Risk factors for scrub typhus and typhoid in the Kurseong sub-division of Darjeeling district, West Bengal, India, 2005 - 2006

By

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DECEMBER 2006

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Dissertation project submitted in partial fulfillment of the requirements for the degree of Master of Applied Epidemiology (M.A.E) of

Sree Chitra Tirunal Institute for Medical Sciences and Technology,

Thiruvananthapuram Kerala -695 011.

This work has been done as part of the two year Field Epidemiology Training Programme (FETP) conducted at

National Institute of Epidemiology,

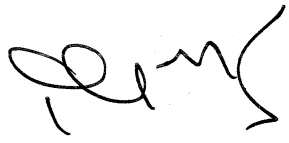
(Indian Council of Medical Research),

Mayor V.R. Ramanathan Road, Chetput, Chennai,-600 031.

DECEMBER 2006

CERTIFICATION

This is to certify that this dissertation, titled **'Risk factors for scrub typhus and typhoid in the Kurseong sub-division of Darjeeling district, West Bengal, India, 2005 - 2006'**, submitted by Dr. Puran Kumar Sharma, in partial fulfillment of the requirements for the degree of Master of Applied Epidemiology, is the original work done by him and has not been submitted earlier, in part or whole, for any other publication or degree.



Date 26.02.07

DIRECTOR

ACKNOWLEDGEMENTS

During the course of my dissertation work several persons have advised, guided, helped and supported me. I would like to express my gratitude sincere thanks to:

Prof. M.D. Gupte, Director National Institute of Epidemiology (NIE), Chennai for his valuable guidance amidst his busy schedule.

Dr. R Ramakrishnan, Deputy Director, NIE, my mentor and Dr. Yvan Hutin, Resident Advisor (WHO), NIE, for their constant guidance, support and encouragement.

Dr. Manoj Murhekar, Deputy Director & Course coordinator, Dr. Vidya Ramachandran, Assistant Director, Dr. P Manickam, Research Officer, Mr.L.Sundarmurthy and Dr.Vasna Josuha of NIE, Chennai for their guidance.

Mr. S.K.Lama, Executive Officer (Health), DGHC, Dr.T.N.Chattoraj, Zonal Malaria Officer, Jalpaiguri division, Dr.Subir Bhowmick, CMOH, Darjeeling and Dr.S.K.Mallick, Superintendent, Kurseong sub-divisional hospital for support and cooperation

Dr. Anup Kumar Barui, Physician, Kurseong sub-divisional hospital for his constant guidance, support and encouragement.

Dr. Manish Kakkar, WHO Consultant (Laboratory surveillance), India and Dr.Veena Mittal, Joint Director & Head of the Department, Zoonosis Division, NICD, New Delhi for laboratory support for confirmation of scrub typhus.

Mr. S. Satish, librarian, and Mrs. Uma Manoharan, secretary to the FETP, for their support.

All my colleagues at the Kurseong sub-divisional hospital and the BPHCs of Sukna and Mirik for their unstinted support.

All those staff of the Kurseong sub-divisional hospital who constantly helped me in my work.

My family for their understanding, patience, unstinted support and encouragement.

Finally, all the respondents who spared their valuable time and extended cooperation.

Date: **Dr. Puran Kumar Sharma**

PREFACE

Scrub typhus and typhoid are public health problems in the Kurseong sub-division of Darjeeling district, West Bengal, India. My dissertation work is on the study of risk factors for scrub typhus and typhoid in the Kurseong sub-division. Apart from taking healthy neighbourhood controls for both scrub typhus and typhoid, I have taken scrub typhus and typhoid cases admitted in the same hospital as unmatched hospital based controls for each other in my study. As the study identifies risk factors for two separate diseases, both presenting with fever, similar in severity and occurring during the rainy season but with different vehicles of transmission, I have presented their findings separately. I have also done a review of the literature on both the diseases and have provided them herein in the appendix.

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TABLE OF CONTENTS

Risk factors for scrub typhus in Kurseong, Darjeeling, West Bengal, India

1. Introduction

Scrub typhus - also known as mite-borne typhus or tsutsugamushi disease - is an acute, zoonotic, febrile illness of humans[1]. The etiological agent, *Orientia tsutsugamushi* (formerly, *Rickettsia tsutsugamushi*), is transmitted through the bite of infected, free-living, larval *Lepotrombidium* mites, or chiggers[1]. The mites are parasitic only in the larval stage. *O. tsutsugamushi* is maintained in the vector mites by transovarial transmission [2, 3]. The chiggers normally feed upon only a single vertebrate host and do not transmit infection from one host to the other[4]. Rodents are critical to the maintenance of the disease as they harbour chiggers[3]. *Leptotrombidium* mites are all primarily parasites on rats, field mice, shrews and rabbits, all of which have been found naturally infected with the agent [5]. Thus, the chigger distribution often directly reflects the distribution of the rodent host. Foci of infection known as typhus or mite islands have been reported in some areas as sharply localized and irregularly scattered spots where transmission is common[6]. Rodents are the usual vertebrate host while humans are accidental hosts[7]. Fever typically begins from six to 21 days following the bite and is accompanied by a maculopapular rash, headache and lymphadenopathy [8]. Frequently, a typical focal lesion or eschar develops at the site of the bite[1]. Clinical manifestations of scrub typhus range from mild fever with few other symptoms to a fatal syndrome characterized by multiple-organ failure[9]. If the disease is unrecognized or untreated, case fatality up to 60% have been reported[10]. The treatment of

choice is doxycycline[9]. Fatalities are rare among treated patients although there have been reports of antibiotic resistant strains and deaths among appropriately treated patients[11].

Scrub typhus is widespread extending from Japan in the North to tropical Queensland (Australia) in the South and from India in the West to Solomon Islands and Vanuatu in the East[12]. The disease was reported from various ecosystems including seashores, mountainous regions, rain forests, semiarid deserts, riverbanks and terrain undergoing secondary vegetation growth[13]. Although 25 – 50 % of scrub typhus cases occur in children, most cases occur through agricultural exposure, among paddy field workers of Thailand, Japan, or Korea and among oil palm and rubber plantation workers in Malaysia[13]. Scrub typhus was endemic in the Asia Pacific region during World War II and re-emerged as outbreaks and sporadic cases since the 1980s in various countries including Japan, Australia and in south Asia[14, 15, 16, 17]. In India, scrub typhus was also reported during the second World War especially in the Eastern regions[18]. Outbreaks of scrub typhus then occurred in Punjab in the West, Shimla in the North, Assam and Bengal in the East. Then, the incidence declined after the war. Factors that may have contributed to this decline include wide spread use of insecticide, effective antibiotics and changes in life style[18]. Since 2000, there have been reports of scrub typhus outbreaks from the Indian state of Tamilnadu in the South and the states of Himachal Pradesh and Sikkim in the Northern Himalayan region[19, 20, 21]. This re-emergence suggests that changes in ecology may have taken place that need to be investigated.

Kurseong sub division is located in the Darjeeling district of West Bengal, India, in the Himalayan region, close to the state of Sikkim where scrub

typhus re-emerged in 2004. In 1969, *Leptotrombidium deliense*, a vector of scrub typhus was reported from the hilly and foothill areas of Darjeeling district up to altitudes of 3,840 metres [22]. *Rattus rattus* was the main host of the mites[22]. However, there were no reports of scrub typhus. In 2000, patients with fever of unknown origin were admitted to the Kurseong hospital. Of these, some died of multi-organ failure. Subsequently, in 2001, 2002 and 2003, similar cases continued to occur, particularly between June and October, during the rainy season, affecting people above thirty years of age who resided in peri-urban and rural areas. Absence of laboratory diagnostic facility prevented the identification of an etiological agent. In 2004, a new cluster of patients presenting with a typical eschar led to the clinical suspicion of scrub typhus that was confirmed by Weil Felix test at the National Institute of Communicable Diseases in Delhi. As the disease was now recognized, lack of information on risk factors made it impossible to formulate recommendations. In 2005, we conducted a case control study to recommend appropriate prevention measures. The objectives of this study were to (1) estimate the strength of association between potential risk factors and protection measures and scrub typhus, (2) estimate the fraction of cases attributable to selected exposures and (3) estimate the fraction of cases of scrub typhus that could be prevented through potential protection measures.

2. Methods

Study population and design

We defined the study population as the residents of Kurseong sub division of Darjeeling district, West Bengal, India. We conducted a case-control study with two control groups: one matched and one unmatched. First, we recruited

cases of scrub typhus at the Kurseong sub-divisional hospital. Second, we recruited an age and neighborhood matched control group from the community. Third, we recruited an unmatched control group from patients admitted for a condition of equivalent severity (typhoid) in the same hospital.

Cases and controls

We defined a case of scrub typhus as an acute onset of fever > 38^0 C with eschar and laboratory confirmation of IgM antibody specific for scrub typhus at ≥1: 128 titer by Immunofluorescent Antibody Test in a patient admitted at the Kurseong Sub Division hospital in 2005. We recruited all cases meeting the clinical case definition during the study period. For the first control group, we recruited one healthy neighbour per case, matched for age. For the second unmatched, hospital-based control group, we recruited patients suffering from typhoid (confirmed by Widal test[23] with four fold rise in 'O' antibody titre, blood samples taken 10 days apart) in the same hospital at the same time.

Laboratory investigations

We collected blood samples from scrub typhus and typhoid patients to confirm the diagnosis as part of routine hospital procedures for their treatment. A qualified laboratory technician collected 10ml of blood from scrub typhus and typhoid cases by venepuncture from the cubital vein using sterile disposable syringes and needles and following the standard aseptic procedures. We sent the blood samples for confirmation of scrub typhus by estimation of the titre of IgM antibodies to scrub typhus infection using the Immunofluorescent Antibody test to the National Institute of Communicable Diseases, New Delhi,

India. We considered IgM titres ≥ 1:128 as positive for scrub typhus[24]. We did not draw any blood sample from the healthy neighbourhood controls.

Data collection

We collected information on demographic characteristics, place of residence, activities, (including occupation) as well as exposure to forests, bushes, domestic animals, rodents and ectoparasites. We defined the referent exposure period as the 21 days prior to the appearance of clinical signs and symptoms (for case-patients and hospital-based controls) or prior to recruitment (for neighborhood controls). To obtain the information we trained health personnel to conduct interviews using structured, standardized, close-ended, pre-tested questionnaires written in Nepali, the local language.

Sample size

Assuming a prevalence of exposure of 10% among controls and aiming at detecting odds ratios of at least three with a 95% confidence interval and 80% power, we needed to recruit 112 cases and 112 controls. To allow for non-responses, we planned a sample size increased by 10%. Thus, our target was to recruit (1) 123 cases and (2) 123 controls in each of the matched and unmatched control groups.

Data analysis

We described the incidence of scrub typhus in terms of time, place and person for the year 2005 using census data as denominators. We conducted two analyses of the case control study: one matched and one unmatched. We calculated matched odds ratio (MOR) using discordant pairs for all exposures

for the neighborhood control group. We calculated unmatched odds ratio (OR) for all exposures for the hospital based control group. We calculated the fraction of cases attributable to various exposures when causality was suspected. We used the following formulae: Attributable fraction in the population (AFP) = Proportion of cases exposed x {(OR – 1) / OR}. For exposures associated with a lower risk of illness, we calculated the fraction of cases attributable to the failure to use the prevention measure. We stratified to eliminate confounding and to identify effect modifications. After checking that the matched and unmatched odds ratios for some significant exposures were similar, we broke the match and examined the dose response relationship for those exposures by calculating the odds ratios according to increasing gradients of exposure. We analyzed the data using Epi-info 2005.

Human subject protection

We explained the objectives, methods, risks and benefits of the study to the participants and took written informed consent. We did not write the names of the study subjects on the questionnaires and used a confidential code instead. We approached healthy neighbourhood controls using precautions to maintain the confidentiality of the matched case patients. The ethical committee of the National Institute of Epidemiology, Chennai (under Indian Council of Medical Research) approved the study.

