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Scrub Typhus: An Emerging Neglected Tropical Disease in Nepal

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ABSTRACT

Background: Scrub typhus is a neglected tropical disease and is under reported from Nepal. The objective of this study was to investigate the sero-epidemiology of scrub typhus in patients suffering from acute febrile illness.

Methods: A total of 434 specimens collected from July to November 2015 at National Public Health Laboratory (NPHL) were investigated for detection of immunoglobulin M (IgM) antibody to Orientiatsutsugamushi. The Scrub Typhus Detect™ kit (InBios, USA) was used to detect the antibodies to O.tsutsugamushi in human serum. Randomly selected 10% positive specimens were used for confirmation by dot- enzyme-linked immunosorbent assay and indirect immunofluorescence assay.

Results: Of the total, 175 (40.3%) were positive for IgM antibodies to O. tsutsugamushi. Positive results of scrub typhus were highest among female in 11-20 year followed by males in 41-50 years age group. The IgM antibodies to O. tsutusugamushi were positive in specimens of various geographical regions including 30 districts of Nepal. Positive cases were found in various ecological regions of Nepal.

Conclusions: Scrub typhus is one of the neglected tropical diseases in Nepal. Patients with acute febrile illness should be investigated for scrub typhus with high priority. There is an urgent need of reliable and affordable diagnostic tests at all level of health facilities of Nepal. Surveillance and public health awareness about the disease transmission and preventive measures needs to be initiated.

Keywords: Epidemiology; Nepal; orientia tsutsugamushi; scrubtyphus.

INTRODUCTION

Scrub typhus iscaused by Gram negative obligate intracellular coccobacillus, Orientia tsutsugamushi, which is transmitted by the bite of larval trombiculid mites.[1,2] Illness varies from mild, self-limiting to fatal. Onset is characterized by fever, headache, myalgia, cough and gastrointestinal symptoms.[3] The signs and symptoms are non-specific; resemble other infectious diseases like malaria, enteric fever, dengue or leptospirosis.

Tropical rickettsial illnesses especially scrub typhus and murine typhus are increasingly recognized as a leading cause of treatable undifferentiated febrile illness in Asia but remain severely neglected and under appreciated diseases in many areas.[4] Scrub typhus is an important cause of febrile illness with an estimated one million infection occurring each year in Asia.[2] To date, no effective and reliable human vaccine against scrub typhus is available.[5] The aim of this study was to investigate the sero-epidemiology of scrub typhus in patients suffering from acute febrile illness.

METHODS

This was a descriptive study conducted at National Public Health Laboratory (NPHL), Kathmandu from July to November 2015. A total of 434 specimens were collected from suspected cases presenting withfever (>38^{0}C), myalgia, headache, conjunctival congestion, generalized weakness and abdominal pain for detection of IgM antibody against O. tsutsugamushi. The serum specimens were collected from acute febrile cases of all age group at NPHL and in different hospital laboratories. Briefly, 3-5 ml venous blood was drawn from patients and kept at room temperature for 30 minutes. After that, blood was centrifuged at 2000 RPM for 10 minutes; serum was separated at collection site and transported

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to NPHL in labeled cryo-tube with triple packed cold chain box as per WHO regulations for the transport of infectious substances.[6] Serum samples were stored at refrigerator (4-6^{0}C) before testing.

