

**ORIGINAL RESEARCH ARTICLE****THREAT OF SCRUB TYPHUS IN POST-EARTHQUAKE NEPAL**S Thapa^{1*}, LB Sapkota², P Hamal¹¹Department of Clinical Microbiology and Immunology, Chitwan Medical College, Bharatpur, Nepal.²Department of Biochemistry, Chitwan Medical College, Bharatpur, Nepal.

*Correspondence to: Dr. Sangita Thapa, Department of Clinical Microbiology and Immunology, Chitwan Medical College, Bharatpur, Nepal.
E-mail: drsangitathapa@gmail.com

ABSTRACT

Scrub typhus is a potentially fatal zoonotic infection, reported from many parts of Asia including Nepal. There is increasing reports of outbreak of Scrub typhus, after the earthquake hit Nepal on April 25, 2015. The recent outbreak of Scrub typhus posed problems in diagnosis and treatment of the disease. It may be related to poor awareness of the disease or lack of suspicion for Scrub typhus which often presents with clinical features indistinguishable from typhoid fever. Since, various parts of Nepal appeared to be suitable hubs for Scrub typhus, the clinical suspicion of Scrub typhus in the differential diagnosis of fever of unknown origin (FUO) is of utmost importance to prevent mortality and morbidity. This is a prospective study conducted in Chitwan Medical College (CMC), Chitwan, Nepal. This study was carried out over a period of 4 months extending from June 2016 to September 2016. A total of 410 serum samples were collected from all patients visiting CMC, clinically suspected of having Scrub typhus infection. The samples were processed for the detection of IgM antibodies for Scrub typhus by ELISA. Results: A total of 410 samples from patients suspected with Scrub typhus infection were processed which included 200 males and 210 females. Out of total 410 samples tested, 181 (44.1%) were seropositive for Scrub typhus. Seropositivity was highest 25.9% among the age group 11-20 years of age. Females were infected more than males. This study implies the re-emergence of Scrub typhus in different regions of Nepal. Although the disease is endemic in our country, it is grossly underdiagnosed owing to non-specific clinical presentation and lack of diagnostic facilities. It is thus suggested that high index of suspicion should be maintained for cases presenting with febrile illness. Infection with Scrub typhus was found high and this calls for an urgent need to introduce vaccine against Scrub typhus.

Key words: ELISA, Febrile illness, Scrub typhus.

DOI: <http://dx.doi.org/10.3126/jcmc.v6i4.16707>

INTRODUCTION

Scrub typhus is an acute, febrile, rickettsial disease which has re-emerged as a major public health problem resulting in significant mortality, morbidity and economic loss. It is a potentially fatal bacterial zoonotic infection, reported from many parts of Asia including Nepal.¹ *Orientia tsutsugamushi*, a Gram negative obligate intracellular coccobacilli belonging to the family Rickettsiaceae is the causative agent of Scrub typhus. Scrub typhus is endemic disease confined geographically to the regions of eastern Asia and the southwestern Pacific (Korea to Australia) and from Japan to India and Pakistan.²⁻⁵ Globally, more

than one million cases occur annually and as many as one billion people living in endemic areas may have been infected at some time. Many studies from Nepal reported outbreak of Scrub typhus or isolated reports of Scrub typhus in the past.^{6,7} In Nepal, the infection from Scrub typhus has been documented from Nuwakot, Gorkha, Dhading, Nawalparasi, Sindhupalchowk, Bara, Parsa, Kailali, Dhankuta, Sarlahi, Sindhuli, Ilam, Morang, Makwanpur, Rukum, Sankhuwasabha, Baglung, Chitwan, Bhojpur and other four districts according to Epidemiology and Disease Control Division (EDCD).⁸ Although the disease is spreading

in our country, it is grossly underdiagnosed due to non-specific clinical presentation, low index of suspicion by the clinicians and lack of access to the specific diagnostic facilities.⁹