3. Results

Descriptive epidemiology

The incidence of scrub typhus increased from 2 per 100,000 populations in July 2005 and reached 20 per 100,000 populations in September 2005 and then decreased without any case patients in December (Figure 1).

The overall incidence in Kurseong sub-division was 30 per 100,000 populations. In some areas clustering of scrub typhus cases occurred but there were no outbreaks. These areas were rural, agricultural and included tea plantations.

Females had a higher incidence compared to males. The age group of 30-44 years had the highest incidence (79 per 100,000 populations, Table 1).

Laboratory investigations

Out of the 122 case patients meeting the clinical case definition for scrub typhus who were recruited for the study and whose blood samples were sent for laboratory confirmation, 62 (51%) were positive with IgM titre of 1:128 and above by the Immunofluorescent Antibody test. 52 typhoid cases were confirmed by a four-fold rise in antibody tire using paired sera. The median acute phase antibody tire was 1:160 (range: 1:80 to 1:320).

Characteristics of cases and controls

We included 62 scrub typhus case-patients in the case control analysis. We recruited 62 healthy neighbors as matched controls and 52 typhoid patients as unmatched controls. Compared to matched controls, cases were more likely to be female and more likely to belong to the general caste. Compared

to unmatched controls, cases were less likely to reside in wooden houses and belong to the general caste. However, none of these differences were statistically significant (Table 1).

Living environment

Compared with matched controls, cases were more likely to live in environments with bushes, piles of wood, rodents and domestic animals. The odds of disease increased with the number of bushes located within five meters of the residence, the number of piles wood in the yard of the house and the number of observations of rodents at work premises (Table 2). However, in the unmatched analysis presence of presence of piles of wood in the yard of the house was the only characteristic significantly associated with illness (Table 2). Living in houses within five metres of bushes, presence of piles of wood in the yard and presence of rodents in and around the house and at workplace accounted for 75%, 40%, 65% and 59% of cases in the population, respectively. The association between exposure to rodents at home and illness was stronger among those rearing animals in the yard (OR: 5.6, 95% CI: 1.8 – 20] than among others (OR: 2.1, 95% CI: 0.4 – 16). Similarly, the association between exposure to bushes among those with monthly family income of more than rupees1500 (US$ 30) was stronger (OR: 13, 95% CI: 2.5 – 95) than among others (OR: 2.6, 95% CI: 0.6 – 12).

Activities and occupation

Compared to matched and unmatched controls, case patients were more likely to be farmers (Table 2). However, the association was not significant in the unmatched analysis.

Exposure to vectors

Compared to matched controls, case-patients were more likely to report itching (Table 2) However, there was no association between itching and illness in the unmatched analysis (Table 2). The association between exposure to itching was stronger among those rearing animals (OR: 12, 95% CI: 1.3 – 300) than among others (OR: 2.5, 95% CI: 0.8 – 7.8).

Hygiene and protective measures

Compared with both sets of controls, cases were less likely to wash after work and to change clothes to sleep (Table 2). Compared with matched controls, cases were less likely to wear gumboots and aprons (Table 2). The use of repellents did not exceed eight percent among cases and controls. There was no association between this practice and illness. The association between the practice of changing clothes to sleep and decreased risk of disease was stronger among those earning more than rupees 1500 (US$ 30, OR: 0.05, 95% CI: 0.01 – 0.3) than among others (OR: 0.4, 95% CI: 0.1 – 1.2).Failure to change clothes to sleep, to wear gumboots at work and to wash after daily work accounted for 45%, 31% and 16% of cases in the population, respectively.

4. Discussion

We identified a number of risk factors for scrub typhus in Kurseong, Darjeeling. These were related to the living environment, daily activities and exposure to rodents and vectors. A review of these risk factors provides some understanding of the ecology of scrub typhus that may be used to recommend interventions. We also identified protective factors. These included wearing

gumboots at work, washing after work and changing clothes to sleep. This information provides useful direction to suggest behaviour change activities to protect the community.

Exposure to environmental factors including bushes, piles of wood, domestic animals and rodents were significantly associated with the disease. Peri-domestic rodents are a risk factor for the scrub typhus because they harbour the trombiculid mites.[25] Bushes and piles of wood are natural habitats of rodents[16, 25, 26, 27]. Two explanations may account for the association between rearing animals and scrub typhus. First, the storage of fodder of domestic animals may attract rodents[28]. Second, domestic animals might also harbour the mites in addition to the rodents[29, 30]. Farmers had higher odds of disease. This may be explained by an exposure to rodents, wood piles and animals[13]. A study in Japan reported that 44% of case-patients engaged in farming, suggesting that the activity in the infected areas increase the duration of exposure and to the possibility of infestation by infected mites[31]. A clean living environment and control of rodents decreased the incidence of scrub typhus significantly in troops in an island in China from 2000-2004. [28]Exposure to lice and ticks was a risk factor. These macroscopically visible ectoparasites do not spread the pathogen by themselves. However, they may constitute a surrogate marker for an exposure to the mites that are not easily visible to the naked eye.

Our results suggest that a number of behaviours may be effective at preventing scrub typhus. Unlike gloves, aprons and gumboots were protective against scrub typhus. Unfed *Leptotrombidium* tend to aggregate closely in clusters on twigs and debris a few inches above the ground and await a host[32]. The mites are closer to the ground and the eschars are more

commonly found on the lower part of the body of the patients. In contrast to changing clothes after work, changing clothes to sleep decreased the risk of scrub typhus. This difference may be explained by the longer period of time available during the night for the mite to bite. Transfer of *Orientia tsutsugamushi* from an infected mite to humans takes more than six hours[31]. Bathing after work was also protective against the illness. Once they gain access to the body, the mites seek out areas where clothing is tight against the skin, the waist and ankles being the parts most commonly attacked[27]. The larvae remain attached to the skin of the host for between 36 to 72 hours after which they disengage and drop off the host on the ground[32]. Hence, thorough scrubbing and washing of the body after exposures may decrease the risk of bites by the chiggers and thus the risk of scrub typhus[31]. While washing and bathing in the Kurseong population might not systematically include thorough scrubbing, our results suggest some effectiveness of the local hygienic practices.

Our study had four limitations. First, we recruited case-patients from the hospital. Thus, patients with milder form of the disease in the community may have been excluded. As a result, the study could be biased if patients with more severe form of the disease had a different risk factor profile. However, there are no elements suggesting that the number of bites or that the infectious dose influences the natural history of the disease. In addition, among our patients, the severity of the cases did not vary with the number of eschars. Hence, severe cases are likely to be representative of all cases with respect to exposure and a bias is unlikely to have occurred. Second, the study was conducted in Kurseong and the findings may not be generalizable to other places. For example, the ecology of the disease may differ like in the

'mite islands' of Japan. Our findings about environmental factors may be specific to the Darjeeling ecosystem. However, the protective behaviours that we identified, including wearing gumboots at work, washing after daily work and changing clothes to sleep might be generalizable to other areas. Third, less than eight percent of participants used repellents. A statistical power calculation suggested that our sample size for that specific uncommon exposure would not have had a power of 80% to detect an odds ratio of 0.01. Hence, we did not have a reasonable capacity to document an association between use of repellents and scrub typhus. Thus, an intervention study may provide a better setting to examine the effect of repellents against scrub typhus[13]. Fourth, though our targeted sample size was 123, we could recruit only 62 case patients because the serum samples of the remaining 61 patients were negative for IgM antibody. This low proportion of confirmation may be due to the low sensitivity of the test (84% at a cut off IgM antibody titre of $\geq$1:128 for diagnosis of scrub typhus) [23]. Further, collection of blood samples early in the first week of illness and effects of early chemotherapy following clinical diagnosis might have delayed the appearance of diagnostic titres of IgM antibody in the reported negative cases[33]. While the recruitment of a smaller number of cases is likely to have reduced the power of our study, this lack of power would not have affected the risk factors and protective practices that we identified.

Our study suggests that there may be opportunities to prevent exposures to scrub typhus through a cleaner, rodent-controlled environment. Furthermore, adoption of safe personal protection measures could further reduce the incidence of disease. On the basis of the findings of our study, we can propose a number of recommendations for local health authorities and

municipalities to control scrub typhus. Education of the population could make the living and working environment safer with respect to scrub typhus. This includes clearing the bushes, keeping wood and animals away and controlling rodents. These environmental interventions could be guided by the spatial distribution of the disease so that "mite islands" are cleared. Promotion of protective measures may reduce individual risks. These include wearing gumboots to work, washing after work and changing clothes to sleep. Further studies could characterize the exact vectors and hosts involved. Behavioral intervention studies could also examine options to introduce safer practices, including repellent use. Finally, hospital-based public health surveillance will provide an opportunity to evaluate the effectiveness of the proposed prevention measures.

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Table 1: Incidence of scrub typhus cases by age and sex, Kurseong sub-division, Darjeeling, West Bengal, India, 2005

Characteristics		Population	# of cases	# of deaths	Case fatality ratio (%)	Attack rate (per 100,000 population)
Age groups	0 – 4	19,287	5	0	0	26
	5 – 14	45,737	10	0	0	22
	15 – 29	54,003	16	0	0	30
	30 – 44	26,450	21	0	0	79
	45 – 59	28,287	10	0	0	35
	60+	9,920	0	0	0	0
Sex	Male	97,353	28	0	0	29
	Female	86,331	34	0	0	39
Total		183,684	62	0	0	34

Table 2: Characteristics of scrub typhus cases and controls Kurseong, Darjeeling district, West Bengal, India, 2005

			Control groups					
	Cases (n=62)		Unmatched hospital controls[1] (n=52)			Matched neighbourhood controls (n=62)		
Characteristics	#	%	#	%	P - value	#	%	P - value
Age ≤ than 30 years	31	50	27	52	0.9	29	47	0.3
Female sex	34	55	28	54	0.9	38	61	0.5
Follower of Hinduism	47	76	40	77	0.9	49	79	0.4
Belonging to general caste	43	69	39	75	0.6	40	65	0.6
Residence in rural areas	52	84	44	85	0.9	52	84	N/A
Residence in houses made of wood	45	73	42	81	0.4	48	77	0.4
Monthly income less than Rs.1500	30	48	25	48	0.9	28	45	0.8
Household with members more than four	40	64	35	67	0.9	35	57	0.5

[1] Serologically confirmed typhoid cases

Table 3: Selected exposures for scrub typhus cases and controls, Kurseong, Darjeeling district, West Bengal, India, 2005

				Control groups							
		Cases (n=62)		Unmatched hospital controls[2] (n=52)				Matched neighbourhood controls (n=62)			
Exposure[1]		#	%	#	%	OR[3]	95% CI[4]	#	%	MOR[5]	95% CI
Living environment	Bushes within 5 meters [6]	56	90	45	87	1.4	0.4 – 4.6	38	61	10.0	2.3 –63.3
	Piles[7] of wood in the yard[8]	39	63	21	40	2.5	1.2 – 5.4	24	39	3.5	1.5 – 9.5
	Observation of rodents at home	55	89	50	96	0.3	0.04 – 1.5	42	68	3.6	1.4 – 10.8
	Observation of rodents at work place	51	82	42	81	1.1	0.4 – 2.9	35	57	9.0	2.4 – 57
	Domestic animals in the yard	44	71	39	75	0.8	0.3 – 1.8	33	53	2.4	1.1 – 5.7
Activities	Plucking tea leaves	10	16	7	13	1.2	0.4 – 3.7	9	15	1.2	0.4 – 4.3
	Working in the farm	44	71	32	61	1.5	0.7 – 3.4	26	42	10.0	2.7 – 63
	Working in the forest[9]	15	24	18	35	0.6	0.2 – 1.3	23	37	0.5	0.2 – 1.1
	Working at construction sites	6	10	1	2	5.3	0.8 – 12.9	5	8	1.2	0.4 – 4.3
Exposure to vectors	Presence of ticks/lice on the body	15	24	22	42	0.4	0.2 – 1	10	16	1.6	0.7 – 4.1
	Presence of itching	25	40	22	42	0.9	0.4 – 2	11	18	3.0	1.3 – 7.6
Hygiene and protective practices	Wearing gumboots at work	23	37	27	52	0.5	0.2 – 1.2	36	58	0.5	0.2 – 0.9
	Washing/bathing after daily work	45	73	47	90	0.3	0.08 – 0.8	56	90	0.4	0.1 – 0.9
	Wearing aprons at work	5	8	4	8	1	0.2 – 4.6	13	21	0.3	0.1 – 0.9
	Changing clothes to sleep	27	44	38	73	0.3	0.1 – 0.6	51	82	0.2	0.1 – 0.5
	Using mite repellents on body	4	7	1	2	3.5	0.4 – 18	5	8	0.8	0.2 - 3.2
	Using mite repellents on clothes	5	8	2	4	2.2	0.4 – 16.8	3	5	1.7	0.4 – 8.5

