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SCRUB TYPHUS A REEMERGING DISEASE: A STUDY FROM A TERTIARY CARE HOSPITAL IN CENTRAL INDIA

Clinical Microbiology

Dr. Sharmila Raut Professor and Head, Department of Microbiology, Indira Gandhi Government Medical College, Nagpur.

Dr. Girish Patil* Tutor, Department of Microbiology, Indira Gandhi Government Medical College, Nagpur. *Corresponding Author

Dr. Ravindra Khadse Associate Professor, Department of Microbiology, Indira Gandhi Government Medical College, Nagpur.

Dr. Sandeep Chaudhari Professor and Head, Department of Veterinary Public Health, Nagpur Veterinary College, Nagpur.

ABSTRACT

Background And Objectives: There is re-emergence of scrub typhus in recent years. Diagnosis is difficult because of its non-specific symptoms. Early diagnosis and treatment is extremely important to prevent complications and mortality. IFA is considered gold standard but it has limitations. There is need for rapid, economic and simple point of care test for the diagnosis of scrub typhus. Our objectives were to study clinico-epidemeological characteristics of scrub typhus and to compare immunochromatographic (rapid) test with ELISA and PCR. **Methods:** During the study period of 4 years (Jan 2018 to Dec 2021), a total of 1572 patients with acute febrile illness of five days or more were studied. The demographic and relevant clinical details of the patients were documented and analyzed. Serum samples from the patients were subjected to rapid test and some randomly selected samples were also subjected to IgM ELISA and PCR. Results of ICT were compared with ELISA and PCR. **Results:** Out of 1572 patients, 127 (8.07%) were positive by rapid test. Maximum positivity was observed in 2018. Peak incidence was during monsoon and post monsoon. Positivity was higher in the age group of 21 to 60 years with male preponderance. People living in rural areas having agriculture and related occupations were mostly affected. Patients presented with non-specific symptoms and deranged laboratory parameters. Eschar was observed in only 21.25% patients. PCR was helpful in diagnosis during early seroneagtive stage. **Conclusion:** Every patients having acute undifferentiated febrile illness should be evaluated for scrub typhus. ICT has promising results as point of care test in resource limited setting but require regular monitoring and quality check. PCR is useful for diagnosis in early seroneagtive stage.

KEYWORDS

Scrub typhus, ICT, ELISA, PCR.

INTRODUCTION:

Scrub typhus is a re-emerging zoonotic bacterial infection in the region known as the 'tsutsugamushi triangle' of South and Southeast Asia, the Asian Pacific Rim, and Northern Australia.[1] The infection is transmitted through the larval mites (chiggers) belonging to the family *Trombiculidae.* Small rodents particularly wild rats of the subgenus Rattus are natural hosts for scrub typhus.[2] The causative organism, *Orientia tsutsugamushi,* is a Gram-negative, obligate intracellular bacterium that infects various cells, including endothelial cells and phagocytes.[3] Scrub typhus is common in areas of scrub vegetation consisting of low lying trees and bushes.[4]

Clinical manifestations are non-specific, most common is acute fever often associated with myalgia, headache, nausea, vomiting, cough and breathlessness.[5] Eschar is a pathognomic sign of scrub typhus. It represents localized necrosis at the site of chigger bite. The presence of eschar is highly variable ranging from 7-97%.[6] Disease severity ranges from mid, self-limiting disease to a fatal illness with many complications. Various complications are jaundice, renal failure, acute respiratory distress syndrome (ARDS), myocarditis, meningo-enchephalitis, multiorgan dysfunction (MODS), etc.[7] If untreated, the case fatality rate can be as high as 30-45%.[8] Early diagnosis and prompt treatment reduces complications and mortality.[9]

Various laboratory tests are available for diagnosis of scrub typhus. Indirect immunoperoxidase assay (IPA) and immunofluorescence assay (IFA) are considered gold standard but are available only in higher laboratories and are costly and require technical expertise. PCR and ELISA are available at medical college level. Results of PCR are best within the first week. ELISA techniques are probably the most sensitive tests available for diagnosis and the presence of IgM antibodies, indicates recent infection. Weil-Felix test lacks sensitivity and specificity.[10]The rapid diagnostic test which can be used as point of care test in primary health centers showed varying sensitivity and specificity.[11]

Our objectives were to study clinico-epidemiological characteristics of scrub typhus and to compare results of immuno-chromatographic test (ICT) with ELISA and PCR.

