

# Seroprevalence of Scrub Typhus in Patients Attending Dhulikhel Hospital, Kavre

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## ABSTRACT

### Background

Scrub typhus is a largely ignored tropical disease and a leading cause of undifferentiated febrile illness. It is caused by *Orientia tsutsugamushi*. Scrub Typhus is frequently observed in South Asian countries. However, clear epidemiological information of this disease is lacking in case of Nepal. Nepal has shown steady increase in cases of Scrub Typhus since 2015. The epidemiological data related to this disease would support the decision making and surveillance design for early outbreak detection and immediate responses including prevention and treatment of scrub typhus in Nepal.

### Objective

To understand prevalence of Scrub Typhus in subjects who had visited outpatient department at Dhulikhel Hospital.

### Method

In this study, we have studied antibody test data (n=784) for Scrub Typhus from 2019 to 2021. The tests were performed on serum samples of patients who had visited OPD at Dhulikhel Hospital with fever lasting more than 5 days. The kit used in analysis was Scrub Typhus Detect™ IgM ELISA Kit from InBios International.

### Result

Out of the total subjects (n=784), 133 were positive (16.9%) for IgM antibody of Scrub Typhus. The positivity in female (18.6%) was higher than the male subjects (15.3%). The positivity rate was variable among the different age groups, with highest positivity for age group 0-14 years (25%). The seasonal variation was also observed among the seropositive cases.

### Conclusion

Scrub Typhus being a neglected tropical disease has high prevalence. It can be postulated that female subjects and subjects of age group 0-14 years are vulnerable to the infection with Scrub Typhus. There is need to increase the surveillance of Scrub Typhus to add the knowledge for diagnosis and treatment.

## KEY WORDS

ELISA, IgM, Scrub Typhus, Seroprevalence

## INTRODUCTION

Scrub Typhus is a rickettsial disease which has been reported in numerous Asian countries such as Indonesia, Pakistan, Myanmar India and China including Nepal.<sup>1,2</sup> It is caused by a bacteria called *Orientia tsutsugamushi* and spread to people through the bites of infected larval mites.<sup>3</sup> The onset of disease is characterized by cough, fever, myalgia, gastrointestinal symptoms and pyrexia.<sup>4</sup> The infection is also known to cause vascular leakage and injury of lungs, heart and kidney.<sup>5</sup>

However, the symptoms and their severity has been found to vary according to the susceptibility of the host as well the virulence of the bacteria.<sup>6</sup> In some instances, eschar is formed during manifestation of the symptoms, occurrence of which is rare in South-East Asia.<sup>7</sup> Various tests have been developed to diagnose this disease, while serology remains the mainstay for diagnosis. In primary infection, IgM antibodies are observed at the end of 1<sup>st</sup> week while IgG are seen at the end of 2<sup>nd</sup> week.<sup>8,9</sup> In Nepal, Weil-Felix (WF) test and ELISA are more frequently used, while Immuno-Florescent Assay (IFA) remains the gold standard for the test.<sup>10</sup>

As Nepal is an agricultural country, the exposure to larval mites, rodents and ticks is highly prevalent.<sup>11</sup> Occurrence of scrub is influenced by rainfall as during this season, the chiggers are attached to rodents, causing burden of scrub in rainy seasons.<sup>12</sup> According to a report from NHRC, in 2015, 101 cases were reported from 16 different districts in Nepal with 8% mortality.<sup>1</sup> Subsequently, Nepal also had another outbreak in 2016 with 831 reported cases from 47 districts and 1271 cases in 2019. In Nepal, Scrub typhus was first reported in 1981, however, until 2015, Scrub was not explored due to burden of other fibrile illness with similar clinical signs and limited diagnosis technology.<sup>13,14</sup> The epidemiological data related to Scrub typhus is minimum as it is not considered a priority disease in Nepal compared to avian influenza, rabies, and other zoonotic diseases.<sup>15</sup> Nevertheless, EDCC (Epidemiology and Disease Control Division) of Nepal has developed the guidelines for prevention and control of scrub typhus but these guidelines have rarely been implemented.<sup>16</sup> In 2021, various districts in Nepal saw rise in Scrub Typhus cases with 28 reported cases in Bajura District and 22 cases in Baitadi until mid of September.<sup>17,18</sup>

Thus, with this study, we aim to provide the seroprevalence and epidemiological data related to scrub typhus that would eventually support decision making and surveillance for early outbreak detection and implementation of immediate responses in Nepal.