Scrub typhus is common in particular geographical locations which are conducive to the survival and multiplication of the mite population as well as the reservoir hosts. These regions abundant with scrub jungles, offer the best ecological niche for the existence of a sylvatic cycle among the chiggers and rodents. It is also known as chigger borne typhus, because humans and other vertebrate hosts usually get infection by the bite of infected larval trombiculid mites (also known as chiggers) harboring *Orientia tsutsugamushi*.¹⁰ The disease is transmitted from mites to 'rats and mice' and the mites in their larval stage contract the disease organism by biting these rodents. Man is an accidental host. The disease is not directly transmitted from person to person. Chigger is the only form in the life cycle of the mite that bites the warm blooded animals for its blood meal, whereas the other life forms i.e. nymph and adults live on soil and plants. The chiggers after getting infected exhibit high degree of potential for the transmission of *Orientia tsutsugamushi*. This happens because of the transfer of the causative agent, *O. tsutsugamushi* in-between different stages of the vector (transstadial transmission) as well as transmission of the agent from adult to the offspring (transovarial transmission). In this way, the vector is able to maintain the infectivity for a prolonged time period. Therefore, it is now believed that mites, apart from being vectors may behave as reservoirs as much as the rodents do.¹¹

The clinical manifestations can range from sub-clinical disease to multiorgan failure. It affects people of all ages; out of these paediatric Scrub typhus is quite common and reported in past.¹² Febrile illness typically begins after the bite of an infected chigger and lasts for 7-10 days presenting with high fever, chills, headache, rash, and eschar usually develop in infected persons. The chigger bite is painless and may become noticed as a transient localized itch. A necrotic eschar at the inoculating site of the mite is pathognomonic of Scrub typhus. Bites are often found on the

groin, axilla, genitalia or neck. An eschar is often seen in humans at the site of the chigger bite.¹³ Eschars are rare in patients in countries of South-East Asia and persons of typhus-endemic areas commonly have less severe illness, often without rash or eschar.¹⁴ Mortality rates in untreated patients range from 0% to 30% and tend to vary with different geographical regions.¹⁵

Scrub typhus that has been identified in various districts across the Nepal since, post-earthquake last few months might be a true reflection of the above ecological niche and epidemiologic behavior of the vector due to altered environmental factors. The growth of the disease has been suspected due to direct human contact with rats that have come out of their usual underground habitat after many houses collapsed during the massive earthquake on April 25, 2015. Following tragic earthquake, many people were homeless and shifted to temporary shelters. This was further accompanied by poor sanitation and overcrowding which provided the close proximity between humans, vectors, and reservoir hosts.¹⁶

Fever remains one of the commonest reasons for seeking medical advice in Nepal. Although there are many reports on cases of FUO, these remain underdiagnosed due to lack of diagnostic facilities. Since, various parts of Nepal appeared to be suitable hubs for Scrub typhus, the clinical suspicion of scrub typhus in the differential diagnosis of FUO is of utmost importance to prevent mortality and morbidity.¹⁷

Scrub typhus, which has been growing as an epidemic threat in various parts of the country, has indicated laboratory confirmation as essential tool to prevent and restrict the spread of disease. Currently, serology continues to be the mainstay for diagnosis of Scrub typhus. In Nepal, the burden of rickettsiosis is under estimated as there is lack of both community based studies and availability of specific laboratory tests. Probably this is the first study done on the seroprevalence of Scrub typhus in Chitwan. Therefore, this study was undertaken to determine the prevalence of Scrub typhus in all patients suspected with Scrub typhus infection.

MATERIAL AND METHODS

STUDY DESIGN AND PARTICIPANTS

This is a prospective study conducted in Chitwan Medical College, Chitwan, Nepal. Quantitative study was carried out to access infection with Scrub typhus. Blood samples were collected from patients visiting CMC clinically suspected to be suffering with Scrub typhus. The samples were processed for the detection of IgM antibodies for Scrub typhus by ELISA.

Sample collection and storage

About 2-3 ml of blood was collected from patients clinically suspected of having Scrub typhus, using strict aseptic precautions and serum was separated using standard methods. Aliquots for ELISA were made and stored at 2-8°C until tested.

Ethical committee approval

Ethical approval was obtained from the Chitwan Medical College Institutional Review Committee (CMC-IRC) before starting the study.

Data analysis

The data were first entered in the Microsoft Excel and were analyzed by SPSS version 20. A series of statistical tables have been used to present the result.

