

# Risk factors associated with scrub typhus infection: A case-control study from Luhe, China

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## Research article

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# Abstract

**Background:** Globally, Scrub typhus (ST) is a serious public health issues in the rural of Asia-Pacific area. This study aimed to determine the risk factors associated with ST infection in Luhe District, China.

**Methods:** The case-control study was conducted which involved (N=116) cases consisted of a patient who diagnosed with scrub typhus, and nearest neighbours control (N=232) subjects were selected by matching for occupation, place of residence and age (within 5-years) and lacked a history of scrub typhus. Statistical analysis was performed using SPSS version 25.0 for Windows.

**Results:** The mean age of confirmed cases was 58.1(10.15) years, while that of control subjects were 56.14 (11.57). The ratio of farmers in cases and controls subjects was 90 (77.6%) and 187 (80.6%) respectively. There are no significant differences in the demographic characteristics of the study population ( $P>0.05$ ). Factors including residential site ( $\chi^2=8.57$ ,  $P=0.05$ ), raising small animal and livestock ( $\chi^2=31.53$ ,  $P<0.001$ ), living in the house near to grassland and vegetable ( $\chi^2=16.61$ ,  $P<0.001$ ), piling weeds in the yard ( $\chi^2=15.48$ ,  $P<0.001$ ), piling weed inside the house and around the house ( $\chi^2=56.64$ ,  $P<0.001$ ), and presence of mouse activities in the house ( $\chi^2= 3.88$ ,  $P<0.054$ ) are significant associated with developing of ST infection. The logistic regression analysis showed that Two out of five general exposure factors were identified which include morning exercise in the park (AOR=3.848, 95% CI=1.018-4.544,  $P=0.047$ ), and working as labourers in vegetable fields (AOR =1.792, 95% CI=1.12-2.87,  $P=0.016$ ) posed a significant association with ST infection.

**Conclusions:** The people who tend to involved in outdoor activities are exposure to ST infection. The findings derived from this study provide an information in the control and prevention of ST in Luhe, China.

## Introduction

Globally, scrub typhus (ST) remains one of the major public health problems. Every year millions of people are infected with ST [1].

It was estimated approximately 55% of the world's population lives in areas where ST is endemic [2]. Epidemiological data shows that in areas where ST is an endemic, the risk of infection is mainly associated with farming and outdoor activities, particularly in rural areas [3]. Previously published studies report that the risk of ST outbreak are presented in bundling or moving waste straw, living in the edge of the village, living in a house near to grassland. In addition, individuals working in vegetable fields and hilly areas, ditches, rice fields, and stacking waste straw indoors are also highly affected [3–5]. In China, the disease is mainly prevalent in tropical and subtropical regions [5, 6]. Recently conducted studies reported an increase of ST in China [7, 8]. Therefore, this present study was undertaken to explore risk factors associated with ST infection in Luhe District, Nanjing, China.

## Patients And Methods

### *Study area*

The study was conducted in Luhe district (figure 1), with a population of more than 927,400 (2014), in the Northern part of Nanjing (longitude, 118°~119°E; latitude, 32°11' ~32°27' N). The district covers an area of 485.50 km<sup>2</sup>. This region has been considered as the main endemic area of ST in Nanjing. Luhe has a subtropical monsoon climate, with an estimated average annual temperature of 16°C to 36.4°C in July, with the lowest temperature being –8.2°C in December. Average annual precipitation ranges between 821mm to 938 mm, while the average annual sunshine is 1,722 hours and the average annual evaporation stands at about 1253.8 mm. The native's population in Luhe are mainly employed in industries and agricultural sectors.

### *Study design*

A case-control study was conducted using data of reported ST cases during the period of October 2015 to December 2017. The cases inclusion criteria were ST reported in Nanjing network of the surveillance system of Nanjing Municipal Center for Disease Prevention and Control, China during the seasonal outbreak of ST seasonal (October, November and December). In total, 116 positive subjects infected with *Orientia tsutsugamushi* (*O. tsutsugamushi*) were confirmed based on one of the laboratory tests performed Nanjing Municipal Center for Disease Prevention and Control, China within the past three years. These confirmed cases are classified confirmed based on one of the laboratory tests performed based on China Information System for Disease Prevention and Control (China CDC) guideline: an increase in the indirect immunofluorescent assay IFA IgM titer against *O. tsutsugamushi* to 1:16; an increase in the IFA IgG titer against *O. tsutsugamushi* to 1: 256; and a 4-fold increase in immunoglobulin G (IgG) using indirect immunofluorescent assay (IFA) with specific IgM, IgG antibodies.

