%
Labour (n=23)	03, (13.04%)	01, (4.34%)	04, 17.38%
Employed (other job) (n=22)	01, (4.54%)	00, (0%)	01, 4.54%
Students (n=31)	00, (0%)	00, (0%)	0, 0%
Mine workers or Drivers (n=10)	4, (40%)	0, (0%)	4, 40%

Clinical features of scrub typhus were observed in mortality patients. Mainly presenting symptoms was fever with chills 49 patients (14%), myalgia 49 patients (14.28%), and headache 49 patients (15.80%). Followed by rashes 39 patients (14.82%), shortness of breath 36 patients (16.28%), lymphadenopathy 20 patients (19.23%), vomiting and conjunctival suffusion each 9 patients (15%), (24.32%), and diarrhea were present in 8 patients (15.09%) [**Table 6**].

Table 6: Correlation of mortality and presenting complaints among Scrub Typhus patients.

Presenting complaints	Death	Recovered
	No. of patients, %	No. of patients, %
Fever with chills (n=350)	49, (14%)	301, (86%)
Rashes (n=263)	39, (14.82%)	224, (85.18%)
Vomiting (n=60)	9, (15%)	51, (85%)
Lymphadenopathy (n=104)	20, (19.23%)	84, (80.77%)
Headache (n=310)	49, (15.80%)	261, (84.20%)
Myalgia (n=343)	49, (14.28%)	294, (85.72%)
Shortness of breath (n=221)	36, (16.28%)	185, (83.72%)
Diarrhea (n=53)	8, (15.09%)	45, (84.91%)
Conjunctival suffusion (n=37)	9, (24.32%)	28, (75.68%)

Mortality was correlated with laboratory parameters among scrub typhus patients. The mean value of ESR in mortality group were 31.75 SD±12.28, Platelet counts 49571 SD±28696, white blood cells 12846.93 SD±3213, Urea 69.79 SD±23.73, serum creatinine 3.11 SD±0.64, SGOT 155.48 SD±67.90, SGPT 220.30 SD±97.01, and bilirubin 2.64 SD±2.60. (p-value <0.05, a statistically significant difference was seen in mortality and laboratory parameters) [**Table 7**]

Table 7: Correlation of mortality and laboratory parameters

Presenting complaints	**Mortality present (n=49)**		**Mortality absent (n=301)**		
	mean	**SD**	**mean**	**SD**	**p- value**
ESR	31.75	±12.28	17.12	±10.75	<0.0001
Platelet counts	49571	±28697	159807	±76285	<0.0001
WBCs	12846.93	±3213	8274	±3144	<0.0001
Blood Urea	69.79	±23.73	32.39	±13.48	<0.0001
S. Creatinine	3.11	±0.64	0.87	±0.62	<0.0001
SGOT	155.48	±67.90	42.40	±29.86	<0.0001
SGPT	220.30	±97.01	50.68	±43.10	<0.0001
S. Bilirubin (Total)	2.64	±2.60	0.95	±0.79	<0.0001

In the presented table, Kato-like gene were present in 70% of dead patients, while 30% in alive cases. Karp-like gene (80%) were present in dead patients. Presence of Gilliam were not identified in all samples processed for sequencing (**Table 8**).

Table 8: Genotypes in scrub typhus patients

Genotype (n=59)	**Recovered %, (No.)**	**Mortality %**
Kato-like (n=24)	30%, (07)	70%, (17)
Karp-like (n=11)	20%, (02)	80%, (09)
Gilliam	0%	0%

Discussion

Scrub typhus is a significant but often overlooked infectious disease that is considered as one of the most common emerging and re-emerging diseases globally. It poses a considerable risk to a large population, with over one billion people estimated to be at risk, leading to approximately one million cases of scrub typhus annually [27]. In certain regions of India, scrub typhus is experiencing a resurgence and has been reported to account for up to 50% of cases of undifferentiated fever presenting to hospitals [28]. Without prompt diagnosis and treatment, the mortality rate from scrub typhus can be substantial, ranging from 30% to 45% in endemic countries.