[1] Referent period of 21 days
[2] Serologically confirmed typhoid patients
[3] Odds ratio
[4] Confidence interval
[5] Matched odds ratio
[6] Bushes stand for bushes/shrubs/bamboo groves
[7] Stacks of wood measuring 5' x 5' x 5,
[8] A yard would be an enclosure near a residence. On an average, it would be an area of around 25 – 50 feet around the residence
[9] Working in the forest for food/fodder for domesticated animals

Figure 1: Incidence of scrub typhus cases by months of occurrence in Kurseong sub-division, Darjeeling district, West Bengal, India, 2005

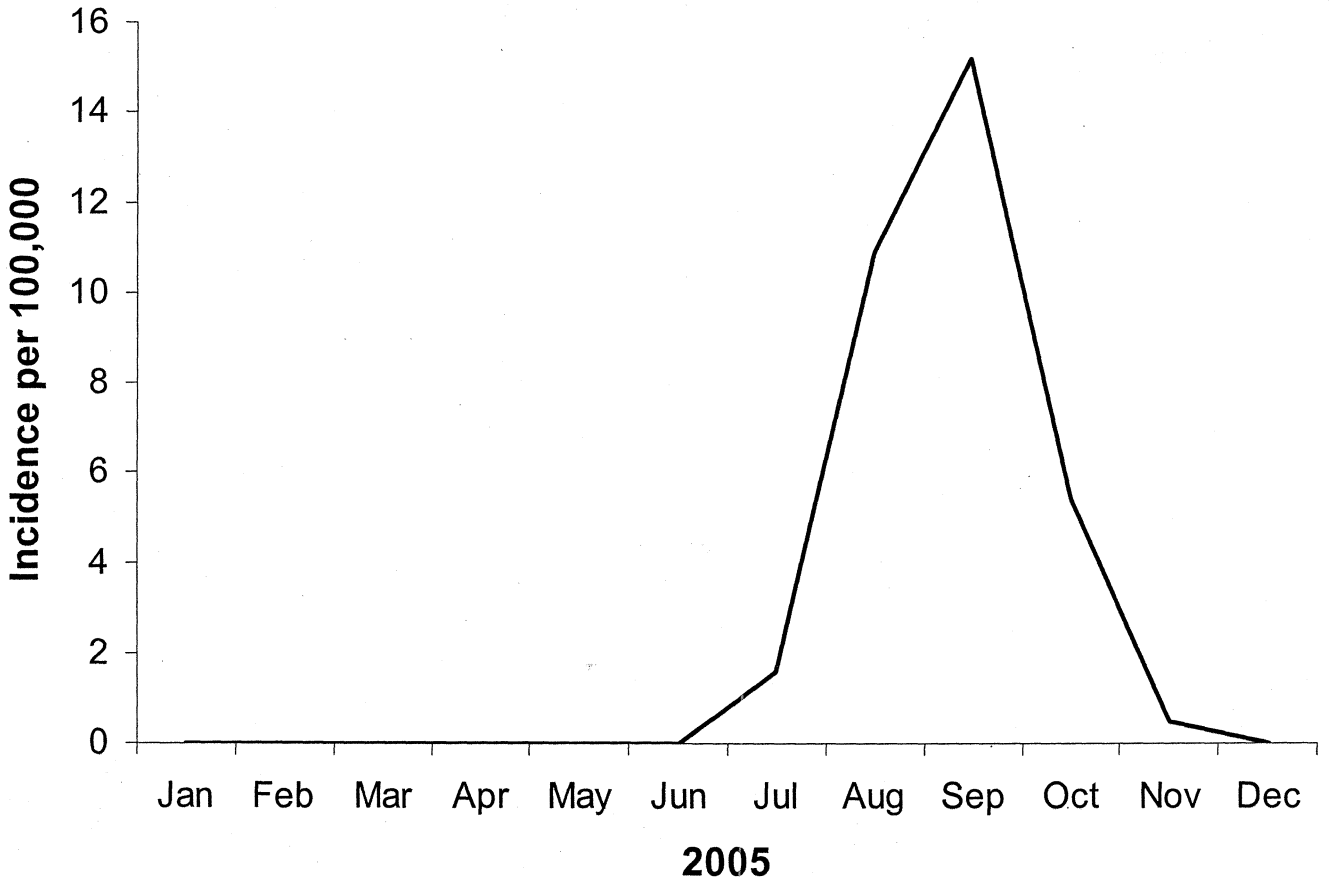

Table 4: Odds of scrub typhus according to increasing gradients of exposure to selected risk factors, Kurseong, Darjeeling district, West Bengal, India, 2005

Exposure		Cases (n = 62)		Controls (n = 62)			
	Level	#	%	#	%	OR	95% CI
Bushes within five metres of house	None	6	10	24	39	1	
	One to six	24	39	26	42	3.7	1.1 – 12.2
	More than six	32	51	12	19	10.7	3.1 – 38.5[1]
Piles of wood in the yard	None	23	36	38	61	1	
	One to two piles	29	46	19	31	2.4	1 – 5.7
	More than two piles	11	18	5	8	3.6	1 – 13.9[2]

[1] Chi-square for trend: 19.8; P-value: 0.0001
[2] Chi-square for trend: 7.2; P-value: 0.007

Risk factors for typhoid in Kurseong, Darjeeling, West Bengal, India, 2005 -2006

1. Introduction

Typhoid is a bacterial infection caused by *Salmonella typhi*[1]. Worldwide, in 2004, typhoid affected 21.7 million people and caused 216,510 deaths[2]. It is endemic in countries where there is neither a safe water supply nor adequate sanitation[3]. Complications occur in about 30 percent of untreated cases and account for 75 percent of all deaths due to typhoid fever[4]. Without effective treatment, the case fatality is 10 percent[4]. Following recovery, up to five percent of patients become chronic carriers of *Salmonella typhi*[4]. Humans are the only reservoir of infection[4]. Typhoid spreads through faecally contaminated water or food. Food handlers are an important source of transmission as they have potential to infect many persons[5]. Primary sources of infection are faeces and urine of carriers or patients. Secondary sources are contaminated water, food, fingers and flies[4]. Risk factors for typhoid in Europe, Central Asia and the Americas include contamination of municipal piped water, faecal contamination of water and food, drinking unboiled tap water, purchasing lunch at school, sharing food items with friends, eating flavoured ice purchased outside home and eating cheese, raw onions and tomatoes [5,6, 7, 8].

Asia reports high incidences [10] of typhoid with almost 80 percent of the cases and deaths of typhoid in the world [9]. Risk factors for typhoid in south-east

[10] High incidence means > 100/100,000 cases/year

Asia include recent contact with a typhoid patient, absence of education, absence of latrines at home, drinking untreated water, eating various food items, including shellfish, ice-cream and food from roadside vendors.[1, 10, 11] . The estimated attack rate of typhoid among travelers to the Indian sub-continent (India, Pakistan and Bangladesh) for travelers is 10 per 100,000 trips[12]. Since 1990, outbreaks of *Salmonella typhi* were reported from different parts of India[13, 14, 15]. In the state of West Bengal, the reported incidence of typhoid was 104 per 100,000 populations in 2004[16]. In 2003, children between 2 and 3 years of age were the most susceptible age group for *Salmonella typhi* infection in Kolkata [13]. However, in a prospective community based study in the same city, patients with typhoid fever had a mean age of 15 years[17].

In 2003, typhoid ranked third among the ten commonest diseases in the Darjeeling district of West Bengal with an incidence of 808 per 100,000 population[18]. Despite this public health burden, little was known about *Salmonella typhi* transmission or local risk factors. In the district, less than 50% of people access potable water and sanitation [19]. In the Kurseong sub-division of the district, water is scarce and people buy it untreated from vendors. It is a hilly area with a temperate climate and seasonal rainfall where the people mostly depend on streams and natural springs for their water requirements. People often have to travel get distances to get water from these water sources which are often exposed to contamination from people washing and bathing there. In the urban areas a public water supply system exists but supply of water is limited in terms of both quantity and quality. In the rural areas public water supply system is virtually non-existent. Identifying risk factors for typhoid in this area is required to use of limited resources available

for control and prevention. In 2005, we conducted a study to (1) estimate the strength of association between potential risk factors and protection measures and typhoid, (2) estimate the fraction of cases attributable to selected exposures and (3) estimate the fraction of cases that could be prevented through these potential protection measures.

2. Methods

We defined the study population as the residents of the Kurseong sub division of Darjeeling district, West Bengal, India. We conducted a case-control study using two control groups: one matched and one unmatched. First, we recruited cases of typhoid in the sub-divisional hospital. Second, we recruited an age and neighborhood matched control group from the community. Third, we recruited an unmatched control group from patients admitted for a condition of equivalent severity in the same hospital (scrub typhus).

Cases and controls

We defined a case of typhoid as the occurrence of fever of at least 38^0C for three or more days with a positive Widal test (i.e., four fold rise in 'O' antibody titre in blood samples taken 10 days apart). We tested all patients meeting the WHO clinical case definition[20] during 2005-October 2006. For the first matched, population based control group, we recruited one healthy neighbour per case, matched for age. For the second unmatched, hospital-based control group, we recruited suspected scrub typhus patients (negative for typhoid using the Widal test) admitted at Kurseong Sub Division hospital in 2005-2006.

Laboratory investigations

We collected blood samples from typhoid case-patients and scrub typhus control-subjects to confirm the diagnosis as a routine hospital procedure for their treatment. A qualified laboratory technician collected 10ml of blood from the cubital vein using sterile disposable syringes and needles and following the standard aseptic procedures. The hospital laboratory tested the blood samples for confirmation of typhoid by the Widal test using paired sera. A four-fold rise in antibody titre using paired sera confirmed the diagnosis of typhoid.

Data collection

We collected information on demographic characteristics and various potential exposures. These included drinking water (sources, treatment, storage and usage), consumption of food items (unwashed fruits, raw vegetables, milk and milk products) and sanitation (availability of latrines at home and sewage disposal practices). We defined the referent exposure period as the 14 days prior to the appearance of clinical signs and symptoms (for case-patients and hospital-based controls) or prior to recruitment (for neighborhood controls). To obtain the information, we trained health personnel to conduct interviews using structured, standardized, close-ended, pre-tested questionnaires written in Nepali.

Sample size

Assuming a prevalence of exposure of 10% among controls and aiming at detecting odds ratios of at least three with a 95% confidence interval and 80% power, we needed to recruit 112 cases and 112 controls. To allow for non-

responses, we planned a 10% increase in sample size. Thus, our target was to recruit 123 cases and 123 controls in each of the matched and unmatched control groups.

