Emergence of *Schoengastiella ligula* as the Vector of Scrub Typhus Outbreak in Darjeeling: Has *Leptotrombidium deliense* Been Replaced?

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Abstract

Background: Following a suspected outbreak of scrub typhus in Kurseong, Darjeeling, the Armed Forces Medical College, Pune was requested by the National Institute of Epidemiology and the State authorities to undertake investigation of the ongoing scrub typhus outbreak and suggest containment measures. **Materials and Methods:** The epidemic team undertook clinical, entomological and serological studies to understand the local disease pattern and delineate high risk areas, host diversity by rodent trapping using Sherman traps, mite fauna diversity, abundance and vector species identification by phase contrast microscopy for preparation of electronic database and rodent and human serological studies by Weil Felix and PCR. **Results:** The results indicate no association of scrub typhus with age and sex (P=0.37 and 0.74 respectively). The maximum cases occurred amongst the tea garden workers (73%) in the age group of 25-44 years. The predominant clinical presentation was fever (100%) with headache (75%), lymphadenopathy (45%) and presence of eschar (76.7%). The dominant host species (50% of trapped rodents) was shrew *Suncus murinus*, the index animal for scrub typhus, which contributed maximally to the vector abundance (52.96%) with a chigger index of 61.56. The trombiculid mite *Schoengastiella ligula* was the vector species much against the expected mite vector *Leptotrombidium deliense*, in the area. The study found the presence and abundance of vector species which corroborated well with the occurrence of cases in the various localities within the subdivisions. **Conclusion:** The study thus establishes *Schoengastiella ligula* as the vector of scrub typhus outbreak in Kurseong, Darjeeling. Preventive and containment measures with emphasis on reduction of man – vector contact were suggested to the state authorities to contain the outbreak.

Key words: *Leptotrombidium, Schoengatiella ligula*, Scrub typhus, Trombiculid mite

Introduction

Scrub typhus also known as '*tsutsugamushi disease*' has a world-wide distribution with special activity over a triangular area bound by Japan in the east, India in the west and Australia in the south.[1] Approximately one million cases of scrub typhus occur each year and more than a billion people are at risk worldwide.[2,3] It is a febrile disease, endemic to the Asia-Pacific region, with case fatality rates up to 50% in untreated patients.[4] It is a common cause of disease among indigenous people where up to 19.3% of all illnesses and up to 23% of febrile illnesses among hospitalized patients are due to scrub typhus.[5,6] The signs and symptoms of the disease are consistent with a systemic infection in which fever, severe headache, and rash are commonly reported. Other signs and symptoms such as lymphadenopathy, eschar, myalgias, cough, sore throat, abdominal pain, stupor, and central nervous system involvement may also be seen.[7,8] Scrub typhus is not only an important health risk factor for individuals living in the endemic region, but it is especially problematic for military personnel operating in endemic areas.[7,9-11]

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The disease is caused by infection with *Orientia tsutsugamushi* (formerly *Rickettsia tsutsugamushi*), which is transmitted to humans by the bite of an infected *Leptotrombidium* mite (chigger stage). Amongst the vector species, *Leptotrombidium deliense* is the most important vector transmitting scrub typhus worldwide. The other trombiculid vectors of importance are *L akamushi, L scutellare, L pallidum, L dihumerale, L subintermedium* and *Schoengastiella ligula*.[12-15]

In India, Scrub typhus came into prominence as a war disease especially in the eastern regions and thereafter a series of outbreaks were reported from various parts of the country. The post war era, however, saw a declining interest in the disease and very soon it was relegated to the pages of public health textbooks as a disease of interest to the Armed forces. This continued up to the recent past, when reports of a series of scrub typhus outbreaks from various parts of the country ranging from up north, west, east to southern India established the re-emergence of scrub typhus in the Indian subcontinent.[16-20]

The outbreaks of scrub typhus reported in Darjeeling district of West Bengal in 2005 and subsequently were thoroughly investigated epidemiologically.[21] The investigations, however, lacked entomological inputs due to the absence of specialised entomological services in the state. It is in this backdrop, that the Armed Forces Medical College was called by the National institute of Epidemiology and the State authorities to undertake entomological survey in Kurseong, Darjeeling and complement the epidemiological investigation of the scrub typhus outbreak.