MATERIALAND METHODS

This cross sectional study was carried out from January 2018 to December 2021 at microbiology department of a tertiary care teaching hospital of central India. A total of 1572 suspected patients were recruited for this study. A suspected case is defined as a patient with acute undifferentiated febrile illness of five days or more with or without eschar.[10] A written informed consent was obtained prior to their inclusion into the study. Demographic details of the patients were noted. A thorough history and examination were carried out and the patient's signs and symptoms were documented using a predesigned proforma. Basic laboratory investigations were performed, including complete blood count, kidney function tests, liver function tests, chest X-ray; additional investigations were performed when indicated. Blood specimen (5ml each) was collected from each patient in sterile plain bulb and EDTA bulb. This study was approved by Institutional Ethics Committee.

Immunochromatographic (Rapid) Test:

All the blood specimens were first subjected to scrub typhus rapid kit – SD Biloline Tsutsugamushi rapid kit (Standard Diagnostics, Seoul, South Korea) with manufacture endorsed sensitivity of 99% and specificity of 96%. This detects IgM, IgG and IgA antibodies to the major surface antigen 56 kDa of representative strains of *O. tsutsugamushi* (Karp, Kato and Gilliam). The test was performed as per manufacturer's instruction and the result was observed after 15 minutes. Violet band appearing in the control 'C' line as well as a test 'T' line indicates that the sample is positive. Absence of violet band in test line indicates negative test. The test is invalid if there is no development of violet band in the control line.

Enzyme-linked Immunosorbent Assay (ELISA):

We compared results of rapid test with ELISA by subjecting few samples to both the tests. A total of 50 rapid test positive samples and 50 rapid test negative samples were subjected to ELISA. The test was performed using Scrub typhus detect IgM ELISA Kit (InBios International, USA). The test detect IgM antibodies to recombinant 56 kDa type specific antigen. Serum samples were diluted at 1:100 dilution as per manufacturer's instructions. Samples with OD values above cut off 0.5 were considered positive and those below it were taken as negative.

Polymerase Chain Reaction (PCR):
We compared results of rapid test with ELISA by subjecting few samples to both the tests. A total of 25 rapid test positive and 25 rapid test negative samples were sent to Nagpur Veterinary College, Department of Veterinary Public Health, and Center for Zoonosis. Nested Polymerase chain reaction targeting the 47 kDa surface antigen gene was performed.

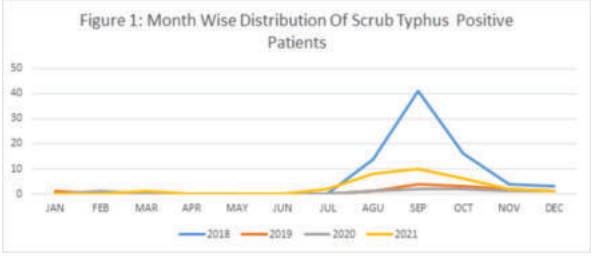
Statistical Analysis:
Data analysis was done using Microsoft Excel. Tables and charts were prepared using Microsoft Word and Excel. Study variables were expressed as percentages. Results of ICT, ELISA and PCR were compared by preparing 2×2 table and calculating sensitivity and specificity using OpenEpi software.

RESULTS

Out of 1572 suspected cases, 127 (8.07%) were positive by rapid test. We observed maximum positivity in 2018 at the end of monsoon season in the month of September. (Table 1, Figure 1) Males (53.54%) were affected more than females (48.45%). Maximum positive patients were in the age group of 41-60 years (46.45%), followed by 21-40 years (33.67%). People living in rural areas having agriculture and related occupation were mostly affected. Baseline demographic, clinical and laboratory parameters of positive patients are shown in Table 2.