Scrub typhus exhibits a seasonal trend, primarily prevalent during the post-monsoon season, likely due to increased growth of scrub vegetation facilitating favorable conditions for mite populations. In the present study, male patients constituted in number of 155 (44.28%) and females were 195 (55.72%). Female patients were outnumbered over males. The average mean age of male patients were 41.4 years and females 41.44 years. Varghese et al [29] reported that median age of scrub typhus patients in the southern part of India was 36.5 years (range: 12-75 years) nearly equal representation of men (48%) and women (52%). In my study, the highest number of patients were seen in the age group 41–50 years 29.42%, followed by age group of 31–40 years 27.14%. Majority of patients were females.

My study observation was consistent with the findings of Nadda N et al. [30] found that 31.03% of patients fell into the age group of 31-40 years, with a majority being females (72.4%). Sharma et al.,[31] also reported a higher incidence of scrub typhus in the age group of 30-40 years, especially among females.

Rajendra Prasad Takhar et al. [32] reported findings similar to this study, with 36% males and 64% females, mostly aged 20-50 years. Gupta et al. [33] found 54.6% males and 45.4% females, aged 4-69 years, with 48.4% in the 10-30 age group. Mallick SK et al. [36] noted most cases under 40 years, with 8.5% male and 12.15% female patients.

In my study, according to occupations majority of the patients were farmers 68.57% followed by housewife (6.85%), students (8.85%), and labour (6.57%). G M Varghese et al, [29] showed that among the total cases, farmers comprised 41%. Vijay Kumar et al [34] reported housewives as 28 (51.85%) patients.

In my study, most scrub typhus patients were from rural (44%) and tribal (42%) backgrounds, with a history of fieldwork. Housewives and school children were the major occupational subgroups. Vijay Kumar et al. [34] found a male predominance (56.1%) in 189 cases, with most patients from rural areas. Bihari Shyam et al. [35] reported 87.5% of cases from rural areas, where dense vegetation and agricultural work increase exposure to chigger mites, and limited healthcare access contributes to higher prevalence.

In my study, scrub typhus cases peaked in September-October (57.14%), followed by July-August (24.57%) and November-December (10.28%). Sporadic cases were observed in May-June (6%), March-April (1.14%), and January-February (0.85%). These patterns align with Mallick SK et al., [36] while Nadda et al. [37] and Sharma N et al. also reported the highest cases in September (39.66% and 31%, respectively).

In my present study, fever with chills was observed in majority (100%) of the patients which is similar to study conducted by Philomena J et al.[38] In my study, Myalgia were observed in 98%, followed by headache in 88.57%. other associated symptoms were rashes in 75.14% of the patients, breathlessness 63.14%, vomiting 17.14%, GI symptoms 15.14% and conjunctival suffusion 10.57%. Lymphadenopathy was observed in 29.71%. According to study done by Varghese GM et al [29], the most frequently reported symptoms among patients were fever, noted in all cases (100%), followed by nausea/vomiting in 54% of cases, shortness of breath in 49% of cases, headache in 46% of cases, cough in 38% of cases, and paresthesia in 26% of the cases.

My findings on scrub typhus align with Atry SK et al. [39] (2020), where fever was the most common symptom (100%), followed by shortness of breath (40%), nausea and vomiting (10%), and diarrhea (7.5%). Eschar was present in 15% of cases. SK S et al. [40] observed fever (100%), lymphadenopathy (93%), hepatomegaly (73%), and eschar (68%), among other signs. Pattnaik et al.[41] also found fever (100%) in their study, consistent with other regional studies, highlighting common clinical features of scrub typhus across different areas, similar to my study.

In my study, eschar was observed in 83 (23.71%) patients. The common sites of eschar finding were groin, axilla, chest, legs and other sites of the body. Some studies have been reported a wide range of eschar prevalence, ranging from 7% to 97% globally. For instance, a study conducted in South India by Varghese GM et al [29] reported an eschar incidence of 55%. A study done by Vivekanandan et al. 42 (46%), Chrispal et al. (45.5%) and studies from Vietnam, Taiwan, and Korea reported slightly higher incidences of eschar probably due to fair-skinned population of these studies, which increases the chances of finding eschar. [43]

In my study, rashes were observed in 75.14% of cases, while the highest incidence was seen in Japan (93%) and China (90.4%) [44-45]. This variation may be influenced by the O. tsutsugamushi genotype [46]. Regional lymphadenopathy was found in 29.71% of cases, similar to Zhang et al. [47] report of 26.5%. However, higher prevalences were noted by Mahajan et al. [48] (52.3%) and Hamaguchi S et al. (64.1%) [49].