Data analysis

We described the incidence of typhoid cases in terms of time, place and person for the year 2005 (as data were available for the whole of that year). We conducted two case-control analyses, one matched and other unmatched. We calculated matched odds ratio (MOR) using discordant pairs for all exposures for the neighborhood control group. We calculated unmatched odds ratio (OR) for all exposures for the hospital based control group. We calculated the fraction of cases attributable to various exposures when causality was suspected. We used the following formulae: Attributable fraction on the population (AFP) = Proportion of cases exposed x {(OR – 1) / OR}. For exposures associated with a lower risk of illness, we calculated the fraction of cases attributable to the failure to use the prevention measure. We stratified to eliminate confounding and to identify effect modifications. After checking that the matched and unmatched odds ratios for some significant exposures were similar, we broke the match and examined the dose response relationship for those exposures by calculating the odds ratios according to increasing gradients of exposure. Because we expected a number of exposures to be closely associated with each other, we also controlled for confounding through a multivariate analysis. We placed all the exposures with a P-value $\leq$ 0.05 on univariate analysis into a conditional logistic regression model (that is valid for the matched analysis, not for the unmatched). We analyzed the data using Epi-info 2005.

Human subject protection

We explained the objectives, methods, risks and benefits of the study to the participants and took written informed consent. We did not write the names of the study subjects on the questionnaires and used a confidential code instead. We approached healthy neighbourhood controls using precautions to maintain the confidentiality of the matched case patients. The ethical committee of the National Institute of Epidemiology, Chennai (under Indian Council of Medical Research) approved the study.

3. Results

Descriptive epidemiology

We recruited 123 typhoid cases from January 2005 to October 2006. Of these, 52 occurred in 2005 (Attack rate: 28.3 per 100,000). There were no deaths. In 2005, the incidence increased from 2.7 per 100,000 populations in July, reached a peak of 13.6 per 100,000 populations in September during the rainy season and decreased to 2.7 per 100,000 populations in December (Figure 1).

In 2005, the Kurseong municipality and the rural areas of Mirik and Kurseong had an overall incidence of 24.8, 40.7 and 11.2 per 100,000 populations respectively. Typhoid cases were evenly distributed except for an area each in Mirik and Kurseong blocks where spatial clustering was present. However, there were no outbreaks of typhoid identified.

Females and males were equally affected. Persons under 30 years of age had a higher incidence (Table 1).

Laboratory investigations

We identified 123 typhoid cases during the course of the study. The median acute phase antibody tire was 1:160 (range: 1:80 to 1:320). We recruited 177 clinically suspected scrub typhus patients admitted at Kurseong hospital as controls-subjects. Of these, 122 were tested for the presence of IgM antibody to scrub typhus and 62 were positive for scrub typhus with IgM antibody titre $\geq$ 1:128 by Immunofluorescent Antibody test.

Characteristics of cases and controls

We included 123 typhoid case-patients in the case control analysis. We recruited 123 healthy neighbors as matched controls and 177 clinically suspected scrub typhus patients as unmatched controls. Compared to matched controls, cases were more likely to be followers of Hinduism, belong to the general caste, have a monthly income less than rupees 1,500 (US $ 30) and to live in houses made of wood (Table 1). These associations were not explained by any other exposure in the stratified analysis.

Environmental factors

Compared to unmatched controls, cases were more likely to have travelled out of Kurseong in the 14 days preceding onset or recruitment and to have more than four members in the household (Table 2).

Drinking water

Compared with matched controls, cases were more likely to drink stream water at home but this was not statistically significant (Table 3). Compared to unmatched controls, cases were more likely to have piped water supply at

home [OR: 2, 95% C.I.: 1.2-3.4]. However, when compared to matched controls, cases were less likely to drink piped water at home [Matched OR: 0.4, 95% C.I.: 0.2-0.9]. Compared with both sets of controls, cases were less likely to store drinking water in narrow mouthed containers (Table 3). Similarly, compared with both sets of controls, cases were less likely to take out water by tilting the container (Table 3). Compared to both sets of controls, cases were more likely to scoop out water from the container with a cup (Table 3). None of the cases and controls drank chlorinated water. Failure to store water in narrow mouthed containers and drawing water by tilting the container accounted for 31% and 27% of typhoid cases in the population. Scooping out water from the container using a cup accounted for 29% cases in the population.

Consumption of raw vegetables

Compared with both sets of controls, cases were more likely to consume raw onions, raw carrots and raw cabbages (Table 3). Compared to unmatched controls, cases were more likely to eat raw tomatoes [OR: 3.6, 95% C.I.: 1.7-8.4]. The odds of disease increased with the increase in consumption of raw onions, cabbage and carrots (Table 4). The association between consumption of raw onions and illness was stronger among those with monthly income less than rupees 1500[(US$ 30 (OR: 4.7, 95% CI: 1.8-13) than among others (OR: 1.3, 95% CI: 0.6-2.7). Consuming raw onions, cabbages and carrots accounted for 35 %, 49% and 27% typhoid cases respectively. Compared to both sets of controls, cases were more likely to eat unwashed guavas, unwashed grapes, and unwashed papayas (Table 3). The association between consumption of unwashed guavas and illness was stronger among those having monthly income less than rupees 1500.00 [(US$

30), OR: 5.2, 95% CI: 2-14.4] than among others (OR: 1.2, 95% CI: 0.5-2.4). Consuming unwashed guavas, papayas and grapes accounted for 31%, 22% and 25% typhoid cases respectively.

Consumption of milk/milk products

Compared with both sets of controls, cases were more likely to eat butter and yoghurt (Table 3). The odds of disease increased with increasing consumption of butter (Table 4). Consuming butter and yoghurt accounted for 27% and 40% typhoid cases in the population respectively. The association between consumption of butter and illness was stronger among those having monthly income less than rupees 1500 [US$30, (OR: 2.1, 95% CI: 2.1-20] than among others (OR: 1.4, 95% CI: 0.7-2.8). Similarly, the association between consumption of yoghurt and illness was stronger among those having monthly income less than rupees 1500 [US$ 30, (OR: 9.1, 95% CI: 3.2-28] than among others (OR: 1.7, 95% CI: 0.8-3.5).

Sanitation

Compared to both sets of controls, cases were less likely to have latrines at home (Table 3). Failure to use latrines at home accounted for 26% typhoid cases in the population. Compared to both sets of controls, cases were more likely to dispose off sewage in the nearby stream but the association was not statistically significant (Table 3).

4. Discussion

We identified three groups of risk factors for typhoid in Kurseong, Darjeeling. The first was related to the supply, storage and use of drinking water, the

second to the consumption of raw vegetables and unwashed fruits and the third to milk products. We also identified factors associated with a lower risk of illness. These included storage of water in narrow mouthed containers, drawing water by tilting the containers and having latrines at home. A review of these risk factors provides some understanding of the practices that expose the community to typhoid and provide useful direction to suggest behaviour change interventions to protect the community.

Consumption of raw vegetables and unwashed fruits was significantly associated with illness. Raw vegetables and vegetables fertilized with sewage have been at the source of typhoid outbreaks[21]. Consumption of papayas and raw vegetables including tomatoes and onions were risk factors for typhoid in Bangladesh and Florida, USA respectively[22, 23]. In the Kurseong sub-division, the disposal of sewage in nearby streams is a common practice (45% cases and 39% and 37% unmatched and matched controls respectively reported it). This exposed the community to increased risk of infection with pathogens transmitted through the fecal oral route. Consumption of butter and yoghurt was strongly associated with typhoid. While cows themselves do not harbour the pathogens, dairy products may be a particularly effective media for growth of S.typhi if they become contaminated[24]. In Kurseong, most people, especially in the rural areas do not consume pasteurized milk. The greater relative risk of disease in low income groups for consuming butter and curd may be explained by poor food hygiene and contamination of food items in that sub-group of the population. Ice cream was associated with typhoid infections in Karachi, Pakistan[11]. Milk and ice cream were reported to have been the vehicle of transmission in many typhoid outbreaks[11].

The association between consumption of raw vegetables, unwashed fruits and milk products and illness was not explained by socio-economic factors in the in the stratified analysis. However, there was an association between low monthly income and disease. Low family income is often related to poor level of education, poor housing and living in unsanitary conditions without adequate water supply[25]. The greater relative risk of disease in low income groups for consuming raw vegetables and unwashed fruits may be explained by poor food hygiene in that sub-group of the population. Poor food hygiene could be a surrogate marker of poor personal hygiene. Poor housing and inadequate food and personal hygiene are associated with typhoid[25].

Our results suggested that a number of practices may be effective in preventing typhoid. Use of narrow mouthed containers for storage of water may decrease the risk of disease. Narrow mouthed containers prevent secondary transmission of typhoid[21]. Similarly, drawing of water from the containers by tilting decreased the risk of disease as opposed to scooping out of water using a cup. Replacing unsafe water storage vessels with safer ones led to lower rates of cholera transmission in households in Kolkata[26]. In a South Asian urban setting with extremely heavily contaminated source water, a safe water storage vessel (narrow mouthed water containers with lids and taps) and in-home chlorination reduced the amount of thermotolerant coliforms and E. coli in stored drinking water by more than 99%[27]. Progressive expansion of improved water supplies is important but fails to address the immediate needs of the most dis-advantaged[28]. Boiling kills pathogens but provides no residual protection after cooling and can be easily recontaminated[28]. Options such as point-of-use water treatment with hypochlorite coupled with storage in narrow mouthed containers targets the

most affected and directly enhances health benefits[28]. Use of latrines for defecation also decreased the risk of disease. Results of a population-based case-control study in Dhaka, Bangladesh indicated that using a latrine for defecation reduced the risk of typhoid[22]. Our study had two limitations. First, this study was not blinded. The validity could have been compromised by the interviewer. The interviewers in this study knew whether a study subject was a case patient or a control subject. It is possible that interviewers might have elicited exposure with more insistence from control subjects than case patients and thus introduced bias. It is also possible that cases reflected upon their exposure prior to illness more carefully than controls, and so may have more fully reported exposures than controls. However, the questionnaire was standardized so that questions were asked of cases and controls in the same way. Further, we ensured that the same person interviewed a case-control set. As we asked the cases and controls about exposures in the two weeks prior to the onset of their first symptoms and within two weeks of interviewing the cases respectively, we expected them to remember the food items they ate outside within the time frame. Further, most of the other questions were about practices which they were currently involved in and so the problem of recall bias did not arise. Second, being a Hindu, belonging to the general caste and residing in rural areas are associated with increased risk of illness in the matched analysis. We stratified to eliminate confounders and identify effect modification but were unable to explain this through this study. Third, the discordant findings of piped water supply being a risk factor in the unmatched analysis and protective in the matched analysis cannot be explained in this study. However, complete absence of chlorination may explain piped water at home being associated with increased risk of disease in the unmatched analysis.

Our study suggests that there may be opportunities to prevent exposures to typhoid through better food hygiene, safe drinking water and sanitation. This includes eating of only cooked vegetables and properly washed raw vegetables and fruits. We need to promote the adoption of hygienic practices in the preparation and storage of milk and milk products. We need to advocate chlorination of drinking water at home, storage of the treated water in narrow containers and drawing out of water from containers without less chances of contamination like by tilting the container or using taps. We also need to use latrines at home and dispose off wastes in closed sewerage systems. Further studies could characterize the quality of drinking water available to the community. Behavioral intervention studies could examine options to introduce safer practices, including storage of water in narrow mouthed containers, use of low cost hypochlorite for treating drinking water at home, food and personal hygiene, use of latrines and disposal of sewage. Finally, hospital-based public health surveillance will provide an opportunity to evaluate the effectiveness of the proposed prevention measures.