## METHODS

This is a hospital-based retrospective study conducted at Dhulikhel Hospital, Kathmandu University Hospital from

2019 to 2021. The subjects, of all age groups, who visited the out-patient department with suspected Scrub Typhus infection and tested for Scrub Typhus, through serology, are selected for this study. Additional demographic data such as age and gender were obtained from the hospital system. The ethical approval was obtained from IRB of Kathmandu University, School of Medical Sciences.

The blood samples collected by the hospital lab are subjected to test of IgM antibody using Scrub Typhus Detect IgM ELISA Kit, which is a qualitative, membrane-based immunoassay to detect IgM antibodies against *O. tsutsugamushi* (OT).<sup>19</sup> The serum separation was performed through centrifugation at 1500 rpm for 10 minutes. The obtained serum was diluted in InBios sample diluted and added to analysis well of the ELISA plate. After incubation and washing, the sample wells were treated with IgM enzyme conjugate (HRP) leading to second incubation. After subsequent washing, the wells were incubated with TetraMethylBenzidine (TMB) substrate. Finally, an acidic stop solution is added and degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nm using iMark BioRad ELISA Plate Reader. The absorbance measured is directly proportional to the concentration of IgM antibodies to OT present. Additionally, a set of positive and negative controls were used as internal controls.

The cutoff calculation included determining average absorbance added with three times the Standard Deviation (SD) of normal human serum and/or human sera with unrelated infections. The cutoff of 0.370 is used.<sup>19</sup> Samples with absorbance (OD, Optical Density) greater than the cutoff are considered to be reactive or positive. The positive data are studied against gender and age stratification as well as seasonal variation. The age group as stratified by WHO is used.

Trained research associates collected the socio-demographic and other information related to the illness. The data were reviewed and entered in computerized database generated in SSPS v 16.0.

## RESULTS

During the study period, a total of 784 individuals were tested for Scrub Typhus using serology method. Among them, 133 (16.9%) subjects tested positive for IgM antibody against Scrub. Additionally, 54.8% (n=73) of the positive subjects were female as shown in table 1.

**Table 1. Gender distribution of subjects with positive IgM antibody against Scrub Typhus**

Gender	Total (n=133)	Positivity (%)
Male	60	15.3
Female	73	18.6

Additionally, the highest seroprevalence was found in age group 0-14 (25%) as shown in the table 2. The average age was  $36.8 \pm 19.4$  with age ranging from 2 years to 79 years.

**Table 2. Seroprevalence of Scrub Typhus among different age groups.**

Age Groups	Total (n=133)	Positivity (%)
0-14	18	25
15-47	79	16.4
48-63	16	11.5
> 64	20	21.2

The appearance of sero-positive cases was studied against weather/season as presented in table 3.

In 2019, the highest number of sero-positive cases (n=35) was observed in September. While in 2020 and 2021 both had highest number of sero-positive cases in the month of August as seen in table 3.

**Table 3. Sero-positive cases in different months from year 2019-2021.**

Year	Month	Number of Positive Cases
2019	Aug	1
	Sep	35
	Oct	22
	Nov	10
	Dec	1
2020	Jan	0
	Feb	1
	Mar	0
	Apr	0
	May	1
	Jun	0
	Jul	6
	Aug	20
	Sep	8
	Oct	2
	Nov	2
	Dec	0
2021	Jan	1
	Feb	0
	Mar	0
	Apr	0
	May	1
	Jun	0
	Jul	1
	Aug	20
	Sep	1

The positivity of the study years have been different: with 17.2% of the total tested cases being sero-positive in 2019, while 19.4 % in 2020 and 13.5% in 2021 (until September). The total tested subjects were also different in the study period as shown in the table 4.