The study team set out for the investigation with the following objectives:

- To undertake epidemiological analysis of existing data of the scrub typhus cases.
- Conduct entomological survey in outbreak area to determine the vector species, its abundance and distribution.
- Determine Rickettsial activity amongst rodents in the proposed study areas.
- Analyze the factors contributing to vector abundance and man-vector contact.
- Recommend preventive and vector control strategies.

Materials and Methods

Outbreak site: Kurseong, Darjeeling: Kurseong subdivision is located in Darjeeling district of West Bengal, India, in the Himalayan region, close to the state of Sikkim, spanning over an area of 5.05 Sq. Km [Figure 1]. It has hilly terrain and its height from mean sea level varies from 4864 ft in Kurseong block to 5800 ft in Mirik block. The area is full of dense vegetation, has high humidity with an annual rainfall of 160 inches. In 1969, *Leptotrombidium deliense*, the known mite vector, was reported among rats in the district up to altitudes of 3840m. However, there were no reports of scrub typhus till 2004.

Scrub typhus outbreak (2005-2007): First confirmed report of scrub typhus outbreak from Kurseong was in 2005. The cases were reported especially during rainy season, when many patients were admitted to Kurseong hospital with fever of unknown origin. It was because of the fever associated with eschar that a clinical diagnosis of scrub typhus was considered and was subsequently confirmed by Weil Felix (OX- K) test in 2005. Since then, 61 cases of scrub typhus were reported till 2007. The cases though were distributed throughout Kurseong but clustering of cases was seen in Ambootia, Kurseong Municipality, Makaibari, and Soureni which contributed maximally to the scrub typhus outbreak.

Methodological framework: For the investigation of the ongoing epidemic, the epidemic investigation team, at the onset, interacted with health authorities at Sub-divisional hospital and took stock of the situation in the entire Sub-division. It also collected the relevant data available with the Superintendent, Health department. The area for the entomological survey i.e. the area from where maximum number of cases came, were also identified. These included Ambootia, Makaibari and Soureni.

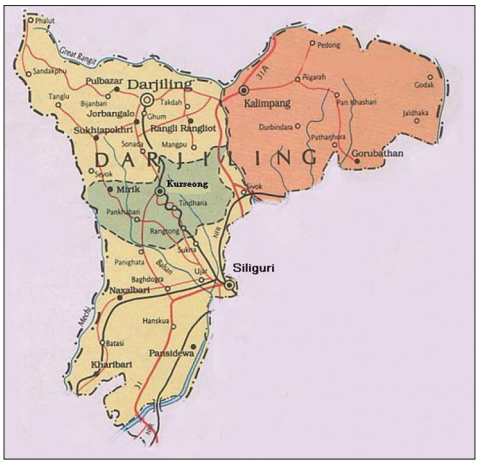

Figure 1: Map of Darjeeling district showing Kurseong

For the purpose of the epidemiological investigation, scrub typhus was defined as all cases of acute onset of fever with chills, severe headache, typical punched- out ulcer covered with a blackened scab (eschar) associated with tender lymphadenopathy which was confirmed by Weil Felix test.

The entire investigation was conducted in three phases:

In the first phase, data of all the suspected cases admitted in the hospital in the years 2005-07 were investigated to understand the determinants of the disease in the study area. Visits were also made to the houses of those patients who were admitted with the diagnosis of scrub typhus and were recently discharged. A thorough clinical examination with emphasis on the presence of eschar was conducted and samples taken wherever possible. Blood sample for serological confirmation and smears from the lesions were also taken. Visit to community was also utilized to search for more cases. Simultaneously details about rodent infestations and garbage disposal were obtained. An assessment of the surrounding, esp. with respect to mite habitat was also made at the same time.