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Table 1: Incidence of typhoid cases by age and sex, Kurseong sub-division, Darjeeling, West Bengal, India, 2005

Characteristics		Population	# of cases	# of deaths	Case fatality ratio (%)	Attack rate (per 100,000 population)
Age groups	0 – 4	19,287	1	0	0	5.2
	5 – 14	45,737	13	0	0	28.4
	15 – 29	54,003	20	0	0	37
	30 – 44	26,450	7	0	0	26.4
	45 – 59	28,287	8	0	0	28.8
	60+	9,920	3	0	0	28.1
Sex	Male	97,353	27	0	0	27.7
	Female	86,331	25	0	0	28.9
Total		183,684	52	0	0	28.3

Figure 2: Incidence of typhoid cases over time in Kurseong, Darjeeling district, West Bengal, India, 2005

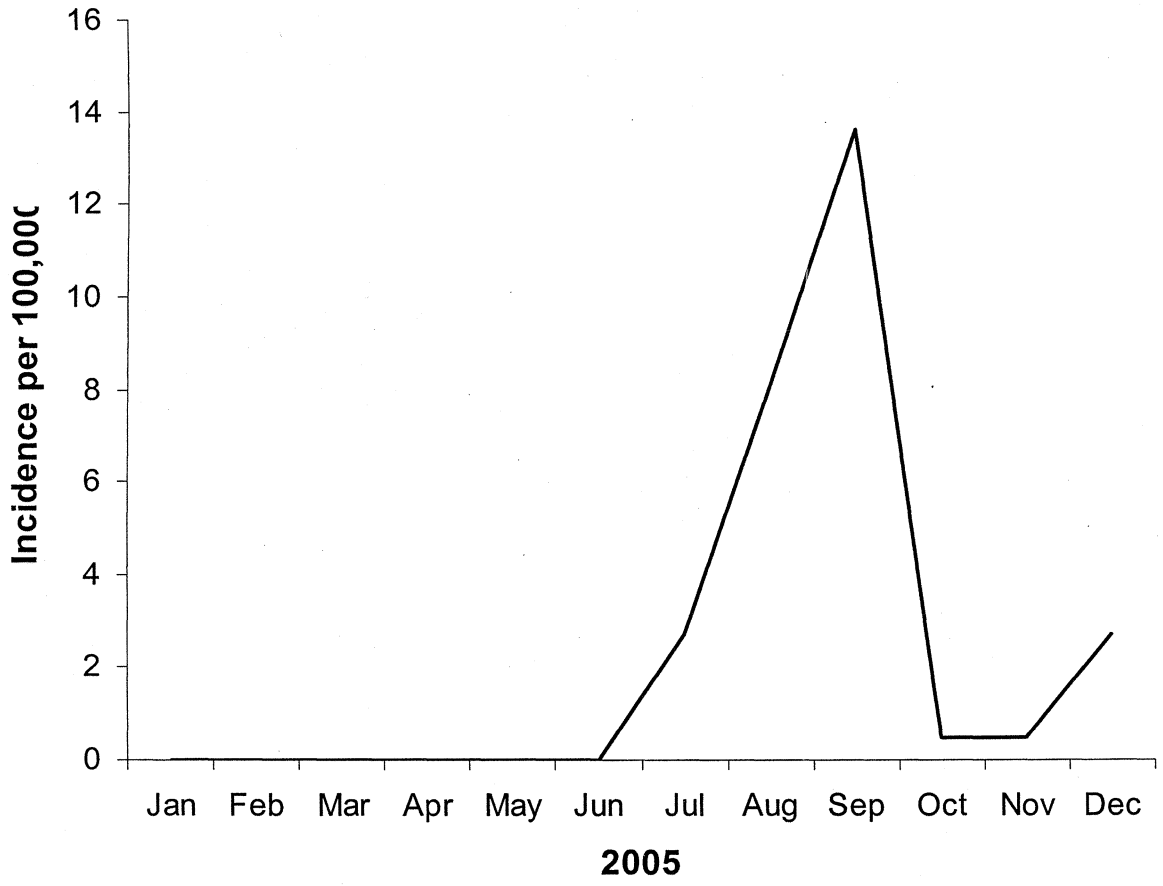

Table 2: General characteristics of typhoid cases and controls in Kurseong, Darjeeling district, West Bengal, India, 2005 – 2006

			Control groups					
	Cases (n=123)		Unmatched hospital controls[1] (n=177)			Matched neighbourhood controls (n=123)		
Characteristics	#	%	#	%	P - value	#	%	P - value
Age ≤ than 30 years	71	57	31	50	0.3	73	59	0.3
Female sex	58	47	34	54	0.07	54	44	0.6
Follower of Hinduism	95	77	47	76	0.5	73	59	0.0004
Belonging to general caste	98	80	43	69	0.06	55	45	0.0000
Residence in rural areas	99	81	52	84	0.3	97	80	0.1
Residence in houses made of wood	88	72	45	73	0.6	73	59	0.04
Monthly income less than Rs.1500	59	48	30	48	0.3	38	31	0.006

[1] Serologically confirmed scrub typhus patients

Table 3: Selected exposures for typhoid cases and community based controls matched for age and neighbourhood and hospital based unmatched scrub typhus controls in Kurseong, Darjeeling district, West Bengal, India, 2005 – 2006

				Control groups							
		Cases (n=123)		Unmatched hospital controls[1] (n=177)				Matched neighbourhood controls (n=123)			
Exposure		#	%	#	%	OR[2]	95% CI[3]	#	%	MOR[4]	95% CI
Environmental factors	Household members > 4	42	34	40	65	0.5	0.3 – 0.8	39	32	1.1	0.7 – 2.0
	Travelled outside 14 days ago	48	39	6	10	3.7	2.1 – 6.5	38	31	1.4	0.8 – 2.4
	Piped water supply at home	42	34	36	20	2	1.2 – 3.4	53	43	0.4	0.2 – 0.9
	Stream water at home	81	66	130	73	0.7	0.4 – 1.1	70	57	1.6	0.9 – 2.6
Drinking water	Drinking boiled water	107	87	158	89	0.8	0.4 – 1.7	102	83	1.3	0.6 – 2.6
Storage of water	Narrow mouthed container	59	48	124	71	0.4	0.2 – 0.6	84	68	0.4	0.2 – 0.7
	Tip of the container	67	55	130	77	0.4	0.2 – 0.7	90	73	0.4	0.2 – 0.7
Water taken out of container	Scooping out with a cup	45	36	38	22	2	1.2 – 3.4	25	20	2.5	1.3 – 4.7
	Tomatoes	47	38	34	19	2.6	1.5 – 4.3	56	46	0.7	0.4 – 1.2
	Onions	81	66	58	33	4	2.4 – 6.4	59	48	2.1	1.2 – 3.9
	Cabbages	85	69	5	3	75	30 – 228	48	39	2.8	1.7 – 4.8
Raw vegetables	Carrots	74	60	27	15	8.3	4.8 – 14.5	56	46	2.1	1.2 – 3.9
	Guavas	66	54	31	17	5.4	3.2 – 9.2	41	33	1.9	1.2 – 3.0
	Papayas	66	54	1	1	200	38 – 4151	49	40	1.8	1.1 – 3.1
Unwashed fruits	Grapes	59	48	1	2	159	30 – 3311	38	31	2.2	1.3 – 4.0
	Butter	62	50	53	30	2.3	1.5 – 3.8	39	32	2.3	1.3 – 4.1
	Ice cream	8	6	10	6	1.2	0.4 – 3	6	5	1.5	0.4 – 6.0
Milk/Milk products	Yoghurt	74	60	61	34	2.9	1.8 – 4.6	41	33	2.3	1.4 – 3.7
Toilet facilities	Latrine at home	58	47	126	71	0.4	0.2 – 0.6	79	64	0.5	0.3 – 0.8
	Closed system	54	44	105	59	0.5	0.3 – 0.8	60	49	0.8	0.5 – 1.4
Sewage disposal	Nearby stream	55	45	66	37	1.3	0.8 – 2.2	43	35	1.5	0.9 – 2.7

[1] Serologically confirmed scrub typhus case
[2] Odds ratio
[3] Confidence interval
[4] Matched odds ratio

Table 4: Odds of typhoid according to increasing gradients of exposure to risk factors, Kurseong, Darjeeling district, West Bengal, India, 2005-2006

		Cases (n = 123)		Neighbourhood controls (n = 123)			
Exposure	Level	#	%	#	%	OR	95% CI
Raw onion	One to two servings a week	19	23	28	34	1	
	Three to four servings a week	25	31	27	61	1.4	0.6 – 3.3
	More than five servings a week	38	46	8	5	7	2.5 – 21[1]
Raw carrots	One to two servings a week	14	19	34	61	1	
	Three to four servings a week	23	31	19	35	2.9	1.1 – 7.7
	More than five servings a week	37	50	3	4	29.9	7.1 – 146[2]
Raw cabbage	One to two servings a week	18	21	29	60	1	
	Three to four servings a week	28	33	16	33	2.8	1.1 – 7.3
	More than five servings a week	39	46	3	7	20.9	5.1 – 99[3]
Butter	One to two servings a week	13	21	17	44	1	
	Three to four servings a week	19	31	16	41	1.6	0.5 – 4.7
	More than five servings a week	30	48	6	15	6.5	1.9 – 24[4]

[1] Chi-square for trend: 16.8; P-value: 0.0004
[2] Chi-square for trend: 35.1; P-value: 0.0000
[3] Chi-square for trend: 28.4; P-value: 0.0000
[4] Chi-square for trend: 11.3; P-value: 0.0008

Scrub typhus – a review

Historical aspects

Scrub typhus, also known as chiggerborne rickettsiosis, tsutsugamushi disease, or tropical or rural typhus is a rickettsial disease dating back to 313 AD with clear descriptions found in Chinese texts[1]. It was first described in modern literature in Japan in 1810[1]. It appeared in the Western literature in 1878 as a disease in river valleys that affected farmers working during July and August[1]. Because of this association, it is also known in Japan as tsutsugamushi ("noxious" or "dangerous mite") disease, Kedani ("hairy mite") river fever, or "Japanese river fever." Identification of the causative agent was attributed to Norio Ogata (among others) in the late 1920s[1]. Outside Japan, Lewthwaite and Savoor, working at the Malaysian Institute for Medical Research in 1940, established the etiologic agent of scrub typhus as that causing disease in Japan[1]. During the 20 years before World War II, careful research work, notably in Malaya and Sumatra, had indicated that tsutsugamushi disease was not limited to Northwestern Japan, Formosa, and the Pescadores Islands, where the Japanese had by 1931 definitely established its rickettsial etiology[2]. The clinical picture, pathology, and epidemiology were shown to be fundamentally the same in Sumatran mite fever, in the scrub typhus of Malaya, the endemic typhus of New Guinea, the coastal fever of North Queensland, Australia, and in cases reported from Burma and Indo-china[2]. The common pattern could be traced in spite of wide variations in mortality and frequent failures to find the primary eschar[2]. Scrub typhus, which is reported to have sometimes caused more casualties than actual combat during the World War 11, continued to be of military

significance during the Indo-Pakistan conflict in 1965 (Padbidri, 1978), the Malayan Emergency (McCrumb et al. 1957), the Vietnam War (Berman & Kundin, 1973) and among soldiers in the Pescadores islands of Taiwan [1, 3, 4]. Prior to the experience of World War II, however, the only species of mite proved by animal experiments to be the vector of tsutsugamushi disease was the trombiculid mite identified by Japanese workers[2]. Other mites had been highly suspected in Sumatra and Malaya, but conclusive proof had not been furnished. Similarly, the principal reservoir host of the trombiculid mite had not been incriminated elsewhere than in Japan and Malaya[2].

Ancient Indian literature has no reference to the presence of typhus or allied disease in the country[5]. In 1933, the army headquarters in India directed all army laboratories to carry out Weil-Felix test on all cases of fevers[5]. From the results of one year's study they reported scrub typhus was present in different parts of India including Srinagar, Jammu, Kumaon hills, Manipur, Kolkata, Jamshedpur, Bangalore and Mysore[5]. Scrub typhus came into prominence as a war disease in India, especially in the eastern regions including Assam[4]. The rapid increase in incidence amongst the troops form 1942 onwards led to the formation of the Army Headquarters Field Typhus Research teams[3]. Outbreaks of scrub typhus were reported in different parts of India including Jamshedpur, Shimla, Punjab and Kolkata before 1960[3]. The rigid application of DDT brought down the morbidity and mortality and with the use of antibiotics and other pesticides, the disease came under control[3]. However, the disease is still present with reports of its emergence and re-emergence in different parts of the world including India.