Table 1: Year Wise Distribution Of Scrub Typhus Positive Patients

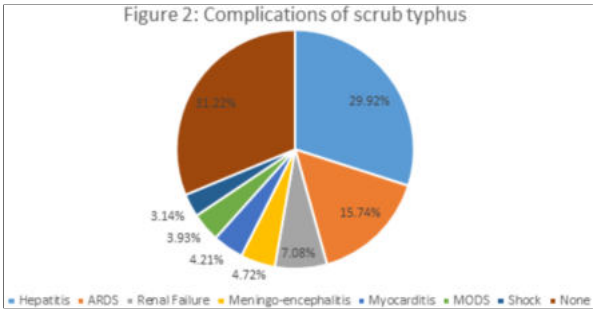
Year	Total samples tested	Number of scrub typhus positive patients	Percentage (%)
2018	422	78	18.48
2019	381	11	2.88
2020	374	8	2.13
2021	395	30	7.59
Total	1572	127	8.07



Most common presenting symptom was fever (100%), followed by headache (61.41%), myalgia (55.11%) and nausea and vomiting (40.15%). Eschar was observed in only 21.25% patients. Some patients had respiratory complaint on admission like cough (26.77%) and dyspnea (31.49%). An abnormal chest radiograph was evident in 30% patients. ARDS was diagnosed in 15.74%. There was also evidence of liver involvement with hepatitis in 29.92%. Central nervous system manifestations were uncommon with altered sensorium in 4.72% and convulsions in 3.14%. Many patients were having deranged laboratory parameters. (Table 2 and figure 2) The case fatality rate was 10.23%

Table 2: Baseline Demographic, Clinical And Laboratory Parameters Of Scrub Typhus Positive Patients

Age (years)	1-20		8 (6.24)
	21-40		42 (33.67)
	41-60		59 (46.45)
	>60		18 (14.17)
Gender	Male		68 (53.54)
	Female		59 (46.45)
Occupation	Agriculture and related		77 (60.62)
	Others		50 (39.37)
Residence	Rural		70 (55.11)
	Urban		57 (43.30)
Clinical presentation	Fever		127 (100)
	Headache		78 (61.41)
	Myalgia		70 (55.11)
	Eschar		27 (21.25)
	Nausea And Vomiting		51 (40.15)
	Jaundice		36 (28.34)
	Cough		34 (26.77)
	Dyspnea		40 (31.49)
	Altered sensorium		6 (4.72)
	Convulsions		4 (3.14)
Laboratory & other investigations	Hemoglobin	< 11mg/dl	40 (31.49)
	Total leukocyte count	>11,000/µl	51 (40.15)
	Platelets	< 1.5 lakhs	33 (25.98)
	Sr. Bilirubin	>1.2 mg/dl	52 (40.94)
	Aspartate transaminase	>70 IU	62 (48.81)
	Alanine Transaminase	>70 IU	65 (51.18)
	Alkaline phosphatase	>150 IU	70 (55.11)
	Urea	>20 mg/dl	26 (20.47)
	Creatinine	>1.2 mg/dl	28 (22.04)
	Chest X ray infiltrates		37 (29.13)
	ECG changes		6 (4.72)



Comparison Of Rapid Test And ELISA
We compared our results of rapid test with ELISA as a reference test. The Sensitivity and specificity of the rapid test were 92.30% and 95.83% respectively. False positive antibody detection was found in only 2 patient. (Table 3) Both the patients were dengue IgM positive.

Table 3: Comparison of Rapid test and ELISA

Rapid test	IgM ELISA	
	Positive (52)	Negative (48)
Positive (50)	48	02
Negative (50)	04	46
Sensitivity = 92.30%, Specificity = 95.83%		

Comparison Of Rapid Test And PCR
We compared our results of rapid test with PCR. Among 50 samples, 24 were PCR positive. Six cases were found to be rapid test positive but PCR negative. The duration of illness among these patients was more than 10 days. Because of late hospitalization and previous administration of antibiotic give rise to low antigen level which could not be detected by PCR. Five samples were PCR positive but rapid test negative. The duration of illness was less than 6 days indicating that the antibody conversion was not detectable at that time. (Table 4)

Table 4: Comparison of Rapid test and PCR

Rapid test	PCR	
	Positive (24)	Negative (26)
Positive (25)	19	06
Negative (25)	05	20