**Table 4. Sero-prevalence in each year from 2019 to 2021.**

Year	Total Subjects Tested	Positivity (%)
2019	401	17.2
2020	206	19.4
2021	177	13.5

The majority of sero-positive cases were from Kavrepalanchowk district (n=69, 51.8%), followed by Sindhupalchowk (n=19, 14.2%), Sindhuli (n=18, 13.5%), Bhaktapur (n=6, 4.5%), Ramechhap (n=5, 3.7%) and Dolakha (n=3, 2.2%).

## DISCUSSION

The prevalence of scrub typhus is higher (16.9%) than other two studies done in Kathmandu Valley, 12.2% in 2021 and 3.2% in 2001.<sup>20,21</sup> The rise in sero-prevalence could be due to increased awareness leading to easy availability of diagnostic tools and improved testing facilities. However, some other studies have reported higher prevalence in Nepal: 22% in 2004 and 40.3% in 2015.<sup>22,23</sup> The variation in prevalence could be because of adaptation of different diagnostic tools and techniques with different specificity and sensitivity.

The sero-positivity was seen slightly higher (18.6%) in female subjects than male subjects (15.3%), with 73 testing positive out of 392 female subjects tested. Additionally, as presented in the result, 54.8% (n=73) of the positive subjects were female. This result agrees with another study done in Kathmandu which presented higher percentage (51.9%) of seropositive female compared to male.<sup>19</sup> In Nepal, female are more involved in household and agricultural activities (harvesting or cultivation) where there is increased chances of exposure to the vector, leading to relatively high seropositivity.

Additionally, the age group of 0-14 have the highest sero-prevalence compared to other age groups. Some studies have shown higher seroprevalence in older age groups but have used IgG antibodies for investigation of seroprevalence. IgG antibodies are developed for longer period of time and retained with repeated exposures.<sup>24,25</sup> Another study performed in S. Korea, did not show significant variation in seroprevalence among different age groups.<sup>26</sup> The higher seroprevalence in age group 0-14 could be explained by increased exposure opportunity for this age group during playing or accompanying the working mother during work.

Studies have shown the higher prevalence of Scrub Typhus in Nepal from the month of July to November.<sup>1,11</sup> This coincides with the results of our study, where the cases were highest in September in 2019, while August in 2020 and 2021. This might be because the incidence of *Leptotrombidium deliense* is influenced by rainfall, with more chiggers attached to rodents during the rainfall and damp months of the year.<sup>12,27</sup> Similar pattern was seen in India, with scrub typhus outbreak in season with highest rainfall.<sup>12</sup> Similarly, in Bangladesh there was increase in scrub typhus cases before and at the end of rainy season and minimal cases during the dry season.<sup>28</sup> Additionally, majority of cases were from Kavre district followed by Sindhupalchowk and Sindhuli, which is expected. Dhulikhel Hospital, located in Kavre district, hosts patients majority of patients from Kavre. Moreover, Sindhupalchowk, Sindhuli, Bhaktapur, Ramechhap and Dolakha district are neighbouring to Kavre and it is expected for patients from those districts to visit Dhulikhel Hospital for diagnosis and treatment.

The seropositivity was lower, in 2021, compared to the preceding years. This could be due to fact that individuals were more cautious in hygiene and sanitation due to COVID-19 pandemic. Also, the analysis data contained data only until September, 2021.

The limitation of this study could be similarity of the symptoms to other febrile illness, as a result of which not all subjects could have been properly identified.

Also, the collected blood samples were tested for presence of IgM antibodies. The investigation of IgG antibodies

would have depicted the long-term exposure as well as decay of the antibody response over time.

## CONCLUSION

Scrub Typhus is an important disease of public health concern in countries with tropical regions like Nepal. The female population and age group 0-14 are in high risk for infection with Scrub Typhus. Nepal being an agricultural nation, increase the risk of exposure to the vectors.

However, variable studies with different approaches of investigating seroprevalence have put depicted different categories of high-risk individuals. Nevertheless, the increment in reported cases every year confirms the increasing transmission of *O. tsutsugamushi*.

Therefore, it is of dire need to increase the surveillance of Scrub Typhus either through serology and ecological, microbiological and molecular characterization of *O. tsutsugamushi*. Such studies will add to the knowledge regarding effective diagnosis and treatment. Additionally, it would provide data for proactive management strategies improving sanitation, hygiene as well as in rodent control.

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