Original Article

Sero prevalence of Scrub Typhus among Suspected Cases in Selected Area of Nepal

Gautam R, Parajuli K, Sherchand JB

Department of Microbiology, Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal

Corresponding author: Rajendra Gautam, PhD scholar (IOM)

Email: gautamrajen@gmail.com

Abstract

Introduction: Scrub typhus is an mite-borne acute, febrile, infectious illness that is caused by *Orientia* tsutsugamushi The causative organism, Orientia *tsutsugamushi*, is transmitted to the human beings by the bite of larval stage (chiggers) of the trombiculid mites, Leptotrombidium delience. This disease is most common in rural areas of low income countries. It is difficult to differentiate clinically from other tropical infections such as malaria, dengue, enteric fever and leptospirosis. Hence the study aim was to determine the seroprevalence of scrub typhus among the acute febrile illness patients attending in different hospitals of Chitwan district.

Methods: The study was based on patients with suspected scrub typhus cases in Chitwan district from March 2017 to August 2017. Blood samples were collected from the suspected patients of scrub typhus, having acute febrile illness. IgM antibody to *Orientia tsusugamushi* was detected by using Scrub Typhus Detect™ Kit, In Bios International, USA.

Results: A total of 451 samples, 112 (24.80%) were positive for IgM Antibodies. Multivariate analysis demonstrated that the following factors were significantly associated with the scrub typhus. 1. Females (odd ratio [OR] =2.088, P=<0.004, confidence interval [CI] =1.268-3.438) 2. House near grassland (odd ratio [OR] =10.380, P=<0.001, confidence interval [CI] =3.615-29.802), 3. Piling weeds in house (odd ratio [OR] =0.498, P=0.011, confidence interval [CI] =0.290-0.855), 4. Presence of mouse inside the house (odd ratio [OR] =2.157, P=0.003, confidence interval [CI] =1.290-3.608), 5. Working in the field (odd ratio [OR] =10.295, P=0.002, confidence interval [CI] =2.309-45.905). All cases enrolled have presented fever (100%) followed by headache (56.2%), nausea (55.4%), shortness of breath (40.2%), abdominal pain (14.3%), jaundice (8.9%), hypertension (8.9%), COPD (8.0%), tachypnoea (4.5%), diabetes (4.5%), eschar (2.7%) and seizure (1.8%).

Conclusion: In the study the proportion of Scrub typhus infection was 25% among acute febrile cases. Our finding also explored the burden of scrub typhus in relation to demographic trend, risk factors, clinical characteristics and findings of different laboratory parameters. Early diagnosis, appropriate treatment along with health education and vector control measures are best way to prevent and management of scrub typhus.

Key words: Scrub typhus, ELISA, *Orientia tsutsugamushi*, Nepal

Introduction

Scrub typhus is an mite-borne acute, febrile, infectious illness that is caused by *Orientia tsutsugamushi* which is most common zoonotic bacterial infection in the region known as the tsutsugamushi triangle which extends from northern Japan and far-eastern Russia in the north, to northern Australia in the south, and to Pakistan in the west[1]. The causative organism ,*Orientia tsutsugamushi*, is transmitted to the human beings by the bite of larval stage (chiggers) of the trombiculid

mites, Leptotrombidium delience [2]. As these mites can be found in many different types of vegetation e.g. forest, rice paddies and plantation, farmers and people who engage in outdoor activities have higher risk of contracting scrub typhus[3].

Clinical manifestations are non specific which include acute febrile illness, fever, nausea headache, shortness of breath, myalgia. Recent studies on scrub typhus have reported the various clinical manifestations with abnormal laboratory findings[4].

This disease is most common in resource limited settings such as rural areas and is difficult to differentiate clinically from other tropical infections such as malaria, dengue ,enteric fever and leptospirosis[5].

The aim of this study was to determine the seroprevalence of scrub typhus among the acute febrile illness patients of Chitwan district of Nepal.

Methods

Cross sectional descriptive study was conducted on acute febrile illness patients with suspected scrub typhus cases in Chitwan district from April 2018 to September 2018. Blood sample was collected from the suspected patients of scrub typhus, having acute febrile illness. IgM antibody to *Orientia tsusugamushi* was detected by using Scrub Typhus Detect™ Kit, In Bios International, USA. An optical density (OD) > 0.50 was considered positive.