In my study, hepatomegaly was observed in 41.14% of the patients, and splenomegaly in 41.42% of the patients. Further analysis of the patients revealed that 89.51% of the patients with hepatomegaly and 91% of those with splenomegaly also had MODS. A statistically significant difference was noted between these groups, indicated by a p-value of less than 0.05. Kumar Vivek et al. [50], reported hepatomegaly in their study was 69% of the cases, while splenomegaly was noted in 41% of the cases.

Hepatomegaly and splenomegaly in scrub typhus-infected patients are primarily caused by the systemic inflammatory response triggered by the *O. tsutsugamushi* bacterium. The bacterium's invasion leads to widespread endothelial cell damage and inflammation in various organs, including the liver and spleen. This inflammatory response results in increased vascular permeability, vascular leakage, and accumulation of immune cells in these organs. Additionally, direct invasion of the liver and spleen by the bacterium contributes to organ enlargement. The combination of these factors leads to hepatomegaly and splenomegaly, which are commonly observed clinical manifestations in patients with scrub typhus.

In my present study, thrombocytopenia was observed in 36.85% of scrub typhus positive patients. My observations regarding thrombocytopenia in scrub typhus are consistent with the findings of Zhang et al. in the Chinese population, who reported a prevalence of 25.4%. [47] Additionally, the mild nature of thrombocytopenia aligns with the description by Chrispal et al. [51] In contrast, higher rates of thrombocytopenia (79.0%) were reported by Varghese et al. [29] and Razak et al. [52], indicating significant variability in the prevalence of this condition across different studies.

Leucocytosis was seen in my study 29.71% of the scrub typhus positive patients and leukopenia in 1.42% of the patients. Vivekanandan et al. [42] reported leucocytosis at 30% in their study. Kim et al. also observed associated leucocytosis to severity of the disease by increased mortality [53].

In my study, elevated AST was observed in 32% of patients, ALT in 33.14%, and conjugated bilirubin in 14.57%, with hypoalbuminemia present in 55%. Rajapakse S et al. [54] reported higher liver enzyme levels, with elevated SGOT in 78%-89%, SGPT in 64%-92%, and ALP in 27%-84%. Lee et al. [55] found that elevated liver enzymes are linked to more complications, longer hospital stays, higher costs, and the need for more efficient management.

Complication in scrub typhus positive patients was observed in my study. MODS is the most common complication in my study patients 141 (40.28%) followed by ARDS in 134 patients (38.28%), Jaundice in 109 patients (31.14%), AKI in 61 patients (17.42%). A similar study was also done by Verghese GM et al, [56] reported ARDS a proportion of 43.5% of patients in their study. Razak et al. [52] documented ARDS in 10% of their scrub typhus cases. Conversely, Varghese et al. observed a higher prevalence of ARDS, with 33.7% of cases in their earlier study group experiencing this complication. [52]

In my study, acute respiratory distress syndrome (ARDS) incidence was 8%-10%, while acute kidney injury (AKI) was 17.42%, lower than Attur et al. (23.2%) and Mahajan et al. (66.4%) [10,19]. Meningoencephalitis (23.3%) was reported in Mahajan et al., higher than other Indian studies (9.5%-14%), but not observed in this study. Mortality varied, with meningitis cases at 6.2% and renal dysfunction at 57%. Multi-organ dysfunction syndrome (MODS) occurred in 40.28% of cases, similar to Atry SK et al. (37.5%) [39] and Varghese et al. (34%-38.3%) [17,57].

In the pre-antibiotic era, scrub typhus case-fatality rates were 35%-50%.[3,5] In this study, the mortality rate was 14% (49 cases), lower than Bonell et al. (24.1%)[58] and similar to Mahajan et al. (14.2%).[59] However, it was slightly lower than Lai et al. (15%-30%), Kumar et al., [60] and Rungta et al. (17.2%)[61] but higher than Atry SK et al. (10%)[154]. High mortality rates may result from delayed hospital admissions, leading to multiple organ failure before treatment.

In my present study, mortality was observed among non-agricultural workers i.e. mine workers or drivers (40%), labour (17.38%), followed by agricultural workers (15.83%), housewives 9%, other job employees 4.54%

Occupational exposure significantly increases the risk of scrub typhus, particularly for mine workers, drivers, and farmers aged 21-50 in rural areas. Drivers and mine workers face higher exposure due to extended road travel and visits to rural or forested regions, while farmers are at risk due to prolonged agricultural activities. Poor living conditions, delayed medical attention, and the prevalence of virulent strains further exacerbate disease severity and outcomes in these groups.