Results

A total of 410 samples from patients suspected with Scrub typhus infection were processed which included 200 males and 210 females. Number of suspected cases 85/410 (20.7%) and positivity 47/410 (25.9%) was higher among the age group 11-20 years followed by 0-10 years of age. Out of total 410 samples tested 181 (44.1%) were seropositive for scrub typhus as depicted in Table 1.

Table 1: Age wise distribution of Scrub typhus cases.

Age group (years)	Male	Female	No. of suspected cases	No. of positives
0-10	48	31	79 (19.2%)	32 (17.6%)
11-20	49	36	85 (20.7%)	47 (25.9%)
21-30	32	41	73 (17.8%)	30 (16.5%)
31-40	14	28	42 (10.2%)	19 (10.4%)
41-50	12	31	43 (10.4%)	20 (11%)
51-60	22	23	45 (10.9%)	22 (12.1)
>61	23	20	43 (10.4%)	11 (6%)
Total	200	210	410 (100%)	181 (100%)

Table 2 shows that out of total confirmed cases, highest seropositivity was found among females 93/181 (51.3%) as compared to males 88/181 (48.6%).

Table 2: Sex wise distribution of Scrub Typhus cases.

Sex	No. of suspected cases	No. of positives
Male	200 (48.7%)	88 (48.6%)
Female	210 (51.2%)	93 (51.3%)
Total	410 (100%)	181 (100%)

DISCUSSION

Scrub typhus caused by *Orientia tsutsugamushi* is known to be endemic to Asia-Pacific region including tropical countries like Nepal. Many outbreaks have been reported in the past.¹⁸ After the earthquake on April 25, 2015, there is increasing reports of outbreak of Scrub typhus from various parts of the country, particularly from districts affected by earthquake.¹⁹ Although there are several studies which have reported the presence of Scrub typhus in Nepal, the recent outbreak of Scrub typhus after earthquake posed problems in diagnosis and treatment of the disease. It may be related to poor awareness of the disease or lack of suspicion for Scrub typhus which often presents with clinical features indistinguishable from typhoid fever.²⁰

The diagnosis of Scrub typhus is based on clinical features and results of serologic testing. Early diagnosis and appropriate treatment is essential to prevent mortality and morbidity. In developing

countries, Scrub typhus is often unrecognized from acute febrile cases contributing to added financial burden. The clinical features of Scrub typhus overlaps with other infections like, typhoid fever, malaria, brucellosis, meningococemia and viral illness. Therefore, Scrub typhus should be considered in differential diagnosis of any febrile illness.²¹ Diagnosis cannot be made easily due to lack of simple diagnostic tools in many laboratories.

The prevalence of Scrub typhus varies from 0-8% to 60% in different countries.²² A total of 410 patients suspected with Scrub typhus over a period of 4 months were serologically detected for presence of Scrub typhus by IgM ELISA. Out of which 181 (44.1%) were seropositive for Scrub typhus. Similar findings had been reported in earlier studies.^{23, 24, 25} In our study, number of suspected cases 85/410 (20.7%) and seropositivity 47/410 (25.9%) was higher among the age group 11-20 years of age which is almost similar to study conducted by other investigators.^{26,27}

In our study, it was observed that out of total 181 seropositive Scrub typhus cases, female 93 (51.3%) were more affected than male 88 (48.6%). The study by Bithu et al, in their study reported that out of 133 infected with Scrub typhus, 80 (62%) were females and 53 (37.3%) males.²⁸ Vivekanandan M et al, also found female preponderance in their study.²⁹ Scrub typhus positivity was significantly higher among females as compared to males. In contrast, study done by Mahajan et al, found that infection with Scrub typhus was higher among male 24% and female 14.6%.³⁰ Similarly, other investigator Ramyasree et al, found that out of 39 patients, 23 were males and 16 were females.³¹ However, the reports from the study conducted by Kedareshwar P S et al, did not mention any predilection for sex.³² Results of our study showed that females were more commonly affected compared to males. This variation observed in our study could be associated with extensive involvement of females in outdoor activities whether for occupational or recreational purposes. A study done by Ogawa et al, have stressed in the fact that age and sex are known to influence the occurrence of Scrub typhus.³³ Our study population included patients from Chitwan district only, which could

be an important reason for the differences in the results observed in ours and others studies.