Second Phase comprised of extensive and detailed entomological survey. Survey was carried out in two rounds to cover the identified areas. These included Ambootia (Baseri, Godamdhura, Sanu baseri, Thulo Baseri), Makaibari (Karbia TE, Munshidhura, Sanupatley, Karbia Thulopatley) and Soureni (Khaptawali Gaon, Lower Soureni Busty, Sadhugaon). In each round, 100 Sherman traps (specialized rodent traps for live rodent trapping) with bait were laid in various biotypes viz. domestic, peridomestic and areas of thick vegetation. The location of traps were planned in such a way as to ensure the coverage of the area from where the cases were reported or the areas which were topographically conducive for mite infestation. The traps were laid during dusk time and retrieved next day before the first light. After having made the assessment of trap positivity, the trapped rodents were anaesthetized using Ether, identified to species and screened for ectoparasites. For larval trombiculid mites, the common infestation sites i.e. ears, eyes, anal area and rump in case of shrews was screened using stereo microscope. For ticks manual screening of body was undertaken and body combing was used for flea collection. Ectoparasites so collected were preserved in 70% ethanol and brought to AFMC for further investigation and identification. A total of 10% of the trombiculid mites recovered from the rodents were then proposed to be mounted in Hoyer's media for identification using Phase contrast microscope following trombiculid mite identification key of Stan Fernandez.[22] A final electronic database for each mammal and the mite species with abundance with respect to locality surveyed was prepared. After screening for ectoparasites, blood samples were drawn for serology by conventional Weil Felix method and rodent dissection done for making organ smears for microscopic screening. It was also proposed to undertake PCR testing at AFMC, Pune for confirmation of scrub typhus.

While carrying out the entomological survey, the opportunity was also utilized for interviewing the old cases, their relatives and neighbours. Blood samples were also collected from old cases for follow up and relatives/ neighbours for control. The interaction with the community helped the team to impart IEC (health education) activity about garbage disposal, control of rodents and scrub typhus and its prevention.

In the third phase, the team went into the heart of the community to identify all old cases, collected blood samples for serological investigation at AFMC. This also helped as a follow up of these cases as on date. In the third phase, the data of all the suspected/ confirmed cases of scrub typhus which occurred since 2005 was collected from the hospital records. The data was analyzed on Epi Info under the guidance of the statisticians of the department.

Results

Clinical data of scrub typhus cases of Kurseong hospital during 2005-07

As per the available hospital data, a total of 235 patients suspected of scrub typhus were admitted to the hospital during the period of Jul 2005 to Jul 2007. Out of the total of 235, only 120 patients' case sheets could be traced from the hospital records. Of these 120 patients, 61% were females and 39% were males. The minimum age of admission was 2.4 yrs and maximum was 74 years with a median age of 30 yrs. The data of these cases show that the major clinical symptoms were fever in all 120 cases, eschar in 76.7% cases, headache in 75% cases, lymphadenopathy in 45% cases and conjunctival congestion in 9.16% cases. All these cases had symptoms suggestive of scrub typhus i.e. fever, headache, lymphadenopathy with or without eschar, and their blood samples were therefore sent for testing

to NCDC, Delhi for confirmation by Weil Felix and / or IgM testing. Based on these tests (either of the two or both positive), 61 cases (50.1%) were found positive for scrub typhus during the period.

Epidemiological analysis of hospital data

a) Time – The available data suggests that scrub typhus cases started occurring from July and continued till October. However, the maximum number of cases occurred during the month of Sep (30 out of 61 cases).

b) Place –Sub division Kurseong is full of thick vegetation, predominantly tea plantation. This presents a suitable habitat with grassy fields, shrubby areas, forests, tea plantation and cleared forests. Most of the cases came from periurban and rural areas. The data collected from the door to door survey revealed that the predominant group of people (73%) affected were working in the tea gardens. These areas represent an ideal mite island with thick vegetation, suitable temperature range, favorable humidity and rainfall. During summer months, the temperature in the Kurseong block area varies from a minimum of 15^0 C to a maximum of 29^0 C; and humidity during the day varies between 50 and 75% and during early morning from 75 to 95%, thus providing ideal conditions for the mites to thrive.

c) Person – Majority of the cases occurred among adult population. The predominant age group found to be affected was 25-44 yrs age bracket. Amongst the confirmed cases, 48.94 % were males and 52.05% were females. The data presented in Table 1 indicate that there is no significant association between age and scrub typhus and sex and scrub typhus (P=0.37 and P=0.74 respectively). These findings also corroborated with the findings on ground. The main occupation of the people living in affected area was workers of tea plantation and majority of workers were females. It was observed that most of the plantation workers were bare foot and had minimal protection below knees. Majority of the population working in this area belong to lower middle socio-economic status.