In my present study, clinical features of scrub typhus correlate with mortality cases. Mortality was present in (14%) of the patients. Chief complaints were fever 14%, myalgia 14.28%, and headache 15.8%, rashes 14.82%, shortness of breath 16.28%, lymphadenopathy 19.23%, vomiting 15%, conjunctival suffusion 24.72%, and diarrhoea 15.09%. Among recovered patients, common clinical features observed were fever 86%, rashes 85.18%, vomiting 85%, lymphadenopathy 80.77%, headache 84.2%, myalgia 85.72%, SOB 83.72%, diarrhoea 84.91%, and conjunctival suffusion 75.68%.

The 56-kDa protein of O. tsutsugamushi is highly prevalent, with unique and cross-reacting epitopes contributing to genetic diversity. PCR targeting the 56-kDa gene in eschar and blood samples is effective for early diagnosis. While nested PCR is well-studied, conventional PCR, though less explored, shows strong correlation with nested PCR and potential for molecular diagnostics.

Testing for different serotypes, such as Gilliam, Karp, and Kato, is essential because these strains are antigenically distinct (Ching et al., 2001). In my study, out of the 159 PCR positive patient samples, we were selected randomly 59 samples for sequencing and found positive only thirty-five samples further amplified using Karp and Kato specific primers. The remaining samples were not amplified by any other sets of specific primers. One possible reason for this could be the absence of Orientia-specific DNA in the blood samples due to the administration of specific treatment before sample collection, while the IgM antibody was still present in the blood. Another reason might be the lack of primers required to identify the diverse serotypes of this organism that exist in nature. This highlights the complexity and challenges in detecting all possible serotypes of *O. tsutsugamushi* accurately.

No specific data is available from Southern region of Rajasthan based on genomic sequencing of serotype specific antigen. In my study, Karp-like strains were observed in 19% of the cases, in which 80% strains observed from mortality patients. Kato-like strains were observed in 41% of the cases, in which 70% strains observed from mortality patients. My observation showed significant similarity to isolates from Southeast Asian countries including Malaysia (Kato-like 56%), Thailand (Kato-like 65%), Myanmar (Kato-like 46%) strains. [62-64] G.M. Varghese et al 2013, reported 65.3% Kato-like, 30.7% Karp-like and 3.8% Gilliam strains of scrub typhus.[170] Yang H H et al 2012, reported four prototype strains (Karp, Kato, Kawasaki and Gilliam) and eleven different Taiwanese genotype isolates. [65] In endemic areas there is great antigenic and genotypic diversity that complicating scrub typhus diagnosis. These findings show that genotype diversity might not always lead to distinct clinical presentations. Nonetheless, scrub typhus strain distribution knowledge is important for diagnostic purposes, development of future diagnostic tests and vaccine development. [66]

The antigenic and genotypic diversity of O. tsutsugamushi in endemic areas complicates scrub typhus diagnosis, often missed due to nonspecific symptoms and limited lab tests. Kim et al. noted differences in eschars, rashes, and other symptoms between Boryoung and Karp clusters, suggesting genotype-related variations. However, their study had few Karp cases, indicating genotypic variation may not always align with clinical presentations. The diagnostic challenge persists due to nonspecific signs and inadequate testing facilities.

Understanding the geographical distribution of scrub typhus strains is crucial for clinicians to diagnosing the disease. This information is also essential for developing future diagnostic tests and vaccines. Although my study did not find a significant association between scrub typhus strains and disease outcome, likely due to the small sample size. Future research with larger sample sizes could provide more insights of prevalent strains and outcome of the disease.

Conclusion

The study highlights the significant morbidity and mortality associated with scrub typhus, especially among females, agricultural workers, and older individuals. Seasonal trends, rural prevalence, and diverse clinical manifestations emphasize the importance of awareness, early diagnosis, and timely treatment. The ELISA test's high sensitivity and specificity validate its role in diagnosing and assessing disease severity. Effective management strategies, including empirical drug therapy and targeted interventions for high-risk groups, are essential for reducing mortality. The genetic variability of *O. tsutsugamushi* strains, including Karp-like and Kato-like genes, underscores the complexity of scrub typhus. Further genetic sequencing (e.g., Boryong, Kawasaki, Kuroki) is needed to enhance early diagnosis and improve outcomes.