Current scenario

Scrub typhus is widespread extending from Japan in the North to tropical Queensland (Australia) in the South and from India in the West to Solomon Islands and Vanuatu in the East[6]. The disease is reported from various ecosystems including seashores, mountainous regions, rain forests, semiarid deserts, riverbanks and terrain undergoing secondary vegetation growth[1]. Although 25 – 50 % of scrub typhus cases occur in children, most cases occur through agricultural exposure, such as rice field workers of Thailand, Japan, or Korea and oil palm and rubber plantation workers of Malaysia[1]. It has emerged and re-emerged as outbreaks and sporadic cases since the 1980s in various countries including Japan, Australia and in south Asia[7, 8, 9, 10]. In India, since 1990, there have been reports of scrub typhus outbreaks from the Indian state of Tamilnadu in the South, and the states of Himachal Pradesh and Sikkim in the Northern Himalayan region[11, 12, 13]. Occurrence of eight cases of scrub typhus and transmission risk of scrub typhus has been reported in the fringe and sylvatic areas in Pune, Maharashtra[14]. Eight and twelve cases of scrub typhus were reported from Dehradun in 1992 and from Jammu in 2002 respectively[15, 16].

Public health importance

Before the age of antibiotic therapy, patients with scrub typhus had high mortality rates, up to 50-60%, with a clinical course ranging from 10 to 28 days and a protracted convalescence of up to four months. Fatalities are rare among treated cases although there have been reports of antibiotic resistant strains and deaths among appropriately treated patients[17]. Scrub typhus was the most significant rickettsiosis affecting the US troops during World War 11

where it was reported to have disabled more men than in actual combat, the mortality rates ranging from 0.5% to 27.5%. In 1943, an outbreak occurred in US Army troops in Assam producing mortality higher than in any other area during the war[1]. The mortality rate for scrub typhus in the China-Burma-India border during the World War 11 was 14.6 per 100,000[1]. In a recent outbreak in Himachal Pradesh more than 100 people were diagnosed to have scrub typhus and there were 15 suspected scrub typhus deaths[12]. There were six suspected scrub typhus deaths in 2004 in Kurseong and the incidence was 34 per 100,000 in 2005. Due to the low index of suspicion of the disease among physicians and absence of diagnostic facilities in the district the diagnosis of scrub typhus is likely to be missed leading to increased morbidity and chances of deaths.

Pathogen

Scrub typhus is caused by *Orientia tsutsugamushi,* a gram-negative bacteria belonging to the order Rickettsiales[18]. It was initially called *Rickettsia tsutsugamushi*. Phylogenetic data have recently been used to support the reclassification of the agent of scrub typhus into a new genus, because it belongs to a unique and distinct clade within the Rickettsia radius[18]. It is therefore now called *Orientia tsutsugamushi*[18]. The four common serotypes of *Orientia tsutsugamushi* are Karp, Gilliam, Kato and Kawasaki. *Orientia tsutsugamushi* is an obligate intracellular parasite usually 0.8 to 2.0 µm long and 0.3 to 0.5 µm in diameter[18]. *Orientia tsutsugamushi* is maintained in the vector mites by transovarial transmission[19, 20].

Vector and host

The vectors are the trombiculid mites, including species of *Lepotrombidium*, *Gahrliepia* and *Achongastia*[21]. *Leptotrombidium deliense* and *Leptotrombidium akamushi* are the common vectors inhabiting a wide area from India to Japan, south-eastern Asia, New Guinea and Australia[22]. In India, *Gahrliepia* species is reported to be the most common vector[23]. However, in the Darjeeling district, an entomological survey done in 1967, found *Leptotrombidium deliense* to be the predominant vector[24]. There is no typical ecology of the disease. It has been acquired wherever the vector mites are found, from sea level to the mountains and from the sub-arctic areas to the semiarid deserts[1]. It is found in disturbed rain forests, river banks and terrain undergoing secondary vegetation growth[1]. It has been more recently found in urban settings and even in rice paddies[1].

The chigger normally feeds on its host only once, and is subsequently non-parasitic[21]. The unfed chiggers are orange-yellow in colour and so small as to be barely visible to the naked eye. They become active at the close approach of a warm-blooded animal host and, if contact is made, they swarm on to the host and, sooner or later, settle down, usually in a sheltered and characteristic locus, where they attach to the skin and feed[21]. The larvae become greatly distended as they engorge. After about 36-72 hours, the larvae disengage and drop off the host on to the ground[21]. They are usually present in 'mite islands', where they could be plentiful in one spot but apparently absent in another place a few feet away in the same habitat[21]. Unengorged *Leptotrombidium* are tiny, and generally do not cause appreciable itching, even at the site of attachment and therefore it is not surprising that the vectors escape the notice of their victims[21]. The mites act as both reservoir and

vector[21]. Whereas most stages in their life cycle are free living, the parasitic larval chigger stage feeds on humans and rodents. While the rodents are the usual hosts, the humans are accidental hosts[18, 25]. Among the rodent and insectivore hosts, who are equally good hosts, the chief host in the Darjeeling district is *Rattus rattus*[24]. Rodents are critical to the maintenance of the disease as they harbour chiggers[19].

Pathogenesis

When the rickettsia is transmitted by the bite from an infected mite to a human, it begins to grow at the location of the bite and a characteristic lesion known as 'eschar' is formed[26]. The rickettsia then spreads systemically via the haematogenous and lymphatogenous routes and the infected human develops various systemic symptoms[26]. However, partial immunity from prior exposures might alter the response to infection in endemic areas[27]. The pathogenesis of this disease is vasculitis caused by the proliferation of organisms in the endothelial lining of small arteries, veins and capillaries[18]. The main organs affected were the heart, the lungs and the brain[2].

Clinical features

Clinical symptoms occur six to 18 days (incubation period of 21 days) after the bite of an infected chigger. The onset is usually sudden and is characterized by fever, severe headache and myalgia. There is tender lymphadenopathy at the site of the bite or eschar. The presence of a typical eschar is pathognomonic of scrub typhus.[2]. The pathognomonic eschar is present in 87 to 100% of the patients[26, 28, 29]. More than 65 percent of the eschars are located on arms, axilla, neck, and trunk[30]. Temperatures rise quickly in the

first several days of disease to 104 ^{0} F or 105 ^{0} F. Early in the illness, the pulse is relatively slow[25]. Other symptoms at this time may include ocular pain, conjunctival injection, non-productive cough and apathy[25]. The severity of the symptoms depends on the susceptibility of the host and the virulence of the infecting strain[25]. In small proportion of patients, tremors, delirium, nervousness, slurred speech, deafness or nuchal rigidity may develop in the second week[25]. Evidence of peripheral circulatory collapse is common and signs of myocarditis may appear[2]. In the severe cases, cardiovascular abnormalities such as faint systolic murmurs, tic-tac sounds, gallop rhythm, tachycardia, hypotension, and pulsus alternans are occasionally observed[2]. Cyanosis and increased respiratory rate accompanied the development of abnormal physical signs in the lungs may occur in severe cases[2]. Hepatitis and nephritis sometimes complicate the picture[2]. Thromboses and cerebral or gastrointestinal hemorrhage may take place[2]. Death is usually due to heart failure and circulatory collapse or pneumonia[25].

Laboratory investigations

Identification of rickettsial infection is done by three methods: First, identification of a rickettsial isolate may be achieved by microscopic examination after staining with Giemsa or Gimenez stains. However, this requires immunofluorescence microscope and a laboratory with biosafety level 3 containment and personnel with extensive experience in cultivating and isolating rickettsiae from clinical specimens. Hence, this method of identification of rickettsial infection is restricted to research laboratories only[18]. Second, rickettsial infection can be confirmed by molecular biology based method based on polymerase chain reaction (PCR) amplification of rickettsial DNA. However, this too is not commonly available except at reference

laboratories[18]. Third, rickettsial infection can be confirmed by serological tests, including the Weil-Felix test, the complement fixation test, the microagglutination test, the indirect haemagglutination test, the latex agglutination test, the enzyme-linked immunosorbent assay (ELISA), the immunoperoxidse test and the indirect fluorescent antibody (IFA) test[18]. Weil-Felix test, an agglutination test using the OX-K strain of *Proteus mirabilis* is the most commonly used serological test to diagnose *Orientia tsutsugamushi* –related infections in developing countries including India. By this test agglutinating antibodies are detectable after five to ten days following the onset of symptoms, with antibodies being detected being mainly of the immunoglobulin M (IgM) type. However, due to the poor sensitivity and specificity of the Weil-Felix test, it is recommended as the first line of test in peripheral hospital laboratories[18]. For a test to be useful in the diagnosis of an acute rickettsial infection, the most important criteria are sensitivity and the length of delay between the onset and appearance of detectable antibody titers. Conversely, when the test is to be used for seroepidemiologic studies, it should be highly specific to prevent false-positive results due to cross-reacting antibodies. The IFA test is the "gold standard" technique for confirmation of rickettsial infections including scrub typhus[18]. For scrub typhus, the sensitivity of IFA is low if high specificity is required: for titer of $\geq$ 1:100, sensitivity is 84% and specificity is 78%, for a titer of $\geq$ 1:200, sensitivity is 70% and specificity is 92%, and for a titer of $\geq$ 1:400, sensitivity is 48% and specificity is 96%. In cases of primary infection with Orientia tsutsugamushi, IgM antibody appears at the end of the first week while in case of reinfection the IgM titres are variable[18]. In areas endemic for scrub typhus, the chances of reinfection are high and hence there may be difficulty in interpreting the results. Treatment in the early stages of the disease is known to delay the appearance of antibody

titres[25, 31]. Hence, in situations where blood samples are collected early in the illness, antibiotic treatment is instituted very early or chances of reinfections are high, the serological test reports are to be interpreted with caution.

Risk factors

Review of literature on scrub typhus did not reveal specific studies done so far for identification of risk factors for scrub typhus. However, one study conducted in the Republic of Palau to assess the distribution of scrub typhus using serological surveys during 2003-2005, identified that an area where the residents were more exposed to outdoor environments, outdoor occupational activities and whose households were more likely to have evidence of rodents was more affected with scrub typhus[32]. Other studies have associated scrub typhus with woody terrain or grounds that have been cleared of forest within a few years, as well as grassy fields[21]. Similarly, farming and working in oil-palm plantations have been associated with scrub typhus[1]. The presence of rodents and trombiculid mites in these habitats and exposure of humans to them are predisposed to infestation, infection and disease. Densely vegetated areas around human dwellings have been associated in Queensland, Australia[10]. Wood for cooking stored in the backyards near kitchens in the affected areas in the Maldives was associated with scrub typhus[9]. Domesticated dogs are reported to harbour vector chiggers[33, 34]. As the endemic foci for scrub typhus can be so localized that a sharp outbreak may affect only certain groups of people in a highly limited area while sparing others apparently equally and simultaneously exposed in the same locus, it is important we know the identify these so called 'mite islands' and the local risk factors to direct efforts to control the disease. Further, as important as the identification of risk factors

for scrub typhus is the identification of protective practices against it as we may not be able to identify all the foci of infection.