DISCUSSION

Scrub typhus has recently re-emerged as a major public health problem in India and other Asian countries.[12] In recent years, many outbreaks has been reported in Indian states of Maharashtra, Rajasthan, Punjab, sub Himalayan belt and other southern states.[13] The overall positivity of scrub typhus in the present study was found to be 8.07%; with maximum positivity of 18.48% in 2018. Thakur *et al*.[14] reported over all positivity of 12 % in a study form Delhi. A study from Madurai reported positivity of 9%.[15] In Vidarbha region of Maharashtra, in 2018, Bhise *et al*.[16] from Nagpur and Gujar *et al*.[17] from Yavatmal reported positivity of 32.24% and 27.3% respectively. Maximum positivity was observed in the months of monsoon and post monsoon. Similarly, studies by Yaqoob *et al*,[18] Vivekanandam *et al*.[19] and Mahajan *et al*.[20] reported maximum cases in months of monsoon and post monsoon which are the active period of mite.

Regarding age distribution of scrub typhus, most of the positive patients were from 41-60 years (46.45%), followed by 21-40 years (33.07%) which is the working age group of the population. Similarly, Gautam *et al*.[21] found maximum patients (28.5%) in the age group of 51-60 years and Ramyasree A *et al*.[22] found maximum patients (77.7%) in the age group of 40-49 years age group. In contrast, Rajendra PT *et*

al.[23] found maximum patients in 21-50 years of age group and Pote *et al.*[24] in age group of 10 years and less.

In our study, males (53.54%) were found to be more affected than females (46.43%). Similar findings were reported by Yaqoob *et al,*[18] K.S. Roopa *et al*.[25] and Rajoor *et al.*[26] In contrast, Gautam *et al*,[21] Dorji k *et al.*[27]and Rajendra PT *et al.*[23] reported female preponderance and Ramyasree *et al.*[22] reported same proportion of positive cases in both sexes. Male preponderance in our study might be due to more outdoor activities of males.

In our study, people living in rural areas (55.11%) and having agriculture related occupation (60.62%) were mostly affected. This is due to more exposure of people to mites, which are found in the grassland and farms. Similarly, Dorji *et al*,[27] Rajendra PT *et al*.[23] and Gautam *et al.*[21] reported most of the patients from rural area. In our study, 43.30% patients were from urban areas. Recently, reports of scrub typhus from urban areas has increased to a great extent, probably because of rapid urbanization and deforestation of rural areas.[28]

The symptoms of scrub typhus are nonspecific, which includes fever, headache, myalgia, nausea, vomiting and breathlessness. There is no significant variations of symptoms among different studies, however the presence of eschar shows considerable variations. The incidence of eschar in present study was 21.25%, which is similar to findings of Bhise *et al.*[16] (29.11%) in the same geographic location. An eschar prevalence upto 90% was reported from Korea.[29] However, Mahajan *el al.*[20] from northern India reported only 9.5%.

Laboratory parameters were considerably deranged in most of the patients and there was variations among different studies. In present study, anemia (Hb <11g/dl) was observed in 31.49% patients, which is similar to study conducted by Roychowdhury *et al.*[30] (32%). Sharma *et al.*[31] showed anemia in 54% patients. Leukocytosis (>11,000/µl) was found in 40.15% of our patients. In study done by Gupta *et al*.[32] in 2016, leukocytosis was found in 28% patients and by Roychowdhury *et al.*[30] in 35% patients. Thrombocytopenia (< 1.5 lakhs) was found in 25.98% patients, which is similar to findings of Roychowdhury *et al.*[30] (23%). However Gupta *et al*.[32] reported thrombocytopenia in 40 % patients. Liver abnormalities are quite common in our study and is consistent with other studies also. Serum bilirubin (>1.2mg/dl) was found in 40.94% of the patients. This is similar to study conducted by Sharma *et al.*[31] (32%) and Roychowdhury *et al.*[30] (45%). SGOT (>70IU) and SGPT (>70IU) was found in 48.81 % and 51.18% of our patients respectively. Roychowdhury *et al.*[30] reported increased SGOT and SGPT in 73.33% and 62.86% patients respectively. Alkaline Phosphatase was raised in 55.11% of patients. This is similar to study conducted by Roychowdhury *et al.*[30] (59%) and Shubhalakshmi MV *et al.*[33] (62.5%). Kidney functions were also deranged in few patients. Increased creatinine (>1.2mg/dl) and Urea (>20mg/dl) was found in 22.04% and 20.47% respectively. Roychowdhury *et al.*[30] reported deranged kidney functions in quite good number of patients. Increased urea and creatinine in 84% and 43% patients respectively.