Future Prospective of This Study

Ongoing research is essential to understand how different *O. tsutsugamushi* strains influence clinical outcomes. Advanced sequencing technologies can provide insights into genetic diversity, virulence factors, and antimicrobial resistance. Phylogenetic studies may trace strain evolution, identify geographical clusters, and correlate strains with clinical presentations and treatment outcomes. Developing a standardized strain-typing system would enable global collaboration, accurate tracking of emerging strains, and improved disease management. Strain-specific diagnostic assays, combined with machine learning and bioinformatics, can enhance detection accuracy, predict trends, and identify potential hotspots. Monitoring antimicrobial resistance and correlating strain genetics with drug resistance will aid clinicians in early, precise, and effective treatment. A strain specific diagnostic assays can be developed and implemented to enhance detection accuracy and enabling early intervention, reducing morbidity and mortality by applying machine learning algorithms and bioinformatics tools to analyse large-scale strain data, predicting emerging trends, and identifying potential hotspots of scrub typhus in case outcome or for epidemiological surveillance. To establish correlation between presence of scrub typhus gene and drug resistance, so that clinicians can manage patients at early stage of the disease by accuracy & effectively.

References

1. Archibald LK, Reller LB. Clinical microbiology in developing countries. Emerg Infect Dis, 2001; **7**(2):302-5.
2. Susilawati T N, McBride W J. Acute undifferentiated fever in Asia: a review of the literature. Southeast Asian J Trop Med Public Health, 2014; 45(3): 719- 26.
3. Petersdorf R G, Beeson P B, Fever of unexplained origin: report on 100 cases. Medicine (Baltimore), 1961; 40:1-30.
4. Vinetz J M, Detection of leptospirosis in India*.* Arch Dis Child, 2003; 88(12); 1033.
5. Naing C, Kassim A I. *Scaling-up attention to nonmalaria acute undifferentiated fever.* Trans R Soc Trop Med Hyg, 2012;106(6):331-2.
6. Ohashi N, Fukuhara M, Shimada M, Tamura A. Phylogenetic position of *Rickettsia tsutsugamushi* and the relationship among its antigenic variants by analyses of 16S rRNA gene sequences. FEMS Microbiol Lett*;* 1995; 125: 299 – 304.
7. Tamura A, Ohashi N, Urakami H, Miyamura S. Classification of *Rickettsia tsutsugamushi* in a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. Int J Syst Bacteriol; 1995; 45: 589 – 591.
8. Jeong YJ, Kim S, Wook YD, Lee JW, Kim KI, Lee SH. Scrub typhus: Clinical, pathologic, and imaging fi ndings. Radio Graphics 2007; 27:161-72.
9. Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak of scrub typhus in Pondicherry. J Assoc Physicians India 2010; 58:24-8.
10. Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Prasher BS, *et al*. Scrub typhus

in Himalayas. Emerg Infect Dis 2006;12:1590-2.