The case exclusion criteria were similar to previously published study [9]. The matched eligible controls were defined as inhabitants living in Luhe district (more than 6 months) in the same village. The controls were matched for age (within 5 years), occupation, and have lacked a history of ST with three years according to recommended guideline by China, CDC in 2009.

### *Data collection*

#### *Cases and controls selection*

A total of 116 cases of ST from 2015 to 2017 were selected in this study. Trained interviewers from Nanjing Municipal Center for Disease Prevention and Control have visited the cases and controls. All 116 cases were selected according to the following criteria through (i) a diagnosis of ST cases reported to Luhe hospital and notified by Nanjing Municipal Center for Disease Prevention (ii) that all reported cases were within different age groups. A total of 116 out of the 232 identified were matched controls subjects (1:2 pair matching) from their nearest neighbour were recruited at the same time during the study period. Structural questionnaires designed by Chinese Center for Disease Control and Prevention were used to obtained the socio-demographic information (age, gender, occupation, and educational level) of case and

control subjects, factors associated with living environment (residential site, house type, house yard with cement, raising animals, piling weeds in the yard and around the house). Others such as case-control behaviours during the outdoor activities (bundling straw, morning exercise, grazing animals, having travelling history, and types of work in the agricultural field) within the previous month were all recorded during the season of ST reported cases using unified format, was applied to documented cases and control demographic information.

### *Statistical analysis*

Data was entered using Epidata 3.1 (Jens M. Lauritsen, Odense, and Syddanmark, Denmark), and statistical analysis was performed using SPSS version 25.0 for Windows (IBM SPSS, Chicago, IL, USA). Continuous data were expressed as the mean (SD). Categorical data were expressed as the frequency or proportion between the case and control by using the Chi-square test ( $\chi^2$ ) or Fisher exact test, where appropriate to detect the statistical difference in case and control group. Furthermore, multivariate logistic regression model was used for assessing the association between cases and control variables. The two-sided p-value of less than 0.05 was considered statistically significant.

### *Ethical consideration*

The study was conducted after obtaining approval from an Institutional Review Board (IRB) of the ethics committee of Jiangsu Province and Nanjing Municipal Center for Disease Prevention and Control in charge of the Ethics Committee of Research School of Public Health. Further we obtained verbal and written informed consent from all study subjects before conducting the study.

## **Results**

In the period between 2015 and 2017, a total of 116 cases and 232 control were recruited in this study. Among all cases, 55 (47.4%) were male, and 61 (52.6%) were females. The mean age was 58.1 (10.15) years in cases and 56.14 (11.57) in control groups. The age group 41-60 had the predominant number of cases proportion at 61 (52.6%) in the case and 118 (50.9%) in the control group. Occupation-wise, 90 (77.6%) cases and 187 (80.6%) controls were farmers. Out of the total, 73 (62.9 %) of cases and 145 (62.5%) of control were in primary school level. There were no significant differences in age, gender, education level, house type, and occupational between the two groups ( $P > 0.05$ ), as shown in **Table 1**.

The association between ST outcome and risk factors of the living environment is shown in **Table 2**. From our data, most of the cases and control groups were living at the center of village 67 (57.8%) and 170 (73.3%), respectively. Raising small animals and livestock ( $P < 0.001$ ), living in the house near to grass and vegetable fields ( $P < 0.001$ ), piling weed in the yard ( $P < 0.001$ ), piling weeds inside the house and around ( $P < 0.001$ ) having the present of muse activities ( $P < 0.054$ ) are significant factors associated with ST infection ( $P < 0.001$ ).

However, the potential exposure factors associated with ST during the outdoor activities are shown in bundling waste straw ( $\chi^2=7.272$ ,  $P=0.008$ ), morning exercise in the park ( $\chi^2=21.94$ ,  $P<0.001$ ), fishing ( $\chi^2=107.85$ ,  $P<0.001$ ), dry clothes in the grasslands ( $\chi^2=46.31$ ,  $P<0.001$ ), and working as labourers in vegetable fields ( $\chi^2=9.306$ ,  $P=0.003$ ), respectively (**Table 3**).

Our analysis showed a reverse association between the risk factors associated with ST infection such factors presenting in morning exercise in the park (AOR=3.84, 95% CI=1.018~14.54,  $P=0.04$ ), and working as labourers in vegetable fields (AOR=1.792, 95% CI=1.12-2.87,  $P=0.016$ ), respectively as presented in **Table 4**.