Early diagnosis of this disease is essential, because post-earthquake outbreak of Scrub typhus caused dozens of deaths in various districts of Nepal. Therefore, it is highly likely that Scrub typhus may be prevalent in our region as well. To the best of our knowledge, this is probably the first study on seroprevalence of scrub typhus done by ELISA method in Chitwan. Our study provides an evidence for the seropositivity of Scrub typhus which is now prevalent in Chitwan.

CONCLUSION

Scrub typhus has re-emerged as an important cause of febrile illness, making it difficult to differentiate from other infections. Scrub typhus should be included in differential diagnosis for patients with non-specific febrile illness where malaria, dengue and leptospirosis are already endemic infections in this part of our country. Unfortunately, there is no effective vaccine against Scrub typhus. Strains of *O. tsutsugamushi* exhibit extreme antigenic variation, natural immunity acquired against one antigenic type may not be protective against another type. This hampers the efforts to produce a viable vaccine and the disease still persists. Delay in diagnosis can be fatal. Hence, most effective measure for prevention of infection with Scrub typhus is proper diagnosis and prompt institution of effective antibiotic therapy as soon as possible.

Future scope of the study

The findings of this study gives the true picture on seroprevalence of scrub typhus in Chitwan which can be used as a baseline data in future. Some of the anticipated outcomes of the project can be used to determine the need for introduction of vaccines against Scrub typhus.

What does this study adds?

This is the first study on seroprevalence of scrub typhus done by ELISA method in Chitwan. Our study provides an evidence for the seropositivity of Scrub typhus which is now prevalent in various districts of Nepal including Chitwan.

ACKNOWLEDGEMENT

Authors express their sincere gratitude to the Department of Microbiology, CMCTH, Bharatpur. We extend our sincere thanks to all faculty members and staff of CMCTH.

REFERENCES

- Murdoch DR, Woods CW, Zimmerman MD, Dull PM, Belbase RH, Keenan AJ, et al. The etiology of febrile illness in adults presenting to Patan Hospital in Kathmandu Nepal. *Am J Trop Med Hyg* 2004;70:670-5.
- Liu Y, Feng D, Suo J, et al. Clinical characteristics of the autumn-winter type Scrub typhus cases in south of Shandong province, northern China. *BMC Infect Dis* 2009;9(1):82.
- McGready R, Blacksell SD, Luksameetanasan R, et al. First Report of an *Orientia tsutsugamushi* type TA716-related Scrub typhus infection in Thailand. *Vector Borne Zoonotic Dis* 2010;10:191-3.
- 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. *Trans R Soc Trop Med Hyg* 2009;103:1153-8.
- Kramme S, An le V, Khoa ND, et al. *Orientia tsutsugamushi* bacteremia and cytokine levels in Vietnamese Scrub typhus patients. *J Clin Microbiol* 2009;47(3):586-9.
- Basnyat B, Belbase RH, Zimmerman MD, Woods CW, Reller LB, Murdoch DR. Clinical Features of Scrub Typhus. *Clinical Infectious Diseases* 2006; *Clin Infect Dis* 2006;42:1505-6.
- Thompson CN, Blacksell SD, Paris DH, et al. Undifferentiated febrile illness in Kathmandu, Nepal. *Am J Trop Med Hyg* 2015;92:875-8.
- Poudel A. Three dead and 91 infected with Scrub typhus so far. *My Rep*. In press August 17, 2016. retrieved from www.myrepublica.com/news/3945.
- Batra HV. Spotted fevers and typhus fever in Tamil Nadu. *Indian J Med Res* 2007;126:101-3.
- Berman SJ, Kundin WD. Scrub typhus in South Vietnam. A study of 87 cases. *Ann Intern Med* 1973;79(1):26-30.
- Jain N, Jain V. Study on Clinico-Laboratory Profile of Children with Scrub Typhus. *J Nepal Paediatr Soc* 2012;32(2):187-92.
- Brown GW, Robinson DM, Huxsoll DL, Ng TS, Lim KJ. Scrub typhus: a common cause of illness in indigenous populations. *Trans R Soc Trop Med Hyg* 1976;70(5-6):444-8.
- Sindhura M, Meenakshi JK, Kumari KS, Lakshmi M. A study on Seroprevalance of Scrub typhus among clinically suspected patients by Rapid test. *Int J Curr Microbiol App Sci* 2014;3(11):811-6.
- Lerdthusnee K, Khuntirat B, Leepitakrat W, Tanskul P, Monkanna T, Khlaimanee N, et al. Scrub Typhus: Vector competence of *Leptotrombidium chiangraiensis* chiggers and transmission efficacy & isolation of *Orientia tsutsugamushi*. *Ann NY Acad Sci* 2003;990:25-35.
- Watt G. Scrub typhus. In: Warrell DA, Cox TM, Firth JD, eds. *Oxford, Textbook of Medicine*. 5th ed. USA: Oxford University Press, 2010, pp 919-24.
- Nayak N. Scrub Typhus in Nepal. *Nepal J Epidemiol* 2016;6(2):563-4.
- Brown GW, Shirai A, Gan E, Bernthal P. Antibodies to Scrub typhus in Eastern Nepal. *Trans Roy Soc Trop Med Hyg* 1981;75:586-7.
- Kumar K, Saxena VK, Thomas TG, Lal S. Outbreak investigation of Scrub Typhus in Himachal Pradesh (India). *J Commun Dis* 2004;36(4):277-83
- The Kathmandu Post. Rats causing scrub typhus: WHO team. The Kathmandu Post (Kathmandu), Oct 7, 2015. <http://kathmandupost.ekantipur.com/news/2015-10-07/rats-causing-Scrub-typhus-who-team>. html (accessed June 24, 2016).
- Blacksell SD, Sharma NP, Phumratanapapin W, et al. Serological and blood culture