Table 1: Association between age, sex and lab confirmed cases of scrub typhus

Variable	Positive	Negative	Total	Odds Ratio (95% CI)	Chi Square	*P*-Value
Age						
< 35	37	31	68	1.39 (0.63 – 3.07)	0.80	*P* = 0.37
≥ 35	24	28	52			
Gender						
Male	23	24	47	0.88 (0.40 – 1.96)	0.11	*P* = 0.74
Female	38	35	73			
Total	61	59	120			

Entomological survey

The results of the entomological survey conducted in high risk areas of Kurseong are as follows:

Trapping efficiency: A total of 32 rodents were collected from the 11 study sites within 3 subdivisions of Kurseong viz. Ambootia, Makaibari and Soureni in two rounds of trapping. The overall trap positivity was 16% for both survey rounds. The maximum catch (73%) was found in and around the living places reflecting the poor hygiene of most of the houses. This was also contributed by the squash plantation near the houses which appeared to be a favourite feed of the rodents.

Ectoparasite diversity: The total ectoparasites sampled from the rodents during the survey were 1930, which comprised the trombiculid mites, ticks and fleas. The trombiculid mites contributed maximally (96. 37%) to the overall ectoparasite fauna, followed by ticks (3.21%). The rat flea's contribution was just 0.42% of the total.

Relative abundance of different species of mammals and parasitization of small mammals: Five genera of rodents were identified from the 32 rodents trapped during the survey which belonged to two orders viz. *Insectivora:* One genus - Shrew *Suncus murinus* (50%) and *Rodentia:* four genera – *Millardia meltada (15.62%), Rattus rattus rufescens (28.12%), Rattus blanfordi (3.13%)* and *Mus booduga (3.13%)* [Table 2]. About 66% of the rodents were positive for some ectoparasite infestation (mite, ticks or fleas). Amongst the trapped rodents, about 56.25% were positive for mite infestation. While fleas

Table 2: Rodent diversity, trombiculid mite infestation and chigger index

Rodent species	Total rodents trapped No. (%)	Rodents positive for trombiculid mite No. (%)	Trombiculid mites No. (%)	Chigger index
Suncus murinus	16 (50.00)	11 (68.75)	985 (52.96)	61.56
Millardia meltada	5 (15.62)	3 (60.0)	265 (14.25)	53.00
Rattus rattus rufescens	9 (28.12)	3 (33.3)	410 (22.04)	45.55
Mus booduga	1(3.13)	0 (0.0)	0 (0.00)	0.00*
Rattus blanfordi	1(3.13)	1 (100.0)	200 (10.75)	200.00*
Total	32 (100)	18 (56.25)	1860 (100)	

and ticks were found on 25% of the rodents, 5% of the rodents were found to have all the three ectoparasites i.e. fleas, ticks and mites.

A total of 1860 trombiculid mites were collected from the trapped rodents. Maximum collection of mites (52.96%) was from the index animal of scrub typhus i.e. *Suncus murinus* (shrew), followed by *R r rufescens* which contributed 22.04% of the total mites. The chigger index (average no. of chiggers per rodent) as expected was higher for *S murinus* (61.56), whereas the other two rodent species *M meltada* and *R r rufescens* had nearly similar chigger indices (53.0 and 45.55 respectively).

Host-parasite relationship of mites: The host parasite relation of mites is presented in Table 3. Two *Leptotrombidium* species (*fulmentum* and *burmense*) were recorded in the collection with four *Schoengastiella* species (*ligula* – the vector species, *aungasta, punctata*, *uttarkashiensis*) and one species of *Walchia i.e. rustica*. The most abundant species was *Leptotrombidium fulmentum* followed by the vector species *Schoengastiella ligula*. The shrew, *Suncus murinus* contributed maximally to the vector species (*S ligula*) abundance followed by *Millardia meltada* and *R r rufescens*.

Distribution of scrub typhus cases and vector species: The findings on distribution of scrub typhus cases and vector species corroborates very well with the no. of cases reported from various localities of the three subdivisions as is seen from Table 4. Maximum vector species were collected from Soureni followed by Ambootia, which have also reported the maximum no. of cases in Kurseong.