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Typhoid – a review

Public health problem

Typhoid fever is a global health problem. Its real impact is difficult to estimate because the clinical picture is confused with those of many other febrile infections. Additionally, the disease is underestimated because there are no bacteriology laboratories in most areas of developing countries[1]. These factors are believed to result in many cases going undiagnosed. However, a revised estimate of the global burden of typhoid fever was published by Crump et al in 2004. They sought by computer search the multilingual scientific literature on population-based studies using confirmation of typhoid by blood culture. Where there were no eligible studies, data were extrapolated from neighbouring countries and regions. Age–incidence curves were used to model rates measured among narrow age cohorts to the general population. One-way sensitivity analysis was performed to explore the sensitivity of the estimate to the assumptions. The burden of paratyphoid fever was derived by a proportional method. A total of 22 eligible studies were identified. Regions with high incidence of typhoid fever (>100/100 000 cases/year) included south-central Asia and south-east Asia[2]. Regions of medium incidence (10–100/100 000 cases/year) include the rest of Asia, Africa, Latin America and the Caribbean, and Oceania, except for Australia and New Zealand. Europe, North America, and the rest of the developed world have low incidence of typhoid fever (<10/100 000 cases/year)[2]. They estimated that typhoid fever caused 21 650 974 illnesses and 216 510 deaths during 2000 and that paratyphoid fever caused 5 412 744 illnesses[2].

Typhoid fever has a very high social and economic impact because of the hospitalization of patients with acute disease and the complications and loss of income attributable to the duration of the clinical illness[3]. It is important to note that reports from some provinces in China and Pakistan have indicated more cases of paratyphoid fever caused by *S. paratyphi* A than by *S. typhi*[1]. In areas of endemicity and in large outbreaks, most cases occur in persons aged between three and 19 years[1]. In 1997, for example, this age range was reported during an epidemic of the disease in Tajikistan[1]. Nevertheless, clinically apparent bacteraemic S. *typhi* infection in children aged less than three years has been described in Bangladesh, India, Jordan, Nigeria, and elsewhere[4, 5]. In Indonesia there is a mean of 900 000 cases per year with over 20 000 deaths[1]. In Indonesia, people aged three to 19 years accounted for 91% of cases of typhoid fever and the attack rate of blood-culture-positive typhoid fever was 1026 per 100 000 per year[1]. A similar situation was reported from Papua New Guinea[1]. When typhoid fever was highly endemic in certain countries in South America the incidence of clinical typhoid fever in children aged less than three years was low[1]. In Chile, however, single blood cultures for all children aged under 24 months who presented at health centres with fever, regardless of other clinical symptoms, showed that 3.5% had unrecognized bacteraemic infections caused by *S. typhi* or *S. paratyphi*[1]. Enteric fever had not been suspected on clinical grounds in any of the children. In South America the peak incidence occurred in school students aged five to 19 years and in adults aged over 35 years[1]. This kind of study has not been conducted in other areas of endemicity[1]. Between one and five percent of patients with acute typhoid infection have been reported to become chronic carriers of the infection in the gall bladder, depending on age, sex and treatment regimen[1]. The propensity to become a carrier follows the

epidemiology of gall bladder disease, increasing with age and being greater in females than in males[1]. However this may have changed now with the present availability and selection of antibiotics as well as with the antibiotic resistance of the prevalent strains. The role of chronic carriers as a reservoir of infection was studied in Santiago, Chile, where a crude rate of 694 carriers per 100, 000 inhabitants was found[1].

The risk of contracting typhoid is highest for travelers to the Indian sub-continent (India, Pakistan and Bangladesh) where the estimated attack rate for travelers is 10 per 100,000 travellers in this populaton[6]. Since 1990, outbreaks of *Salmonella typhi* were reported from different parts of India, including Kolkata[7, 8, 9]. In the West Bengal state of India, the reported incidence of typhoid was 104 per 100,000 populations in 2004[10]. In 2003, children between 2 and 3 years of age were the most susceptible age group for *Salmonella typhi* infection in Kolkata[7]. However, in a prospective community based study in the same city, patients with typhoid fever had a mean age of 15 years[11]. In 2003, typhoid ranked third among the ten commonest diseases in the Darjeeling district with an incidence of 808 per 100,000 population[12].

The organism, the disease and transmission

The organism

Typhoid fever is caused by *Salmonella typh*i, a Gram-negative bacterium[1]. A very similar but often less severe disease is caused by *Salmonella* serotype *paratyphi* A.. *Salmonella typhi* can be identified in the laboratory by several biochemical and serological tests. The ratio of disease caused by *Salmonella*

typhi to that caused by *Salmonella paratyphi* is about ten to one in most of the countries where this matter has been studied[1].

The disease[1]

During an acute infection, *S. typhi* multiplies in mononuclear phagocytic cells before being released into the bloodstream. After ingestion in food or water, typhoid organisms pass through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either microfold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages that ingest the bacilli but do not generally kill them. Some bacilli remain within macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by macrophages. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion throughout the organs of the reticuloendothelial system (spleen, liver, bone marrow, etc.), where it resides during the incubation period, usually of eight to 14 days. The incubation period in a particular individual depends on the quantity of inoculum, i.e. it decreases as the quantity of inoculum increases, and on host factors. Incubation periods ranging from three days to more than 60 days have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (one to ten bacteria per ml of blood).

Risk factors, contamination and transmission

Humans are the only natural host and reservoir[1]. The infection is transmitted by ingestion of food or water contaminated with faeces[1]. Risk factors for typhoid in Europe, Central Asia and the Americas include contamination of municipal piped water, faecal contamination of water and food, drinking of unboiled tap water, purchasing lunch at school rather than bringing lunch from home, sharing food items with friends, eating flavoured ice purchased outside home and eating cheese, raw onions and tomatoes [13, 14, 15, 16]. Risk factors for typhoid in south-east Asia identified include recent contact with a typhoid patient, absence of education, absence of latrines at home, drinking untreated water, eating shellfish and ice-cream and eating food from roadside vendors[17, 18, 19]. Shellfish taken from contaminated water, and raw fruit and vegetables fertilized with sewage, have been sources of past outbreaks[1]. The highest incidence occurs where water supplies serving large populations are contaminated with faeces[1]. Epidemiological data suggest that waterborne transmission of *S. typhi* usually involves small inocula, whereas foodborne transmission is associated with large inocula and high attack rates over short periods[1]. The inoculum size and the type of vehicle in which the organisms are ingested greatly influence both the attack rate and the incubation period[1]. Family studies were conducted in Santiago, Chile, during an era of high typhoid endemicity in order to ascertain whether chronic carriers were significantly more frequent in households where there were index cases of children with typhoid fever than in matched control households[1]. Other epidemiological studies investigated whether risk factors could be identified for persons with typhoid fever in comparison with uninfected household members. It was concluded that chronic carriers in households did not play an

important role in transmission[1]. Subsequently, it was shown that the irrigation of salad with wastewater contaminated with sewage was the key factor responsible for maintaining the high endemicity of typhoid in Santiago[1]. In developed countries, on the other hand, typhoid is transmitted when chronic carriers contaminate food as a consequence of unsatisfactory food-related hygiene practices[1].

Diagnosis of typhoid fever

Case definition

The World Health Organisation has provided three levels of case definitions for typhoid[20].

Suspected case of typhoid fever

A patient with fever of $\geq$38°C and compatible with the clinical features of typhoid.

Probable case of typhoid fever

A patient with suspected typhoid and positive Widal test with a single titre of 1:160.

Confirmed case of typhoid fever

A patient with suspected case typhoid and laboratory confirmation by either isolation of *Salmonella typhi* from blood, stool or other clinical specimens, or by a fourfold rise in agglutinating titer in paired sera taken 10 days apart using the Widal test.

Chronic carrier

A chronic carrier is a person who excretes *S. typhi* in stools or urine (or repeated positive bile or duodenal string cultures) for longer than one year after the onset of acute typhoid fever[1]. Short-term carriers also exist but their epidemiological role is not as important as that of chronic carriers[1]. Some patients excreting *S. typhi* have no history of typhoid fever[1].

Clinical features

The clinical presentation of typhoid fever varies from a mild illness with low-grade fever, malaise, and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications[1]. Many factors influence the severity and overall clinical outcome of the infection. They include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial treatment, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculum ingested, host factors (e.g. HLA type, AIDS or other immunosuppression) and whether the individual was taking other medications such as H2 blockers or antacids to diminish gastric acid[1]. Patients who are infected with HIV are at significantly increased risk of clinical infection with *S. typhi* and *S. paratyphi*[2]. Evidence of *Helicobacter pylori* infection also represents an increased risk of acquiring typhoid fever[1].

Acute typhoid fever is characterized by prolonged fever, disturbances of bowel function (constipation in adults, diarrhoea in children), headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness[1]. During the period of fever, up to 25% of patients show exanthem (rose spots) on the chest, abdomen and back.

Acute typhoid fever may be severe. Complications occur in about 30 percent of untreated cases and account for 75 percent of deaths in patients with typhoid[21]. Depending on the clinical setting and the quality of available medical care, up to 10% of typhoid patients may develop serious complications[21]. Without effective treatment, the case fatality is 10 percent[21]. Since the gut-associated lymphoid tissue exhibits prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients, and up to three percent may have maelena[1]. Intestinal perforation has also been reported in up to three of hospitalized cases[1]. Abdominal discomfort develops and increases. It is often restricted to the right lower quadrant but may be diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes follow, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and guarding, and subsequent abdominal rigidity. A rising white blood cell count with a left shift and free air on abdominal radiographs are usually seen. Altered mental status in typhoid patients has been associated with a high case-fatality rate[1]. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barré syndrome, cranial or peripheral neuritis, and psychotic symptoms, although rare, have been reported[1]. Other serious complications documented with typhoid fever include haemorrhages (causing rapid death in some patients), hepatitis, myocarditis, pneumonia, disseminated intravascular coagulation, thrombocytopenia and haemolytic uraemic syndrome[1]. In the pre-antibiotic era, which had a different clinical picture, if patients did not die with peritonitis or intestinal haemorrhage, 15% of typhoid fever cases died with prolonged persistent fever and diseases for no clear reason. Patients may also experience genitourinary tract manifestations or relapse, and/or a chronic

carrier state may develop[1]. One to five percent of patients, depending on age, become chronic carriers harbouring S.typhi in the gallbladder[1].

Culture of clinical specimens

The definitive diagnosis of typhoid fever depends on the isolation of *S. typhi* from blood, bone marrow or a specific anatomical lesion[1]. However, blood culture is the mainstay of the diagnosis of this disease[1]. More than 80% of patients with typhoid fever have the causative organism in their blood[1]. A failure to isolate the organism may be caused by several factors[1]: (i) the limitations of laboratory media; (ii) the presence of antibiotics; (iii) the volume of the specimen cultured; or (iv) the time of collection, patients with a history of fever for 7 to 10 days being more likely than others to have a positive blood culture.

Bone marrow aspirate culture is the gold standard for the diagnosis of typhoid fever[22] and is particularly valuable for patients who have been previously treated, who have a long history of illness and for whom there has been a negative blood culture with the recommended volume of blood[23]. Duodenal aspirate culture has also proved highly satisfactory as a diagnostic test[1] but has not found widespread acceptance because of poor tolerance of duodenal aspiration, particularly in children[1] Stools can be collected from acute patients and they are especially useful for the diagnosis of typhoid carriers. The isolation of *S. typhi* from stools is suggestive of typhoid fever. Though blood culture is the mainstay of the diagnosis, its limited availability and low sensitivity[2, 24] is not of much help in making a diagnosis in peripheral health settings.