At the time of admission, a through history taking for the demographic variables, clinical characteristics and laboratory parameters were performed in the patients who were enrolled in this study. Signed informed consent was obtained for each patient before the patients were included in the study. This study was approved from the IRC of Institute of medicine.

The collected data were entered in Epi info 3.5 and exported to IBM SPSS 16.0 version. Association between the different demographic variables and scrub typhus was determined using the chi square test, frequency distribution, univariate logistic regression analysis. Significant variables from the univariate logistic regression analysis were selected for the multivariate logistic regression analysis. The odd ratio with 95% confidence interval is presented to find the statistical significance.

Results

Among the total cases 451, 112 (24.80%) were positive for IgM Antibodies. Highest percentages of scrub typhus positive were students (13.1%) followed by housewife (6.7%). Patients with primary level of education (7.8%) were severely infected which was followed by no education (6.2%) and secondary level of education (6.2). The most common age group was between 11-20 years (6.0%) followed by 1-10 years (5.3%). (Table-1 demonstrate the association of socio demographic variables and ELISA report)

Table-1: Association of Socio demographic variables and ELISA report

Variables	ELISA Report		P-value	Odd Ratio	95% confidence interval
	Positive	Negative			
Occupation					
Students	59 (13.1%)	162 (35.9%)	0.750	0.850	0.312-2.314
Housewife	30 (6.7%)	71(15.7%)	0.979	0.986	0.346-2.810
Farmers	10 (2.2%)	48 (10.6%)	0.229	0.486	0.150-1.573
Other [Business, Job]	7 (1.6%)	44 (9.8%)	0.119	0.371	0.107-1.290
Daily wages	6 (1.3%)	14 (3.1%)		1	
Education					
Primary level	35 (7.8%)	117 (25.9%)	0.496	2.094	0.249-17.604
No Education	28 (6.2%)	89 (19.7%)	0.469	2.202	0.260-18.679
Secondary Level	28 (6.2%)	70 (15.5%)	0.346	2.800	0.329-23.814
Higher Secondary	20 (4.4%)	56 (12.4%)	0.405	2.500	0.289-21.604
Graduate	1(0.2%)	7 (1.6%)		1	
Age Group					
1-10 Years	24 (5.3%)	76 (16.9%)	0.296	2.000	0.544-7.348
11-20	27 (6.0%)	61 (13.5%)	0.120	2.803	0.765-10.278
21-30	20(4.4%)	62 (13.7%)	0.288	2.043	0.547-7.631
31-40	10 (2.2%)	34 (7.5%)	0.386	1.863	0.456-7.607
41-50	11(2.4%)	34 (7.5%)	0.313	2.049	0.508-8.264
51-60	13 (2.9%)	31 (6.9%)	0.165	2.656	0.669-10.548
61-70	4 (0.9%)	22 (4.9%)	0.864	1.152	0.228-5.807
71 and Above	3 (0.7%)	19 (4.2%)		1	

Fever was the most common (100%) clinical characteristic observed in this study, which was followed by headache (56.2%), nausea (55.4%), shortness of breath (40.2%), abdominal pain (14.3%), ,jaundice (8.9%),hypertension (8.9%), COPD(8.0%), tachypnoea (4.5%), diabetes(4.5%), eschar (2.7%) and seizure(1.8%). (Table-2 describes the clinical characteristics of scrub typhus cases)

Table 2: Clinical Characteristics in Scrub typhus cases (n=112)

Clinical Characteristics	Number (%)
Fever	112 (100)
Headache	63 (56.2)
Nausea	62 (55.4)
Shortness of Breath	45 (40.2)
Abdominal Pain	16 (14.3)
Jaundice	10 (8.9)
Hypertension	10 (8.9)
COPD	9 (8.0)
Diabetes	5 (4.5)
Tachypnoea	5 (4.5)
Eschar	3 (2.7)
Seizure	2 (1.8)