Rodent and human Serological Survey

Along with the entomological survey, blood samples of 12 old cases of scrub typhus from different survey locations and blood samples from 13 healthy relative/ neighbours were also collected. Rodent sera of the trapped rodents were pooled as per trapping area and 14 pooled sera from 32 rodents were transported to AFMC, Pune for testing. All these human and rodent samples were brought to AFMC for testing by Weil Felix and PCR. A majority of the samples, however, got haemolysed thus rendering them unfit for PCR as well as Weil Felix testing. A total of seven pooled rodent sera were tested by Weil Felix which however proved inconclusive as the titres were less than 30.

Table 3: Host parasite relationship of mites and mite species diversity in Kurseong

Rodent species	Trombiculid Mite species*							Total
	L f	L b	S l	S p	S a	S u	W r	
Suncus murinus	49	16	44	8	1	1	1	120
Millardia meltada	18	5	11	11	0	1	0	46
Rattus rattus rufescens	12	10	7	0	0	1	0	30
Mus booduga	0	0	0	0	0	0	0	0
Rattus blanfordi	6	1	2	0	0	1	0	10
Total	85	32	64	19	1	4	1	206

** L f – Leptotrombidium fulmentum, L b - Leptotrombidium burmense, S l - Schoengastiella ligula, S p - Schoengastiella punctata, S a - Schoengastiella aungasta, S u – Schoengastiella uttarkashiensis, W r - Walchia rustica*

Discussion

Trombiculid mites, as vectors of scrub typhus, pose a serious threat to civilian and military populations throughout the world. Scrub typhus figures prominently amongst the list of re-emerging diseases globally and in India as well.

The reports of scrub typhus outbreaks from various parts of the country in the recent past have firmly established its reemergence in India.[16-20] The outbreak in Kurseong further reinforces the threat from this disease not only in this part of the country but in other vulnerable areas as well. The study has clearly brought out the importance of recognizing this entity as one of the important causes of pyrexia of unknown origin in known endemic areas. The threat is equally, if not more, pertinent in areas which provide optimum conditions for the propagation of the vector mites and are not yet known for the occurrence of scrub typhus.

Table 4: Distribution of scrub typhus cases and vector species

Area	Cases of scrub typhus	Rodents trapped no. (mite positivity)	Trombiculid mite	Schoengastiella ligula
Ambootia	29	11 (9)	795	11
Godhamdhura	4	5 (4)	365	3
Baseri	3			
Sanu Baseri	5	6 (5)	430	8
Thulo Baseri	2			
Others (9 localities)	15	0	0	0
Makaibari	14	4 (1)	10	2
Makaibari	2	4 (1)	10	2
Others (9 localities)	12	0	0	0
Soureni	18	17 (10)	1055	51
Soureni basti	1	7 (3)		
Soureni TE	2		285	11
Sadhugaon	2	8 (6)	765	40
Others (14 localities)	13	2 (1)	5	0
Total	61	32	1860	64

The present study clearly brings out an important fact that majority of the scrub typhus cases occurred between July-October, which coincides with the monsoon season and the propagation of trombiculid mites - the vector of scrub typhus. The topography, climatic conditions and presence of extensive tea plantations (which provide ideal temperature, humidity and vegetation- the essential requirements for the establishment of mite islands) further adds to the potential of scrub typhus outbreak in the area. The main occupation of the people being workers of tea gardens also increases the chances of man-vector contact thus additionally enhancing the risk of transmission of scrub typhus. The workers were also found working barefoot or were ill clad thus increasing the risk of exposure to the chiggers.

The most predominant presenting symptom was fever found in cent percent cases. Similar findings of presence of fever as the most important complaint reported by over 98-100% of cases have also been reported by other workers.[20,23-25] The presence of eschar in 76.7% of the cases in the study corroborates well with the findings of other studies, which report its presence in 68-87% of cases.[26,27] The presence of eschar was however found in 45.5% of cases reported from south India[28] and 67.3% of cases in a study in China.[25] The presence of eschar in only 45% cases in south India could be due to missing out on detection as it is difficult to detect eschars on darker individuals.