## Discussion

Scrub typhus is known to be an endemic disease in China.<sup>8</sup> The number of cases has been reported in many parts of China [6,8,10–13].

To identify the risk factors associated with ST in the district, we conducted a case-control study. In this case-control study, we found that most of the ST infected cases, 90 (77.6%) and 187 (80.6%) controls were farmers, which suggested that the disease endemicity is associated with agricultural activities and raising animals and livestock. Further, outdoor activities and less use of protection measures are significant factors associated with ST infection.

Factors that may contribute to the increase of ST included outdoor activities, changes in ecological environment, living in an endemic area might be the reason for increasing the chances of being bitten by chigger mites [14]. These findings indicated significant differences between the two groups living in the environment. This is consistent with other findings [9]. The results suggest that age above 40 years is closely associated with risk of ST infection [9]. Similarly, primary school level education is the most risk groups; which is in line with other studies [15].

Further, the results showed the details of the living environment of ST such as residential area (living in the edge of the village and living in the center of the village), raising animals and livestock. Others such as piling weeds in the yard and around the house. In addition to the presence of mouse activities, and living in houses near to grassland and vegetable fields (within 20 meters). If the house builds in the edge of the village and near to grassland, the vegetable field is more likely of growing of chigger mite and makes the disease transmission easy during the endemic period. These findings were supported by other previously published studies [9].

The infection of ST among farmers, those living in the center of the village and completed their primary school level education are exceptionally high, mainly due to their frequent contact with disturbed habitats in their surroundings. Another element is that ST infection in Luhe district is more common in female than male. It may be because most women generally spend more time on the farm and engaged in outdoor activities [11]. A study conducted in Korea reported that females are more likely to be at risk of ST infection than males [16]. Moreover, the rise of farming activities has traditionally been associated with

ST infection. Previous studies conducted in Taiwan and Korea point out that farmers are at a greater risk of ST due to their occupation status. Further, the individual-level of the risk factors associated with ST and outdoors activities showed protective associations of ST infection[14,16].

The daily morning exercise in the park, fishing in the rivers, drying clothes in the grasses, working as labourers especially in the vegetable field are the main factors associated with the risk of exposure; since the mites are widely spread in different types of vegetation, grasses, and scrubland. Factors such as raising of small animal and livestock (dog, pig, cattle, goat, cat, sheep, and rabbit) have been significantly associated with the risk of ST infectious. Similarly, keeping domestic animals in the yard and rodents at home has also been found to be a significant factor associated with the risk of ST in Luhe. Thus, findings similar to this study was reported in Darjeeling city, which is located in northeast India, but not so in Beijing, China[9,17–18]. The data subjected to multivariate logistic regression specified that those having a morning exercise in the park and those working in the vegetable field were significantly associated with the occurrence of scrub typhus.

The presence study outlined the following findings. First, this is the first case-control study in the area to identify the risk factors associated with ST infection in Luhe district for the first time. Second, owing to the growing public health concern for China CDC in the prevention and control of endemic ST infection, this results may assist the health authorities in Nanjing Municipal Center for Disease Prevention and Control for a development programme for health prevention and control ST infection in future.

There are several limitations to this study. First, the sample size of confirmed cases by serological and PCR are small. Possibly there exists some potential for recall bias because of using ST cases that had the infection during the seasonal outbreak 2015 to 2017, which notified by Nanjing Municipal Center for Disease Prevention and Control and it involved a long time from the onset of diseases to be interviewed. Secondly, the study represents only an endemic area in the northern part of the Nanjing city, which only consider an endemic area of ST by Nanjing CDC and they not provide information about others endemic area close to Nanjing.

## Conclusions

The findings indicate that living environment in piling weeds in the yard, the presence of mouse activities in the house, living in the house near to grassland and vegetable (within 20 meters), raising animals and livestock and residential site are associated with develop of ST infection. In addition to outdoor activities such as morning exercise and working as labourers in vegetable, fields are significantly related to ST infection. Therefore, these findings will provide baseline information for ST prevention and control.

## Declaration

### *Abbreviations*

ST: Scrub typhus; IRB: Institutional Review Board (IRB); CDC: Center for Disease Prevention and Control; PCR: Polymerase chain reaction;  $\beta$ : Partial regression coefficient; SE: Standard error; AOR: odds ratio.

#### *Authors' contributions*

THM, WL, YH, QN, YJ, XL and YG contributed to the design of the study. PW, WL, YH, YJ, YG and TA conducted the data extraction. WL, XL, YG, TA, HHM, and THM conducted the data analysis, drafting, editing and revision of the manuscript. All authors have read and approved the final manuscript.