Thrombocytopenia was the most common laboratory finding seen in 64.3% patients. Low level of Hemoglobin was observed in 31.2% patients. Leucocytosis was seen in 18.8% & leucopenia in 8%. Renal function test such as creatinine was raised in 14.3% and elevated urea was observed in 13.4%. Assessment of liver function showed the rise of liver enzymes, ALT was markedly increased in 65.2% cases followed by AST in 63.4%, ALP 33.0%, direct bilirubin 31.2%, and total bilirubin raised in 22.3%. (Table-3 demonstrates laboratory parameter of scrub typhus cases)

Table 3: Laboratory Parameter of Scrub typhus cases (n=112)

Laboratory parameters	Value	Number (%)
Hemoglobin	<11.0gm/dl	35 (31.2)
	>11.0gm/dl	77 (68.8)
Total Leucocyte count	<4000 cumm	9 (8.0)
	4000-11,000cumm	82 (73.2)
	>11,000 cumm	21 (18.8)
Platelet count	<1,50,000/µL	72 (64.3)
	1,50,000-4,50,000/µL	40 (35.7)
Urea	>45mg/dl	15 (13.4)
	<45mg/dl	97 (86.6)
Creatinine	>1.4mg/dl	16 (14.3)
	<1.4mg/dl	96 (85.7)
Bilirubin(Total)	>1.2mg/dl	25 (22.3)
	Upto 1.2mg/dl	87 (77.7)
Bilirubin(Direct)	>0.4mg/dl	35 (31.2)
	Upto 0.4mg/dl	77 (68.8)
AST	>45 mg/dl	71 (63.4)
	Upto 45 mg/dl	41 (36.6)
ALT	>40mg/dl	73 (65.2)
	Upto 40 mg /dl	39 (34.8)
ALP	>192.0 U/L	37(33.0)
	<192.0 U/L	75 (67.0)

Multivariate analysis demonstrated that the following factors were significantly associated with scrub typhus infection. There was female (odd ratio [OR] =2.088, P=<0.004, confidence interval [CI] =1.268-3.438), predominance significantly associated with the scrub typhus.

Most of the patients were residing near the grassland (odd ratio [OR] =10.380, P=<0.001, confidence interval [CI] =3.615-29.802), piling weeds in house (odd ratio [OR] =0.498, P=0.011, confidence interval [CI]=0.290-0.855), presence of mouse inside the house (odd ratio [OR] =2.157, P=0.003, confidence interval [CI] =1.290-3.608),working in the field (odd ratio [OR] =10.295, P=0.002, confidence interval [CI] =2.309-45.905) were significantly associated with scrub typhus. Seropositivity was higher in age group 11-20 years. Students (13.1%) were severely affected scrub typhus followed by housewife (6.7%). (Table-4 describes the multivariate analysis of association of ELISA report with socio demographic variables)

Table 4: Multivariate analysis of association of ELISA Report with socio demographic Variables

Variables	ELISA Report		P-value	Adjusted Odd Ratio	95% confidence interval
	Positive	Negative			
Sex					
Male	44 (9.8%)	181 (40.1%)		1	
Female	68 (15.1%)	158 (35.0%)	0.004	2.088	1.268-3.438
Piling Weeds in House					
Yes	67 (14.9%)	266 (59.0%)	0.011	0.498	0.290-0.855
No	45 (10.0)	73 (16.2)		1	
House Near Grassland					
Yes	108 (23.9%)	253 (56.1%)	<0.001	10.380	3.615-29.802
No	4 (0.9%)	86 (19.1%)		1	
Piling Weeds in Yard					
Yes	89(19.7%)	158(35.0%)	0.249	0.406	0.88-1.880
No	23(5.1%)	181(40.1%)		1	
Presence of Mouse					
Yes	77(17.1%)	153(33.9%)	0.003	2.157	1.290-3.608
No	35(7.8%)	186(41.2%)		1	
Working in the field					
Yes	87(19.3%)	129(28.6%)	0.002	10.295	2.309-45.905
No	25 (5.5%)	923(46.6%)		1	

Discussion

Scrub typhus is a common cause of acute febrile illness in our country Nepal. A Total of 451 patients suspected of having scrub typhus were included in our study to detect the presence of IgM antibody by ELISA. Prevalence of scrub typhus was found to be 24.80% which is similar to the study conducted in Bangladesh and India [6, 7]. During the last three years scrub typhus have started to appear in different district of Nepal with the prevalence rate of 40.3%[8]. In this study seroprevalence of scrub typhus is found high in females which are in congruence with the previous data published from chitwan district of Nepal, Rajasthan India and Korea[9-11]. This might be due to active involvement of females in household and field workers in our country Nepal. In contrast, the study in India found that prevalence rate for Scrub Typhus was higher among male (54.3%) and female (45.7%)[12]. Scrub typhus was most common in young adults of age group 11-20 years which is similar to the study conducted by other investigators [9, 13]. Students were severely affected in our study. This might be due to the large number of infected individuals were from age group 11-20 years.