The complications of scrub typhus usually develops after 1st week of illness. Early diagnosis and treatment is essential to lower the risk of complications. In present study, 69% patents develop some or the other complications. Hepatitis was the most common complication in our study found in 30 % patients. But this is lower than findings of studies conducted by Varghese *et al.*[34] (64.2%) and Sharma *et al.*[31] (61 %). ARDS was found to be associated in 15% of the patients, which is similar to study conducted by Thakur *et al.*[14] (16%). However, Varghese *et al.*[34] reported 43.5% of patients with evidence of ARDS. The incidence of renal failure was 7% in this study, which is similar to findings of Roychowdhury *et al.*[30] (5%) and Varghese *et al.*[34] (13%). However, Mahajan *et al.*[20] reported renal failure in 66.4% patients, which is much higher than our findings. Meningitis and meningo encephalitis were found in 4.72% patients, which is similar to findings of Roychowdhury *et al.*[30] Varghese *et al.*[34] reported meningo-encephalitis in 18.8% patients. Myocarditis developed in 4.21% patients. Thakur *et al.*[14] reported myocarditis in 10.5% patients. MODS was observed in 3.9% of patients, which is similar to findings of Roychowdhury *et al.*[30]. Varghese *et al.*[34] reported MODS in 38.3% of patients. The mortality due to scrub typhus depends on host factors like age, immune status, comorbidities, delay in treatment initiation and possibly on the geographical location and the genotype involved as well.[14] In our study, the overall case fatality rate was 10.23%. this is similar to findings of Yaqoob *et al.*[18] Varghese *et al.*[34] reported CFR of 7.8% and Thakur *et al.*[14] reported CFR of 5%. Higher mortality in our study might have been due to late referral resulting in delayed initiation of effective therapy.

Clinical diagnosis of scrub typhus is difficult because of its nonspecific symptoms. Early diagnosis and treatment is necessary to reduce mortality and morbidity. Laboratory test paly a very important role in diagnosis. In resource limited settings, performing sophisticated laboratory test is not feasible, hence, accurate point of care (POCT) testing for scrub typhus diagnosis would be invaluable for patient diagnosis and management.

We compared results of our rapid test (SD bioline tsutsugamushi) with ELISA (Inbios International) as reference test. We found satisfactory performance of rapid test with sensitivity and specificity of 92.30% and 95.83% respectively. This is similar to findings of Bhise *et al.*[35] in the same geographical region with sensitivity of 98.55% and specificity of 91.66%. Ramyashree *et al.*[22] reported 97% correlation between SD bioline rapid kit and IgM ELISA. In study conducted by Yaqoob *et al.*[18] all positive samples by SD bioline correlated with ELISA results. However, Silpasakorn *et al.*[36] and Blacksell *et al.*[37] observed only moderate sensitivity of 66.7% and 68% and specificity of 98.4% and 73% respectively. This variations in the sensitivity and specificity might be due to diversity of antigenically different strains of O. tsutsugamushi circulating in different geographical locations. More than 30 antigenically different strains are identified other than three prototype strains of karp, kato and gilliam strains. Also, performance of rapid test is affected by temperature during transport and storage.

In comparison with PCR, we found that during early stage of illness serological tests were negative and in later stages PCR came negative in some patients. Similar findings were reported by Gatika *et al*.[38] and Kim *et al.*[39] This is probably due to early seronegative stage in scrub typhus and molecular tests are more effective in this stage due to high antigen load. In such early stage of illness, molecular test could be the only test of choice for diagnosis of scrub typhus.

CONCLUSION

Patients presenting with acute undifferentiated febrile illness during monsoon and post monsoon should be evaluated for scrub typhus irrespective of geographical location. For early diagnosis rapid tests shows promising results especially in resource limited setting. However, there is need of more research to identify the prevalent strains of O. tsutsugamushi in different geographical locations so that accurate rapid tests are manufactured. Since same manufacturer showing different sensitivity and specificity in different locations, regular monitoring and period quality check of rapid tests is necessary. Further work is needed for development of an affordable and accurate antigen detection tests for rapid detection of scrub typhus in early seronegative stage of illness.