11. Blacksell SD, Jenjaroen K, Phetsouvanh R et al. Accuracy of rapid IgM-based immunochromatographic and immunoblot assays for diagnosis of acute scrub typhus and murine typhus infections in Laos. Am J Trop Med Hyg 2010;83:365–369. ttps://doi.org/10.4269/ajtmh.2010.09-0534 PMID: 20682883.
12. Blacksell SD, Paris DH, Chierakul W et al. Prospective evaluation of commercial antibody-based rapid tests in combination with a loop-mediated isothermal amplification PCR assay for detection of Orientia tsutsugamushi during the acute phase of scrub typhus infection. *Clin Vaccine Immunol;* 2012; 19:391–395 https://doi.org/10.1128/CVI.05478-11 PMID: 22219313.
13. Varghese GM, Trowbridge P, Janardhanan J et al. Clinical Profile and Improving Mortality Trend of Scrub Typhus in South India. *Int J Infect Dis*. 2014; 23: 39–43.
14. Watt G, Parola P. Scrub typhus and tropical rickettsioses. Curr Opin Infect Dis. 2003; 16(5) :429–36.
15. Prakash JAJ, Abraham OC, Mathai E. Evaluation of tests for serological diagnosis of scrub typhus. Trop. Dr. 2006; 36:212–213.
16. Xu G, Walker DH, Jupiter D, Melby PC, Arcari CM et al. A review of the global epidemiology of scrub typhus. PLOS Neglected Trop. Dis. 2017; 11: e0006062.
17. Bayne Jones S. Epidemic Typhus in the Mediterranean Area during World War II with Special Reference to the Control of the Epidemic in Naples in the Winter of 1943–1944; Soule MH, Ed; American Association for the Advancement of Science: Washington, DC, USA, 1948; 1–15.
18. Maxcy KF. Scrub Typhus (Tsutsugamushi Disease) in the US Army during World War II; Soule MH, Ed; American Association for the Advancement of Science: Washington, DC, USA, 1948; 36–50.
19. Sharma A, Mahajan S, Gupta ML, Kanga A, Sharma V. Investigation of an outbreak of scrub typhus in the Himalayan region of India. *Jpn J Infect Dis* 2005; 58: 208-20.
20. Varghese GM, Abraham OC, Mathai D, Thomas K, Aaron R, Kavitha ML et al. Scrub typhus among hospitalized patients with febrile illness in South India: magnitude and clinical predictors. *J Infect* 2006; 52(1):56-60.
21. Mahajan SK, Kashyap R, Kanga A, Sharma V, Prasher BS, Pal LS. Relevance of Weil- Felix test in diagnosis of scrub typhus in India. *J Assoc Physicians India* 2006; 54: 619-21.
22. Narvencar KP, Rodrigues S, Nevrekar RP, Dias L, Dias A, Vaz M, Gomes E. Scrub typhus in patients reporting with acute febrile illness at a tertiary health care institution in Goa. *Indian J Med Res* 2012; 136(6):1020-4.
23. Khan SA, Dutta P, Khan AM, Topno R, Borah J, Chowdhury P, Mahanta J. Reemergence of scrub typhus in northeast India. *Int J Infect Dis* 2012; 16(12): e889- 90.
24. Bithu R, Kanodia V, Maheshwari RK. Possibility of scrub typhus in fever of unkown origin (FUO) cases: An experience from Rajasthan. *Indian J Med Microbiol* 2014; 32(4):387-390.
25. Sinha P, Gupta S, Dawra R, Rijhawan P. Recent outbreak of scrub typhus in north western part of India. *Indian J Med Microbiol* 2014; 32 (3):247-50.
26. Singh SP, Ragini Singh, Niaz Ahmad. A study of complications of scrub typhus in a tertiary health care institute of Uttarakhand, India. *Int J Res Med Sci* 2014; 2(1): 246-49.
27. Kelly DJ, Fuerst PA, Ching WM, Richards AL. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of orientia tsutsugamushi. Clin Infect Dis. 2009; 48: 203- 230. 10.1086/596576
28. Issac R, Varghese GM, Mathai E, Manjula J, Joseph I. Scrub typhus: prevalence and diagnostic issues in rural southern india. Clin Infect Dis. 2004; 39:1395-1396. 10.1086/424748
29. John Victor Peter, Thomas I Sudarsan, John Anthony J Prakash, George M Varghese et al, Severe scrub typhus infection: Clinical features, diagnostic challenges and management, World J Crit Care Med August 2015; 4(3): 244-250.
30. Nadda N, Chauhan N, Mahajan A, Chaudhary A, Katoch K, Chamotra S. A descriptive study to quantify severity in patients with severe scrub typhus in hilly city of Northern India. Int J Adv Med [Internet]. 2019;6(4):1182. Available from: http://dx.doi.org/10.18203/2349-3933.ijam20193268.
31. Sharma PK, Ramakrishnan R, Hutin YJ, Barui AK, Manickam P, Kakkar M, et al. Scrub typhus in Darjeeling, India: opportunities for simple, practical prevention measures. Transac Royal Soc Trop Med Hyg. 2009;103(11):1153-8.
32. Velmurugan Anitharaj, Selvaraj Stephen, Jothimani Pradeep, Sungman Park et al. Serological Diagnosis of Acute Scrub Typhus in Southern India: Evaluation of InBios Scrub Typhus Detect IgM Rapid Test and Comparison with other Serological Tests. J Clin Diagn Res. 2016 Nov; 10(11): DC07–DC10.
33. Naveen Gupta, Veena Mittal, B Gurung, U Sherpa, Pediatric Scrub Typhus in South Sikkim, Indian Pediatr 2012;49: PP 322-324.
34. Agarwal VK, Reddy GKM, Krishna MR, Ramareddy G, Saroj P, Bandaru VCSS. Predictors of scrub typhus: a study from a tertiary