Peoples residing in rural area with their house near the grassland and working in the field were more susceptible to scrub typhus. These were the significant risk factors associated with scrub typhus. Rodent populations of these rural areas were responsible to transmit the disease in human beings. Precautionary measures should be taken in the rural area to control the rodent's population.

Fever is the most common clinical characteristic present in scrub typhus patients which must be differentiated from other acute febrile illnesses[6, 14]. The common clinical characteristics seen in this study were fever, headache, nausea, abdominal pain, tachypnoea, shortness of breath and seizures. Our findings are in correlation with other studies conducted in Korea, India, Thailand and Bangladesh[4, 15-18].

Thrombocytopenia is the most common hematological parameter with leucocytosis and low level of Hemoglobin in scrub tyhus patients. These finding are similar with the study conducted in India and Korea[19-21]. Elevated Transaminase with raised bilirubin and renal dysfunction were observed in our study which

were common laboratory abnormalities found in other studies[22-25].

The central pathophysiological derangements of thrombocytopenia, liver function and renal function in scrub typhus is because of wide spread vasculitis and perivasculitis of these organs. This is due to multiplication of the organism in the endothelial cells lining the small blood vessels and consumption of platelets in the process of intravascular microthrombosis[26].

The limitation of this study includes the collection of single serum specimens for the detection of IgM antibodies during the acute phase of the disease. A rise in the antibody titre in paired sera could not be detected in our study. Additional descriptive study of one year duration is required to find the exact scenario of scrub typhus with its monthly distribution and seasonal variation.

Conclusion

Scrub Typhus is an important public health problem in tropical countries like Nepal. Acute febrile illness patients must be investigated for Scrub typhus with other similar types of infections and clinicians should be aware of this disease. The result of this study describes the demographic trend, risk factors, clinical characteristics and laboratory findings in Scrub typhus. Proper confirmation of diagnosis, early institution of therapy, public awareness, and vector control are important factors to be taken into consideration in the prevention and management of scrub typhus.

Acknowledgement

We would like to acknowledge the faculty members from the Department of Microbiology, Maharajgunj Medical Campus, Institute of Medicine. This work was partially supported by NAST Ph.D. Fellowship award.