All reagents and serum were brought to room temperature for enzyme-linked immunosorbent assay (ELISA). Detect TM IgM ELISA (InBios International, Seattle, WA, USA) was used to detect the presence of antibodies to O. tsutsugamushiin serum according to manufacturer's instructions. Briefly, 100 µl of diluted serum (1:100) was dispensed onto micro-well along with control serum. Plate was incubated at 37^{0}C for 30 minutes followed by six times washing with 1x buffer. Enzyme-HRP conjugate (100 µl) was added and incubated at 37^{0}C for 30 minutes. Enwashsolution was added to corresponding micro-well after six times washing and incubated at room temperature (20-25^{0}C) for five minutes. The ELISA plate was washed for six times using 1x wash buffer and incubated at room temperature (20-25^{0}C) in dark place for 10 minutes after addition of substrate. After the incubation, 50 µl stop solution was added, optical density (OD) was measured at 450 nm (Humareder, Germany) and cut-off value was calculated. The results were considered positive when OD is > Cut-off value.The values near to Cut-Off were repeated in triplicate to confirm the result. Negative Control (NC) OD (<0.200), Positive Control (PC) OD (>0.500) and discrimination capacity (RPC/NC) ≥ 5.0 criteria were followed to ensure the assay performance according to manufacturer's instruction protocol. After completion of tests, all serum specimens were stored at -80^0 C freezer (Thermo Scientific, USA) for further study. In addition to scrub typhus IgM ELISA, all specimens positive for scrub typhus were also tested for malaria (Malaria Ag P.f/P.v., Cat.NO-05FK80 and 05FK83, SD Bioline Korea), dengue(Dengue NS1 Ag, Cat. NO-11FK50, SD BiolineKorea), leptospirosis(Leptospira IgM, Cat.NO- 16FK30, SD Bioline, Korea) and typhoid fever (Salmonella typhi IgG/IgM Fast, Cat. NO- 15FK12, SD Bioline, Korea) using rapid diagnostic tests according to the manufacturer's protocol.

Statistical analysis was performed using SPSS-11.5 version, inferential statistics and percentage were generated.

RESULTS

In this study, 220 (50.7%) specimens were from male and 214 (49.3%) were from female and 175 (40.3%) were positive for IgM antibodies to O. tsutsugamushi. Gender-wise distribution of positive cases was varied in different age groups. Positive result of scrub typhus was found highest among female in 11-20 year group followed by 21-30. However in male, scrub typhus was found high among 41-50 year followed by 60 year and older age group (Figure-2).

Figure 1. Geographical distribution of scrub typhus (Dot represents affected districts)

Based on frequency of specimens obtained from different districts, we had spotted highly affected areas in the map of Nepal (Figure-1). The maximum number of positive cases were observed in Dhading (positive/total case: 36/102), Kailali(32/53), Kanchanpur (13/24), followed by Ramechhap (13/16), Khotang (8/13) and Rautahat (7/8) district (Figure 3).

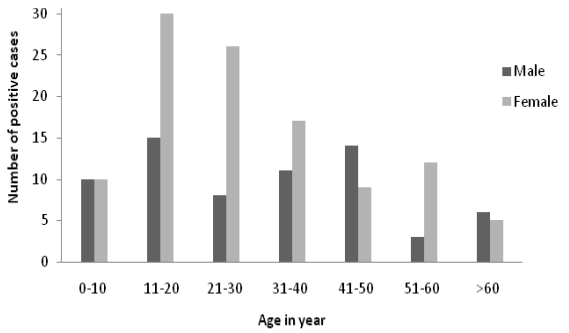

Figure 2. Age & Sex-wise distribution of scrub typhus positive cases (n=175)

In addition to scrub typhus; specimens of acute and undifferentiated febrile illness cases were further tested for malaria, dengue, leptospirosis and typhoid fever which were found negative. Ten percent of positive specimens for scrub typhus were randomly selected for PCR and IFA which is a gold standard method for confirmation. Of the specimens selected for confirmation, five were positive for O. tsutsugamushi by real time PCR assay and 12 were positive by dot-ELISA and IFA (Table-1).

Table 1.Indirect Immunofluorescence Assay (IFA) result of Scrub typhus, Murine typhus and Tick typhus.