care center. Asian Pac J Trop Dis [Internet]. 2014;4: S666–73. Available from: http://dx. doi. org/10.1016/s2222-1808(14)60704-0.
35. Bihari Shyam, Meena KV, Meena SR. A Study On Socio-Demographic, Clinical and Laboratory Profile of Scrub Typhus in Hadoti Region Kota, Rajasthan, August 2018-2019. Indian Journal of Research, 2019; Volume-8, Issue-9, DOI: 10.36106/paripex.
36. Mallick SK, Hazra S, Nandi T, Sarkar A. Scrub typhus: a hospital-based study in the northern districts of West Bengal, India. Int J Res Med Sci [Internet]. 2019;7(6):2403. Available from: http://dx.doi.org/10.18203/2320-6012.ijrms20192537.
37. Nadda N, Chauhan N, Mahajan A, Chaudhary A, Katoch K, Chamotra S. A descriptive study to quantify severity in patients with severe scrub typhus in hilly city of Northern India. Int J Adv Med [Internet]. 2019;6(4):1182. Available from: http://dx.doi.org/10.18203/2349-3933.ijam20193268.
38. Philomena J, Rangaswami M, Prathiba P. A study on demographic, clinical profile and outcome of scrub typhus. Int J Adv Med [Internet]. 2016;586–90. Available from: http://dx. doi.org/10.18203/2349-3933.ijam20162499.
39. Atri SK, Homdutt, Manjari, Yadav D, Dhanda A, Karthik. Scrub typhus in North India: clinical spectrum, laboratory parameters and treatment outcome in tertiary care teaching hospital. Int J Res Med Sci [Internet]. 2020;8(11): 3996. Available from: http://dx.doi.org/10.18203/2320-6012.ijrms20204892.
40. Sk S, Jbk P, Priyadarshini, Choudhury, Dash, Rath, et al. A study on clinical spectrum, laboratory profile, complications and outcome of pediatric scrub typhus patients admitted to an intensive care unit from a tertiary care hospital from eastern India. Int J Curr Res Rev [Internet].2021;13(09):99–102.
41. Sibabratta Patnaik, Natabar Swain, Bandya Sahoo, Reshmi Mishra, Mukesh Kumar Jain. Emergence of scrub typhus in Odisha – A hospital based study. Annals of Tropical Medicine and Public Health. 2017; 10 (3), doi:10. 4103/ATMPH_144_16.
42. Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak of scrub typhus in Pondicherry. J. Assoc. Phys. India 2010;58: 24–28.
43. Chrispal A, Boorugu H, Gopinath KG, Prakash JAJ, Chandy S, Abraham OC, et al. Scrub typhus: an unrecognized threat in South India - clinical profile and predictors of mortality. Trop Doct [Internet]. 2010;40(3):129–33. Available from: http://dx.doi.org/10.1258/td.2010.090452.
44. Liu Y-X, Feng D, Suo J-J, Xing Y-B, Liu G, Liu L-H, et al. Clinical characteristics of the autumn-winter type scrub typhus cases in south of Shandong province, northern China. BMC Infect Dis [Internet]. 2009;9(1):82. Available from: http://dx.doi.org/10.1186 /1471-2334-9-82.
45. Ogawa M, Hagiwara T, Kishimoto T, Shiga S, Yoshida Y, Furuya Y, et al. Scrub typhus in Japan: epidemiology and clinical features of cases reported in 1998. Am J Trop Med Hyg [Internet]. 2002;67(2):162–5. Available from: http://dx.doi.org/10.4269/ajtmh.2002.67.162.
46. Kim D-M, Yun NR, Neupane GP, Shin SH, Ryu SY, Yoon HJ, et al. Differences in clinical features according to Boryoung and Karp genotypes of Orientia tsutsugamushi. PLoS One [Internet]. 2011;6(8):e22731. Available from: http://dx.doi.org/10.1371/journal.pone.0022731.
47. Zhang M, Zhao Z-T, Wang X-J, Li Z, Ding L, Ding S-J. Scrub typhus: surveillance, clinical profile and diagnostic issues in Shandong, China. Am J Trop Med Hyg [Internet]. 2012 ;87(6):1099–104. Available from: http://dx. doi.org/10.4269/ajtmh.2012.12-0306.
48. Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Prasher BS, et al. Scrub typhus in Himalayas. Emerg Infect Dis. 2006;12 (10): 1590-2.
49. Liu Y-X, Feng D, Zhang Q, Jia N, Zhao Z-T, De Vlas SJ, et al. Key Differentiating Features Between Scrub Typhus and Hemorrhagic Fever with Renal Syndrome in Northern China. The American Journal of Tropical Medicine and Hygiene. 2007;76(5):801-5.
50. Kumar Vivek, Kumar Vinod, K Yadav Ashok, Sreenivasa Iyengar et al. Scrub Typhus Is an Under-recognized Cause of Acute Febrile Illness with Acute Kidney Injury in India. PLOS Neglected Tropical Diseases, 2014; 8 (1), e2605.
51. Chrispal A, Boorugu H, Gopinath KG, Chandy S, Prakash JAJ, Thomas EM, et al. Acute undifferentiated febrile illness in adult hospitalized patients: the disease spectrum and diagnostic predictors - an experience from a tertiary care hospital in South India. Trop Doct. 2010;40(4):230-4.
52. Razak A, Sathyanarayanan V, Prabhu M, Sangar M, Balasubramanian R. Scrub typhus in Southern India: are we doing enough? Trop Doct. 2010;40(3):149-51.
53. Kim DM, Kim SW, Choi SH, Yun NR. Clinical and laboratory findings associated with severe scrub typhus. BMC Infect Dis. 2010;10: 108.
54. Rajapakse, S.; Weeratunga, P.; Sivayoganathan, S.; Fernando, S.D. Clinical manifestations of scrub typhus. Trans. R. Soc. Trop. Med. Hyg., 2017;111, 43–54.