REFERENCES:

1. 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:S203-30.
2. Sharma P, Kakkar R, Kaore SN, Yadav VK, Sharma R. Geographical distribution, effect of season & life cycle of scrub typhus. JK Sci. 2010;12:63–4.
3. Kawamura A, Tanaka H, Rickettsiosis in Japan. Jpn J Exp Med 1988;58:169-84.
4. Mittal V, Gupta N, Bhattacharya D, Kumar K, Ichhpujani RL, Singh S, et al. Serological evidence of rickettsial infections in Delhi. Indian J Med Res. 2012;135:538–41.
5. Mathai E, Rolain JM, Verghese GM, Abraham OC, Mathai D, Mathai M, et al. Outbreak of scrub typhus in southern India during the cooler months. Ann N Y Acad Sci. 2003;990:359–64.
6. Paris DH, Shelite TR, Day NP, Walker DH. Unresolved problems related to scrub typhus: A seriously neglected life-threatening disease. Am J Trop Med Hyg. 2013;89:301–7.
7. Mahajan SK. Scrub typhus. J Assoc Physicians India. 2005;53:954–8.
8. Batra HV. Spotted fevers & typhus fever in Tamil Nadu. Indian J Med Res. 2007;126:101–3.
9. Jang MO, Jang HC, Kim UJ, Ahn JH, Kang SJ, Jung SI, et al. Outcome of intravenous azithromycin therapy in patients with complicated scrub typhus compared with that of doxycycline therapy using propensity-matched analysis. Antimicrob Agents Chemother. 2014;58:1488–93.
10. Rahi M, Gupte MD, Bhargava A, Varghese GM, Arora R. DHR-ICMR Guidelines for Diagnosis & Management of Ricketsial Diseases in India. Indian J Med Res, 2015 ; 141(4): 417-422.
11. Saraswati K, Day NPJ, Mukaka M, Blacksell SD (2018) Scrub typhus point-of-care testing: A systematic review and meta-analysis. *PLoS Negl Trop Dis* 2018;12(3): e0006330.
12. Bonell A, Lubell Y, Newton PN, Crump JA, Paris DH. Estimating the burden of scrub typhus: a systematic review. PLos Negl Trop Dis 2017;11:e0005838.
13. Kalal BS, Puranik P, Nagaraj S, Rego S, Shet A. Scrub typhus and spotted fever among hospitalized children in South India: Clinical profile and Serological epidemiology. *Indian J Med Microbiol* 2016;34:293-8.
14. Thakur CK, Chaudhry R, Gupta N, Vinayaraj EV, Singh V, Das BK, et al. Scrub typhus in patients with acute febrile illness: a 5-year study from India. *Int J Med 2020*; 113(6):404–410.