		IFA Result			
S. N.	Sample ID	Scrub Typhus		Murine Typhus	Tick Typhus
		IgM titer	Result	IgM	IgM
1	NP15ST-A	1:6,400	positive	Negative	Negative
2	NP15ST-B	1:400	positive	Negative	Negative
3	NP15ST-C	1:3,200	positive	Negative	Negative
4	NP15ST-D	1:100	Negative	Negative	Negative
5	NP15ST-E	1:800	positive	Negative	Negative
6	NP15ST-F	1:1,600	positive	Negative	Negative
7	NP15ST-G	1:6,400	positive	Negative	Negative
8	NP15ST-H	1:6,400	positive	Negative	Negative
9	NP15ST-I	1:1,600	positive	Negative	Negative
10	NP15ST-J	1:3,200	positive	Negative	Negative
11	NP15ST-K	1:1,600	positive	Negative	Negative
12	NP15ST-L	Negative	positive	Negative	Negative
13	NP15ST-M	1:50	positive	Negative	Negative
	368	100			

These results strongly suggest that there is transmission of scrub typhus infection in Nepal even though small number of specimens was tested. Optical density (OD) of negative control, positive control and discrimination capacity factors were calculated to ensure performance of the assay. The average mean and range of OD of negative control, positive control and discrimination capacity was found 0.07 (0.07-0.12), 1.66 (1.38-2.45) and 11.0 respectively. The discrimination capacity (RPC/NC) value of all IgM positive assays was found greater than 5 (10 to 20) which strongly suggest the evidence of scrub typhus infection in Nepal. The longevity of IgM and IgG antibodies against scrub typhus in human and which isotype appears earlier in naive and exposed populations remain unsolved. Non-human primate time course studies have shown that IgM and IgG can appear almost simultaneously in cynomolgusmacaques.[7] The use of IgM-ELISA to determine an admission diagnosis of scrub typhus based on a single sample with an IFA reciprocal titer cutoff of ≥ 1,600 would translate into an ELISA cutoff at 0.5 OD, resulting in sensitivity of 93% and specificity of 91%.[8]

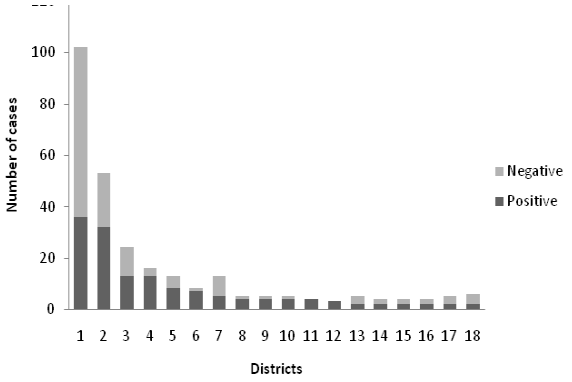

Figure 3. Districts with high number of scrub typhus positive cases. (1-Dhading, 2-Kailali, 3-Kanchanpur, 4- Ramechhap, 5-Khotang, 6-Rautahat, 7-Dadeldhura, 8-Sarlahi, 9-Bara, 10-Chitwan, 11-Makawanpur, 12-Bhojpur, 13-Sunsari, 14-Siraha, 15-Arghakhanchi, 16-Palpa, 17-Bajhang, 18-Doti)

Evidence of morbidity and mortality were not clearly documented due to limited data traceability, geographical difficulty, poor communication andco-ordination. However; eight deaths were reported by Epidemiology and Disease Control Division (EDCD),

Department of Health Services of Nepal.