Conflict of Interest: None

References

1. Kelly DJ, Fuerst PA, Ching W-M, Richards AL. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of Orientia tsutsugamushi. Clinical Infectious Diseases. 2009;48(Supplement_3):S203-S30.
2. Lerdthusnee K, Khlaimanee N, Monkanna T, Sangjun N, Mungviriya S, Linthicum KJ, et al. Efficiency of Leptotrombidium chiggers (Acari: Trombiculidae) at transmitting Orientia tsutsugamushi to laboratory mice. Journal of medical entomology. 2002;39(3):521-5.
3. Tay S, Ho T, Rohani M, Devi S. Antibodies to Orientia tsutsugamushi, Rickettsia typhi and spotted fever group rickettsiae among febrile patients in rural areas of Malaysia. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000;94(3):280-4.
4. Kim D-M, Kim SW, Choi S-H, Yun NR. Clinical and laboratory findings associated with severe scrub typhus. BMC infectious diseases. 2010;10(1):108.
5. Peter JV, Sudarsan TI, Prakash JAJ, Varghese GM. Severe scrub typhus infection: clinical features, diagnostic challenges and management. World journal of critical care medicine. 2015;4(3):244.
6. Bonell A, Lubell Y, Newton PN, Crump JA, Paris DH. Estimating the burden of scrub typhus: A systematic review. PLoS neglected tropical diseases. 2017;11(9):e0005838.
7. Jacob SM, Sekkizhar G, Kanagasabai S, Gopal P, Gopal T, Elumalai S. Seroprevalence and clinical manifestations of scrub typhus infection in Chennai city: A cross-sectional study. International Journal of Health & Allied Sciences. 2018;7(3):201.
8. Upadhyaya B, Shakya G, Adhikari S, Rijal N, Acharya J, Maharjan L, et al. Scrub typhus: An emerging neglected tropical disease in Nepal. J Nepal Health Res Counc. 2016;14(33):122-7.
9. Thapa S, Sapkota L, Hamal P. Threat of scrub typhus in post-earthquake Nepal. Journal of Chitwan Medical College. 2016;6(4):1-6.
10. Bithu R, Kanodia V, Maheshwari R. Possibility of scrub typhus in fever of unknown origin (FUO) cases: An experience from Rajasthan. Indian journal of medical microbiology. 2014;32(4):387.
11. Bang H, Lee M, Lee W. Comparative research on epidemiological aspects of tsutsugamushi disease (scrub typhus) between Korea and Japan. Japanese journal of infectious diseases. 2008;61(2):148.
12. Jyothi R, Sahira H, Sathyabhama M, Bai JR. Seroprevalence of Scrub typhus among Febrile Patients in a Tertiary Care Hospital in Thiruvananthapuram, Kerala. Journal of Academia and Industrial Research (JAIR). 2015;3(11):542.
13. Mahajan SK, Rolain J-M, Sankhyan N, Kaushal RK, Raoult D. Pediatric scrub typhus in Indian Himalayas. The Indian Journal of Pediatrics. 2008;75(9):947-9.
14. Taylor AJ, Paris DH, Newton PN. A systematic review of mortality from untreated scrub typhus (Orientia

tsutsugamushi). PLoS neglected tropical diseases. 2015;9(8):e0003971.

15. Varghese GM, Janardhanan J, Trowbridge P, Peter JV, Prakash JA, Sathyendra S, et al. Scrub typhus in South India: clinical and laboratory manifestations, genetic variability, and outcome. International Journal of Infectious Diseases. 2013;17(11):e981-e7.

16. Bibhuti Saha S, Mitra K, Ghosh S, Naskar A, Ghosh MK, Parui S, et al. Socio-demographic and Clinico-Epidemiological Study of Scrub Typhus in Two Tertiary Care Hospitals of Kolkata. Journal of The Association of Physicians of India. 2018;66:22.

17. Sirisanthana V, Puthanakit T, Sirisanthana T. Epidemiologic, clinical and laboratory features of scrub typhus in thirty Thai children. The Pediatric infectious disease journal. 2003;22(4):341-5.

18. Maude RR, Maude RJ, Ghose A, Amin MR, Islam MB, Ali M, et al. Serosurveillance of Orientia tsutsugamushi and Rickettsia typhi in Bangladesh. The American journal of tropical medicine and hygiene. 2014;91(3):580-3.

19. 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. International Journal of Infectious Diseases. 2014;23:39-43.

20. Silpapojakul K, Varachit B, Silpapojakul K. Paediatric scrub typhus in Thailand: a study of 73 confirmed cases. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2004;98(6):354-9.

21. Jeong YJ, Kim S, Wook YD, Lee JW, Kim K-I, Lee SH. Scrub typhus: clinical, pathologic, and imaging findings. Radiographics. 2007;27(1):161-72.

22. Mahajan SK, Rolain J-M, Kashyap R, Bakshi D, Sharma V, Prasher BS, et al. Scrub typhus in Himalayas. Emerging infectious diseases. 2006;12(10):1590.

23. Vivekanandan M, Mani A, Priya YS, Singh AP, Jayakumar S, Purty S. Outbreak of scrub typhus in Pondicherry. J Assoc Physicians India. 2010;58(1):24-8.

24. Singh S, Singh R, Ahmad N. A study of complications of scrub typhus in a tertiary health care institute of Uttarakhand, India. International Journal of Research in Medical Sciences. 2017;2(1):246-9.

25. Prakash A, Acharya AS, Jain N, Bhattacharya D, Chhabra M. Scrub typhus–An emerging public health problem in Delhi! Indian Journal of Medical Specialities. 2014;5(1):68-72.

26. Seong S-Y, Choi M-S, Kim I-S. Orientia tsutsugamushi infection: overview and immune responses. Microbes and Infection. 2001;3(1):11-21.