55. Lee CS, Min IS, Hwang JH, Kwon KS, Lee HB. Clinical significance of hypoalbuminemia in outcome of patients with scrub typhus. BMC Infect Dis. 2010; 10:216.
56. Varghese GM, Trowbridge P, Janardhanan J, Thomas K, Peter JV, Mathews P et al. Clinical profile and improving mortality trend of scrub typhus in South India. Int. J. Infect. Dis. 2014; 23:39–43.
57. Tamura A, Ohashi N, Urakami H, Miyamura S. Classification of Rickettsiae tsutsugamusgi in a new genus, Orientia gen. nov., as Orientia tsutsugamushi comb. nov. Int J Syst Bacteriol 1995; 45(3):589-91.
58. Bonell A, Lubell Y, Newton P N, Crump J A. Estimating the burden of scrub typhus: A systematic review, PLOS Neglected Tropical Diseases. [2017] 11,(9), 2-s2.0-85030716918, http://doi.org/10.1371/journal.pntd.0005838,e0005838.
59. Mahajan SK, Rolain JM, Kashyap R, Bakshi D, Sharma V, Prasher BS, *et al.* Scrub
60. typhus in Himalayas. *Emerg Infect Dis* 2006; 12**:**1590–2.
61. Kumar K, Saxena VK, Thomas TG, Lal S. Outbreak investigation of scrub typhus in Himachal Pradesh (India). *J Commun Dis* 2004; **36:**277–83.
62. Rungta N. Scrub typhus: Emerging cause of multiorgan dysfunction. Indian J Crit Care Med 2014; 18:489–91.
63. Shirai A, Robinson DM, Brown GW, Gan E, Huxsoll DL. Antigenic analysis by direct immunofluorescence of 114 isolates of Rickettsia tsutsugamushi recovered from febrile patients in rural Malaysia. Jpn J Med Sci Biol 1979; 32:337–44.
64. Blacksell SD, Luksameetanasan R, Kalambaheti T, Aukkanit N, Paris DH, McGready R. Genetic typing of the 56-kDa type-specific antigen gene of contemporary Orientia tsutsugamushi isolates causing human scrub typhus at two sites in north-eastern and western Thailand. FEMS Immunol Med Microbiol 2008; 52:335–42.
65. Bengtson IA. A serological study of 37 cases of tsutsugamushi disease (scrub typhus) occurring in Burma and the Philippine Islands. Public Health Rep 1946; 61:887–94.
66. Yang HH, Huang IT, Lin CH, Chen TY, Chen LK. New genotypes of Orientia tsutsugamushi isolated from humans in eastern Taiwan. PLoS One 2012; 7:e46997.