DISCUSSION

With significant reductions in malaria in South East Asia, the public health importance of understanding the epidemiology of other acute febrile illness has increased.[9] Scrub typhus infection is an important cause of acute undifferentiated fever in South East Asia.[10] The disease is most common in rural areas where there is limited access to healthcare facility, diagnosis and treatment.[2] Currently, three modalities are used for diagnosis of scrub typhus, i.e., culture, nucleic acid and antibody detection. Culture of clinical specimens is insensitive, laborious, and expensive; nucleic acid detection is accurate in the early phase of infection, but its sensitivity falls with fever duration beyond 9 days.[7] IFA is considered as a gold standard which is very expensive.[2]

Scrub typhus presents as an acute febrile illness with non-specific signs and symptoms such as fever, myalgia, headache, conjunctival congestion, generalized weakness, abdominal pain, vomiting, loose motions, and jaundice.[11,12] Similar presentation can be found mainly in typhoid fever, scrub typhus, murine typhus, dengue virus infection and leptospirosis.[13] In our study, fever was the most common clinical symptoms in all cases (100%) in contrast to other studies from India where headache and myalgia were the most common manifestations of scrub typhus followed by fever.[14] Undifferentiated febrile illness (UFIs) are more common in low and middle-income countries like Nepal. Notably, Rickettsia species are important cause of UFIs, which is clinically similar to enteric fever.[15]

The disease is highly endemic in the "tsutsugamushi triangle" extending from Afghanistan to China, Korea, the islands of the Western Pacific, Indian Oceans, northern Australia and several countries of South East Asia including Bangladesh, Taiwan, northern Japan, west of Pakistan, Thailand, and Laos.[14,16] However, epidemics of scrub typhus have been documented worldwide.[17]

Acute undifferentiated febrile illness is one of the public health burdens in Nepal. The cases of scrub typhus were rapidly increased frommid of July to November 2015 and reported in 30 districts of Nepal. Antibodies (IgM)against O. tsutusugamushi were found higher in serum samples collected from Dhading, Kailali, Kanchanpur followed by Ramechhap, Khotang and Rautahat district. Similar findings were reported in northern India between the months of September and November, which follow the rainy monsoon season and coincide with the peak growth of vegetations and mite population.[10] Findings of our study were quite higher than reports of Laos, Bangladesh and Vietnam.[9,18,19]

Scrub typhus affects people of all age groups. During this study period, a total of 8 deaths were recorded from eastern and far western region of Nepal, of them; two cases were from pregnant women. The case fatality rate was 4.6% within a short period of time, similar result was obtained from China.[20] The mortality of scrub typhus in untreated patients range from 0% to 30% and tends to vary with age.[12] Scrub typhus cases were found highest among young and active reproductive age groups of female in Nepal. In a similar study conducted in Jingjiang, China; the majority of reported patients were female farmers which might be explained by the fact that most female in rural area are responsible for farm work.[14]

It had not been regarded as an important cause of febrile illness now a days although, murine typhus, scrub typhus and leptospirosis were previously reported from Nepal.[15] Transmission of disease may vary on time and place depending upon seasonality, geographical location and nature of work. It is not clear whether mixed infection primarily results from multiple mite bites or from colonization of multiple strains within individual mites. The latter possibility is supported by the detection of multiple antigenic strains of O. tsutsugamushi in both naturally infected and laboratory-reared chigger mites "Leptotrombidium spp".[1] Therefore, rapid diagnostic test to distinguish between typhoid, typhus and other UFIs are urgently needed, particularly after the earthquake in Nepal when three million people are still living in shelter.[13]

This study had various constraints such as limited number of specimens were investigated which may not be representative of entire population of affected districts. Because of limited resources and fund; we could not perform IFA for all positive specimens which is considered as a gold standard method. Similarly, the use of rapid diagnostic test for the detection of specific sero-marker against malaria, dengue, leptospirosis and typhoid fever is not considered as gold standard methods. Diverse geography, poor communication and interrupted transportation system due political instability had also narrowed this study. Hence, a comprehensive study together with clinical, epidemiological and laboratory investigations need to be conducted to establish the burden of scrub typhus in Nepal.

CONCLUSIONS

Scrub typhus is one of the neglected tropical diseases

in Nepal. Patients with acute febrile illness should be investigated for scrub typhus with high priority. There is an urgent need for affordable and reliable diagnostic test at all level of health-care facility of Nepal.

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