The study did not find any association between gender and scrub typhus, though a majority of the tea garden workers were females. The reason is obvious as the probability of exposure of a worker to the vector is the same, be it male or female. The reasons for enhanced man-vector contact have already been elaborated in the earlier paragraphs. The vulnerable age group found in the present investigation was 25-44 years, which is similar to the age distribution of the workers in the tea gardens, with majority of the workers falling in this age group, however no association was found between age and scrub typhus.

The entomological survey has revealed very interesting results. The most expected results are the trap positivity of 16% and highest chigger index (61.56) in case of *Suncus murinus* – the index animal for scrub typhus. *Suncus murinus* emerged as the most efficient host for the vector mite in the study settings which might have been due to the trapping areas being mostly domestic and peri-domestic, where shrews abound. The most abundant mite species was *L fulmentum* followed by the vector species - *S ligula*.

The most important vector of scrub typhus throughout the world is *Leptotrombidium deliense*. Besides this vector, a couple of other *Leptotrombidium* species viz. *dihumerale*, *pallidum, scutellare, akamushi, subintermedium* have also been incriminated as vectors of scrub typhus the world over. *Schoengastiella ligula*, in addition to the *Leptotrombidium* species, has also been incriminated as a vector of scrub typhus and especially so in India. However, no outbreak has been attributed to *S ligula* as a vector.

The trombiculid mite fauna of India comprising 204 species of trombiculid mites, boasts the presence of only four vector species namely *L deliense, L dihumerale, L subintermedium* and *S ligula* with outbreaks of scrub typhus always being attributed to the presence of *L deliense*.

The district of Darjeeling has also been historically considered as one of the scrub typhus endemic areas in the country with scrub typhus outbreaks reported until the sixties. *L deliense* was incriminated as the vector of scrub typhus in the area at that time.[21] Thereafter, for a long time no outbreaks were reported which continued till 2005, when the area appeared to have suddenly awakened to the presence of scrub typhus with a series of cases getting reported from the area, thus once again drawing attention to this nearly forgotten disease entity. The diagnosis was confirmed based on clinical and serological studies, however vector studies were lacking in all investigations undertaken in the area until 2008, when the team from Armed Forces Medical College, comprising an entomologist, undertook entomological studies in the area. The results of the studies corroborated the scrub typhus outbreak but surprisingly the vector mite species i.e. *L deliense* was not present in the collections done in the survey. The search for the vector species zeroed on to the presence of *S ligula*, which is also considered as a vector of scrub typhus in India. The absence of *L deliense* in the outbreak area is interesting and equally interesting is the emergence of *S ligula* as the vector of the outbreak in Kurseong. It is a surprising finding and defies explanation as to why and how *L deliense* has disappeared from the area or whether this particular area ever had *L deliense* and if it was present earlier then what climatic or ecological changes have dwindled

its numbers which makes it difficult to be collected or whether it has disappeared from the area altogether or has it been replaced by other species like *S ligula* as the principal vector of scrub typhus in the area. Further long term studies are required to be undertaken in the area which may be able to solve the riddle.

Conclusion

The study thus establishes *S ligula* as the vector of scrub typhus outbreak in Kurseong, however the study suffers from the limitation that it has not been able to undertake serological testing of the rodent sera and testing of vector mite for determining rickettsial presence and strain. Based on the findings of the study, the following recommendations are being made:

i) To reduce man-vector contact and risk of transmission of scrub typhus, the tea plantation workers should be provided with proper barrier clothing with appropriate footwear by the plantation owners and its compliance should be strictly implemented.
ii) The workers should be educated on scientific choice of rodent control method (trapping and baiting) for implementation in their domestic and peri-domestic environments.
iii) The disposal of the dead rodents being very important for reducing vector exposure, the population should be advised not to dispose the dead rodents in the bins outside their houses as was seen as a routine practice, which increases the risk of transmission.
iv) The medical fraternity should be apprised of the presence of the disease in the area to increase their index of suspicion for scrub typhus while dealing with fever cases with associated symptoms (lymphadenopathy, eschar, rashes etc) indicative of scrub typhus.
v) Rickettsial surveillance should be established in the area for early warning as well as adoption of preventive strategies and control action.

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