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#### *Availability of data and materials*

The datasets used in current study are available from the corresponding author on reasonable request.

#### *Ethics approval and consent to participate*

The study was conducted after obtaining approval from an Institutional Review Board (IRB) of the ethics committee of Jiangsu Province and Nanjing Municipal Center for Disease Prevention and Control in charge of the Ethics Committee of Research School of Public Health. Furthermore, written informed consent was obtained from the patients.

#### *Consent for publication*

Not applicable.

#### *Competing interests*

The authors declare that they have no competing interests.

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## Tables

**Table 1:** Demographic characteristics of studied subjects.

Characteristics	Case (n=116)		Control (n=232)		P-value
	No.	(%)	No.	(%)	
Gender					0.762
Males	55	47.4	114	49.1	
Females	61	52.6	118	50.9	
Age group, years					0.359
20~40	5	4.3	19	8.2	
41~60	61	52.6	123	53.0	
>61	50	43.1	90	38.8	
Mean age, year. Mean (SD)	116	58.1(10.15)	232	56.14 (11.57)	0.123
Occupation					
Farmer	90	77.6	187	80.6	0.510
Non-farmers*	26	22.4	45	19.4	
Education level, years					0.517
≤ 6 years	73	62.9	145	62.5	
> 6 years	43	37.1	87	37.5	

\*Non-farmers: Childcare, teacher, civil servants, medical staff, business workers, fishing, and retired officer  
t

**Table 2:** Association between scrub typhus outcome and risk factors of the Living environment among the study population.

Living environment	Case (n=116)		Control (n=232)		$\chi^2$	P-value
	No.	(%)	No	(%)		
Residential site						
Edge of the village	49	42.2	62	26.7	8.57	0.005
Center of village	67	57.8	170	73.3		
Housing types						
Independent house	83	71.6	174	75.0	0.47	0.519
Apartment/others (A cottage house)	33	28.4	58	25.0		
House yard with cement floor						
Yes	103	88.8	203	87.5	0.122	0.826
No	13	11.2	29	12.5		
Raising animals and livestock*						
Yes	85	73.3	96	41.4	31.53	<0.001
No	31	26.7	136	58.6		
Living in the house near to grassland and vegetable (within 20 meters)						
Yes	96	82.8	142	61.2	16.61	<0.001
No	20	17.2	90	38.8		
Piling weeds in the yard						
Yes	23	19.8	14	6.0	15.48	<0.001
No	93	80.2	218	94.0		
Piling weeds inside the house and around the house						
Yes	44	37.9	14	6.0	56.64	<0.001
No	72	62.1	218	94.0		
The presence of mouse activities in the house						
Yes	66	56.9	106	45.7	3.88	0.054
No	50	43.1	126	54.3		

\*Breeding poultry and livestock; dog, pig, cattle, goat, cat, sheep, and rabbit.

**Table 3:** Association between scrub typhus outcome and risk factors of the in outdoor activities

Exposure within one month	Case (n=116)		Control (n=232) †		P-value
	No.	(%)	No.	(%)	
Occupations					
Farmer	90	77.6	187	80.6	0.434 0.510
Non-farmers	26	22.4	45	19.4	
Bundling waste straw					
Yes	22	19.0	76	32.8	7.272 0.008
No	94	81.0	156	67.2	
Morning exercise in the park					
Yes	4	3.4	36	15.5	11.073<0.001
No	112	96.6	196	84.5	
Grazing animals					
Yes	8	6.9	30	12.9	2.895 0.089
No	108	93.1	202	87.1	
Fishing					
Yes	6	5.17	32	13.79	5.908 0.015
No	110	94.82	200	86.20	
Working as labour in sweet potatoes fields					
Yes	16	13.79	35	15.09	0.103 0.872
No	100	86.21	197	84.91	
Drying clothes in the grasses					
Yes	5	4.31	27	11.64	4.973 0.030
No	111	95.68	205	88.36	
Working as labour in vegetable fields					
Yes	51	44.0	142	61.2	9.306 0.003
No	65	56.0	90	38.8	
Working as labour in yellow soybean fields					
Yes	16	13.8	27	11.6	0.332 0.65
No	100	86.2	205	88.4	
Working as labour in the rice fields					
Yes	18	15.5	43	18.5	0.487 0.551
No	98	84.5	189	81.5	
Working as labour in cotton fields					
Yes	6	5.2	25	10.8	2.992 0.110
No	110	94.8	207	89.2	
Have travelling history					
Yes	3	2.6	3	1.3	0.763 0.405
No	113	97.4	229	98.7	