15. Charles J, Ramesh, Rajendran, Vithiya. **Sero surveillance of Scrub typhus in a tertiary care hospital at Madurai, Tamilnadu, South India. IOSR JDMS 2021;20(4):51-55.**
16. Bhise SM, Taiwade PD, Zodpey SS, Agarwal VA. Scrub typhus in rural & suburbs of Vidarbha region of Maharashtra. *Indian J Med Res* 2020; 151(1):104-106.
17. Gujar VM, Deshmukh DG, Domple VK, Tankhiwale SS, Bulle PA. Outbreak of scrub typhus in central India. *Int J Med Micrbiol Trop Dis* 2019;5(2);112-5.
18. Yaqoob S, Siddiqui AH, Shukla P. Scrub Typhus: A Neglected Tropical Disease and A Potential Threat in North India. J Pure Appl Microbiol 2020;14(2):1589-93.
19. Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak ofscrub typhus in Pondicherry. *J Assoc Physicians India.* 2010;58:24-28.
20. Mahajan SK, Rolain JM, Kashyap R, et al. Scrub typhus in Himalayas. *Emerg Infect Dis.* 2006;12:1590-1592.
21. Gautam R, Parajuli K, Sherchand JB. Epidemiology, Risk Factors and Sesonal Variation of Scrub Typhus Fever in Central Nepal. *Trop Med Infect Dis* 2019;4(1). Pii: E27.
22. Ramyasree A, Kalawat U, Rani ND, Chaudhury A. seroprevalence of Scrub Typhus at a tertiary care hospital in Andhra Pradesh. *Indian J Med Micrbiol* 2015;33:68-72.
23. Takhar RP, Bunkar ML, Arya S, Mirdha N, Mohd A. Scrub typhus: A prospective, observational study during an outbreak in Rajasthan, India. Natl Med J India 2017;30:69-72
24. Pote K, Narang R, Deshmukh P. Doagnostic performance of serological tests to detect antibodies against acute scrub typhus infection in central India. Indian J Med Microbiol 2018;36:108-112.
25. KS Roopa et al. Serodiagnosis of Scrub Typhus at a Tertiary Care Hospital from Southern India. *JCDR*. 2015;9(11):DC05-DC07.
26. Rajoor UG, Gundikeri SK, Sindhur JC, Dhananjaya M. Scrub typhus in adults in ateaching hospital in north Karnataka, 2011-2012. *Ann Trop Med Public Health*. 2013;6:614-17.
27. Dorji K, Phuentshok Y, Zangpo T, Dorjee S, Dorjee C, Jolly P. Clinical and Epidemiological Patterns of scrub Typhus, an Emerging Disease in Bhutan. Trop Med Infect Dis 2019;4(2)pii:E56.
28. Ranjan J, Prakash J. Scrub typhus re-emergence in India: contributing factors and way forward. *Med Hypotheses 2018*; 115:61-4.
29. Kim DM, Kim SW, Choi SH, Yun NR. Clinical and laboratory findings associated with severe scrub typhus. *BMC Infect Dis* 2010;10:108
30. Roychowdhury S, Ghosh S, Majumder D, Mukhopadhyay P. A menace without specific feature – Scrub Typhus a reemerging disease. *J Assoc Physicians India* 2021;69:68-71.
31. Sharma N, Biswal M, Kumar A, Zaman K, Jain S, Bhalla A. Scrub typhus in a tertiary care hospital in North India. The American Journal of Tropical Medicine and Hygiene 2016; 95:447-51.
32. N Gupta P, Gurjar U, Sharma BS, Gupta ML. Complication profile of scrub typhus and its association with total leucocyte count. Int J Contemp Pediatric 2017; 4:96-99.
33. Subbalaxmi MV, Madisetty MK, Prasad AK, Teja VD, Swaroopa K, Chandra N, Upadhyaya AC, Shetty M, Rao MN, Raju YS, Lakshmi V. Outbreak of scrub typhus in Andhra Pradesh– experience at a tertiary care hospital. J Assoc Physicians India 2014; 62:490-6.
34. Varghese GM, Janardhanan J, Trowbridge P, Peter JV, Prakash JA, Sathyendra S, Thomas K, David TS, Kavitha ML, Abraham OC, Mathai D. Scrub typhus in South India: clinical and laboratory manifestations, genetic variability, and outcome. International Journal of Infectious Diseases 2013; 17:e981-7.
35. Bhise SM, Taiwade PT, Agrawal VA. Performance of an immunochromatographic assay for rapid diagnosis of scrub typhus in an outlying health care facility. Int J App Res 2020; 6(10): 794-796.
36. Silpasakorn S, Waywa D, Hoontrakul S, Suttinont C, Losuwanaluk K, Suputtamongkol Y. Performance of SD Bioline Tsutsugamushi Assays for the Diagnosis of Scrub Typhus in Thailand. J Med Assoc Thai 2012;95(2):18-22.
37. Blacksell SD, Jenjaroen K, Phetsouvanh R, Wuthiekanun V, Day NPJ, Newton PN et al. Accuracy of Access Bio Immunoglobulin M and total antibody rapid immunochromatographic assays for the diagnosis of acute scrub typhus infection. Clin Vaccine Immunol 2010;17(2):263-266.
38. Gatika A, Swain SK, Mohanty JN, Sarangi R. Comparison of diagnostic performance between serological tests (Scrub IgM-ELISA) and Scrub-PCR for scrub typhus fever in an Indian tertiary care teaching hospital. J Appl Biol Biotech 2020;8(04):065-068.
39. Kim DM, Byun JN. Effects of antibiotic treatment on the results of nested PCRs for scrub typhus. J Clin Microbiol 2008;46(10):3465-6.