- investigations of Nepalese fever patients. *Trans R Soc Trop Med Hyg* 2007;101:686-90.
21. Shah V, Vaidya V, Bang V, Shah I. Spotted fever in a child in Mumbai. *India J Vector Borne Dis* 2009;46(4):310-12.
 22. Chanyasanha C, Kittigul L, Puenchitton S, Sangasuan P. Antibodies titers to rickettsial diseases in blood donor in Bangkok by Indirect immunoperoxidase technique. *Proc Natl Epidemiol Semin Bangkok* 1992;167-9.
 23. Oberoi A and Varghese SR. Scrub typhus- an emerging entity: A study from a tertiary care hospital in North India. *Ind J Pub Health* 2014;58:281-3.
 24. Kumar P, Subbanna A, Dharanitrageda S, Suri K. Multi-organ dysfunction in Scrub typhus. *Ann Trop Med Publ Health* 2012;5(4):393-6.
 25. Chanyasanha C, Kaeburong K, Chenchittikul M, Sujirarat D. Seroprevalence of Scrub typhus infection in patients with pyrexia at some malaria clinics in three western provinces of Thailand. *Asian Pac J Allergy Immunol* 1998;16:119-25.
 26. Mahajan SK, Rolain JM, Sankhyan N, Kaushal RK, Raoult D. Pediatric Scrub typhus in Indian Himalayas. *Indian J Pediatr* 2008;75:947-9.
 27. Nautiyal S, Jauhari S, Goel N, Mahawal BS. Incidence of Scrub Typhus in a Tertiary Care Hospital in Uttarakhand. *Intl J Adv Res* 2016;4(1):144-9.
 28. Bithu R, Kanodia V, Maheshwari RK. Possibility of Scrub typhus in fever of unknown origin (FUO) cases: an experience from Rajasthan. *Indian J Med Microbiol* 2014; 32(4):387-90. doi: 10.4103/0255-0857.142241.
 29. 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.
 30. Mahajan SK. Review Article on Scrub typhus. *J Associat Physician Ind* 2005;53: 954-8.
 31. Ramyasree A, Kalawat U, Rani NB, Chaudhury A. Seroprevalence of Scrub typhus at a tertiary care hospital in Andhra Pradesh. *Ind J Med Microbiol* 2015;33(1):68-72.
 32. Narvencar KPS, 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;136:1020-4.
 33. Ogawa M, Hagiwara T, Kishimoto T, et al. Scrub Typhus In Japan: Epidemiology And Clinical Features of Cases Reported In 1998. *Am J Trop Med Hyg* 2002;67(2):162-5.