† Matched by age and occupation

**Table 4:** Multivariate logistic regression analysis of risk factors for scrub typhus infection, Luhe district\*.

Variables	$\beta$	SE.	Wald $\chi^2$	P- value	AOR	(95%CI)
Morning exercise in the park	1.348	0.678	3.947	0.047	3.848	(1.02-14.54)
Working as labour in vegetable fields	0.583	0.241	5.855	0.016	1.792	(1.12-2.87)
Fishing	0.454	0.568	0.638	0.424	1.575	(0.52-4.79)
Contact with straw	0.424	0.296	2.046	0.153	1.527	(0.85-2.73)
Dry clothes in the grasses	-0.44	0.668	0.434	0.510	0.644	(0.17-2.39)

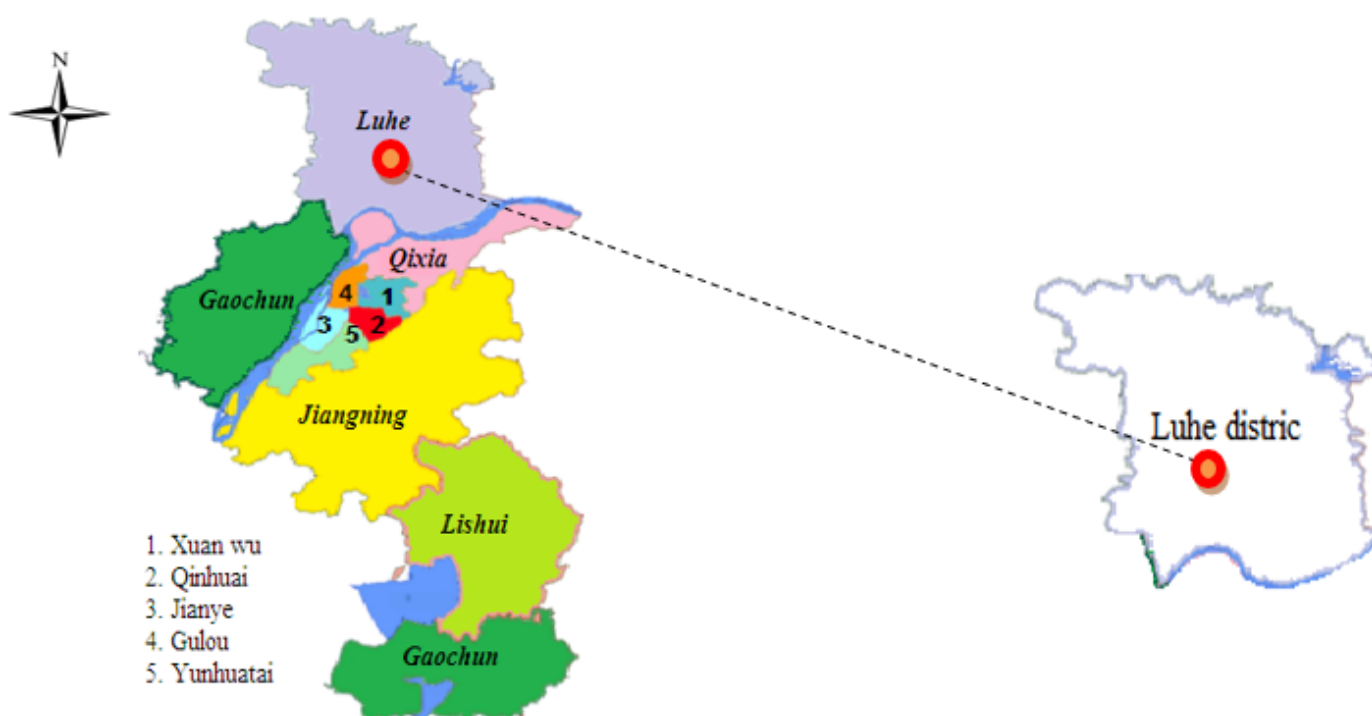
$\beta$ , Partial regression coefficient; SE, Standard error; AOR, odds ratio; CI, confidence interval.

\* Only the significant factors in Table 3 were selected for this analysis.

The significant level for entering the multivariate logistic regression model was set as 0.01 and for staying in it was set as 0.05.

\*Positive correlation factors: Morning exercise in the park and working as labour in vegetable fields

## Figures



## Figure 1

Map of study area (Luhe district, Jiangsu Province, China). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.