Serological identification of Salmonella

The Widal test is the most common serological test available in peripheral health settings. This test measures agglutinating antibody levels against O and H antigens. The levels are measured by using doubling dilutions of sera in large test tubes. Usually, O antibodies appear on days 6-8 and H antibodies on days 10-12 after the onset of the disease. The test is usually performed on an acute serum (at first contact with the patient) whose sensitivity is low (52%) [25]. Then, it can be negative in up to 30% of culture-proven cases of typhoid fever. On the other hand, *S. typhi* shares O and H antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other Enterobacteriacae, and this can lead to false-positive results. Such results may also occur in other clinical conditions, e.g. malaria, typhus, bacteraemia caused by other organisms, and cirrhosis. In areas of endemicity there is often a low background level of antibodies in the normal population. Determining an appropriate cut-off for a positive result can be difficult since it varies between areas and between times in given areas[1]. It is therefore important to establish the antibody level in the normal population in a particular locality in order to determine a threshold above which a single antibody titre is considered significant. A convalescent serum should preferably also be collected so that paired titrations can be performed. If paired sera are available a fourfold rise in the antibody titre between convalescent and acute sera is diagnostic[1]. In a study done in Turkey, Widal test with convalescent sera had sensitivity, specificity, positive predictive value and negative predictive values of 90%, 90%, 88% and 93% respectively[25]. Quality control of the test is achieved by running a standard serum with a known antibody titre in parallel in each batch of assays. The

variations in the standard serum should not exceed one tube, i.e. double dilution.

Newer diagnostic tests are being developed. Recent advances include the IDL Tubex test which reportedly can detect IgM O9 antibodies from patients within a few minutes[1]. Another rapid serological test, Typhidot, is reported to take three hours to perform[1]. A recently developed newer version of the test, Typhidot-M, detects specific IgM antibodies only[1]. The detection of specific IgM within three hours suggests acute typhoid infection. Evaluations of Typhidot and Typhidot-M in clinical settings showed that they performed better than the Widal test and the culture method[1]. Evaluation studies have shown that Typhidot-M is superior to the culture method[1]. Although culture remains the gold standard it cannot match Typhidot-M in sensitivity (>93%), negative predictive value and speed[1]. The high negative predictive value of the test suggests that Typhidot-M would be useful in areas of high endemicity.

Treatment of typhoid fever

General management

Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, the use of antipyretics, and appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with oral antibiotics, reliable care and close medical follow-up for complications or failure to respond to therapy[1]. However, patients with persistent vomiting, severe diarrhoea and abdominal distension may require hospitalization and parenteral antibiotic therapy.

Antimicrobial therapy

Efficacy, availability and cost are important criteria for the selection of first-line antibiotics to be used in developing countries. The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever in adults[1]. They are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first-line drugs, viz. chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole. The majority of isolates are still sensitive. The fluoroquinolones attain excellent tissue penetration, kill *S. typhi* in its intracellular stationary stage in monocytes/macrophages and achieve higher active drug levels in the gall bladder than other drugs. They produce a rapid therapeutic response, i.e. clearance of fever and symptoms in three to five days, and very low rates of post-treatment carriage[1]. Evidence from various settings in Asia indicates that the fluoroquinolones are equally effective in the treatment of typhoid fever in children. However, the emergence of MDR strains has reduced the choice of antibiotics in many areas. There are two categories of drug resistance: resistance to antibiotics such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole (MDR strains) and resistance to the fluoroquinolone drugs. Resistance to the fluoroquinolones may be total or partial. The so-called nalidixic-acid-resistant *S. typhi* (NARST) is a marker of reduced susceptibility to fluoroquinolones compared with nalidixic-acid-sensitive strains. Nalidixic acid itself is never used for the treatment of typhoid. These isolates are susceptible to fluoroquinolones in disc sensitivity testing according to current guidelines. However, the clinical response to treatment with fluoroquinolones of nalidixic-acid-resistant strains is significantly worse than with nalidixic-acid-senstive strains. There are a significant number of MDR strains from the Indian

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subcontinent and some other Asian countries (not Indonesia). Recent data on the use of azithromycin in children indicate that it may be safely given as an alternative agent for the treatment of uncomplicated typhoid fever[1]. Azithromycin in a dose of 500 mg (10 mg/kg) given once daily for seven days has proved effective in the treatment of typhoid fever in adults and children with defervescence times similar to those reported for chloramphenicol. If intravenous antibiotics are required, i.v. cephalosporins like ceftriaxone, cefotaxime, and cefoperazone can be given. Ciprofloxacin, ofloxacin and pefloxacin are also available for i.v. use. There are few data on the treatment of typhoid in pregnancy. The beta-lactams are considered safe[26]. Ampicillin is safe in pregnant or nursing women, as is ceftriaxone in such women with severe or MDR disease. Knowledge of the antibiotic sensitivity of the infecting strain is crucial in determining drug choice. If no culture is available knowledge of likely sensitivity as indicated by the available global data may be useful. Relapses involving acute illness occur in five to 20% of typhoid fever cases that have apparently been treated successfully. A relapse is heralded by the return of fever soon after the completion of antibiotic treatment. The clinical manifestation is frequently milder than the initial illness. Cultures should be obtained and standard treatment should be administered. In the event of a relapse the absence of schistosomiasis should be confirmed.

Management of carriers

An individual is considered to be a chronic carrier if he or she is asymptomatic and continues to have positive stool or rectal swab cultures for *S. typhi* a year following recovery from acute illness. Overall, some one to five % of typhoid fever patients become chronic carriers. The rate of carriage is slightly higher among female patients, patients older than 50 years, and patients with

cholelithiasis or schistosomiasis. If cholelithiasis or schistosomiasis is present the patient probably requires cholecystectomy or antiparasitic medication in addition to antibiotics in order to achieve bacteriological cure. In order to eradicate *S. typhi* carriage, amoxicillin or ampicillin plus probenecid or Trimethoprim-Sulfamethoxazole is administered for six weeks. About 60% of persons treated with either regimen can be expected to have negative cultures on follow-up. Clearance of up to 80% of chronic carriers can be achieved with the administration of 750 mg of ciprofloxacin twice daily for 28 days or 400 mg of norfloxacin[3]. Carriers should be excluded from any activities involving food preparation and serving, as should convalescent patients and any persons with possible symptoms of typhoid fever. Food handlers should not resume their duties until they have had three negative stool cultures at least one month apart[1].

Antimicrobial susceptibility test for typhoid fever organisms

Antimicrobial susceptibility testing is crucial for the guidance of clinical management. Isolates from many parts of the world are now multidrug-resistant (MDR)[1]. Isolates are usually resistant to ampicillin, chloramphenicol, sulfonamide, trimethoprim, streptomycin and tetracycline. Alternative drugs that are used for treatment include: fluoroquinolones (e.g. ciprofloxacin), third-generation cephalosporins (e.g. ceftriaxone, cefotaxime), a monobactum beta-lactam (aztreonam) and a macrolide (azithromycin).

Prevention of typhoid fever

Prevention is based on ensuring access to safe water and by promoting safe food handling practices. Health education is paramount to raise public awareness and induce behaviour change.

Safe water

Typhoid fever is a waterborne disease and the main preventive measure is to ensure access to safe water. The water needs to be of good quality and must be sufficient to supply all the community with enough drinking water as well as for all other domestic purposes such as cooking and washing. Providing safe water for all is a long-term goal. Relying only on time and resource-intensive centralized solutions such as piped, treated water will leave hundreds of millions of people without safe water far into the future[27]. Self-sustaining, decentralized approaches to making drinking water safe, including point-of-use chemical and solar disinfection, safe water storage, and behavioral change, have been widely field-tested[27]. These options target the most affected, enhance health, contribute to development and productivity, and merit far greater priority for rapid implementation[27]. Drinking-water can be made safe by boiling it for one minute or by adding a chlorine-releasing chemical. Narrow-mouthed pots with covers for storing water are helpful in reducing secondary transmission of typhoid fever[1]. In Karachi, an interventional study using specially designed narrow mouthed water containers with lids and taps as well as in-house water chlorination using diluted hypochlorite solution in a highly contaminated environment provided safe water[28].

Food safety

Contaminated food is another important vehicle for typhoid fever transmission. Appropriate food handling and processing is paramount and the following basic hygiene measures must be implemented or reinforced during outbreaks: washing hands with soap before preparing or eating food; avoiding raw food, shellfish, ice; eating only cooked and still hot food or re-heating it[1]. During outbreaks, food safety inspections must be reinforced in restaurants and for street food vendors' activities. Typhoid can be transmitted by chronic carriers who do not apply satisfactory food-related hygiene practices[1]. These carriers should be excluded from any activities involving food preparation and serving[1]. They should not resume their duties until they have had three negative stool cultures at least one month apart[1].

Sanitation

Proper sanitation contributes to reducing the risk of transmission of all diarrhoeal pathogens including *Salmonella typh*i. Appropriate facilities for human waste disposal must be available for all the community. In an emergency, pit latrines can be quickly built. Collection and treatment of sewage, especially during the rainy season, must be implemented. In areas where typhoid fever is known to be present, the use of human excreta as fertilisers must be discouraged.

Health education

Health education is paramount to raise public awareness on all the above mentioned prevention measures. Health education messages for the vulnerable communities need to be adapted to local conditions and translated

into local languages. In order to reach communities, all possible means of communication (e.g. media, schools, women's groups, religious groups) must be applied. Community involvement is the cornerstone of behaviour change with regard to hygiene and for setting up and maintenance of the needed infrastructures. The novel behavior change intervention technique of Motivational Interviewing to prevent diarrhoeal diseases in Zambia had very high rate of adherence and community acceptance of the water intervention as evidenced by the high percentage of water samples with detectable chlorine[29].

The MI content was adapted to be relevant to water and sanitation issues as well as cultural considerations based on information gained from focus groups, individual interviews and guidance from the local nurses[29]. Such techniques might be useful in our setting too.

Vaccination

The old parenteral killed whole-cell vaccine was effective but produced strong side- effects. Two safe and effective vaccines are now licensed and available. It is mainly used by travellers visiting areas at high risk of typhoid fever because of the presence of multidrug-resistant strains. The live oral vaccine Ty2la is available in enteric-coated capsule or liquid formulation. It is approved for use in children aged at least 5 years. Travellers should be revaccinated annually. The protective efficacy of the enteric-coated capsule formulation seven years after the last dose is still 62% in areas where the disease is endemic[1]; the corresponding figure for the liquid formulation is 70%[1]. WHO recommends vaccination for people travelling in high-risk areas where the disease is endemic[1]. People living in such areas, people in refugee

camps, microbiologists, sewage workers and children should be the target groups for vaccination. WHO recommends that the immunization of school-age children be undertaken wherever the control of the disease is a priority[1]. School-based typhoid immunization programmes have been recommended in geographical areas where typhoid fever is a recognized public health problem and to areas where antibiotic-resistant *S. typhi* strains are particularly prevalent[1].

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Consent form for the investigation of the risk factors for scrub typhus and typhoid in the Darjeeling district of West Bengal, India in 2005 – 2006

Greetings,

I am _____ and am working with the sub-divisional hospital, Kurseong to look into factors that may put you at risk for scrub typhus and typhoid or protect you from them. We are doing this investigation as a response to the occurrence of more than forty-four cases of scrub typhus in Kurseong between July and December in the year 2004. Since typhoid is a public health problem and also commonly occurs in the same period in Kurseong, it is also being looked at. The National Institute of Epidemiology, Chennai and the National Institute of Communicable Diseases, New Delhi are also working with us on this investigation.

To find out why people get scrub typhus and typhoid, we need to ask questions to persons who had scrub typhus and typhoid and to persons who did not. Thus, between _____ and _____, we will be asking the same questions to all the persons with scrub typhus and typhoid, as well as to some healthy members of the neighborhood of similar age who have scrub typhus and typhoid. We would like to confidentially ask these few questions to you once. We will not disclose your having the disease or any information provided by you to anyone during the interview of you healthy neighbour. Answering these questions should take about 25 minutes of your time. In this survey, we will ask questions and make a few laboratory tests only on the hospitalized scrub typhus and typhoid cases. 10 ml or two table spoonfuls of

blood will be taken from the scrub typhus and typhoid cases on two occasions, at intervals of ten days, by venepuncture using sterile disposable syringes and needles and following the standard aseptic procedures. However, these laboratory tests for confirmation of scrub typhus and typhoid would be a part of the regular medical care that you need. The blood samples taken for testing for scrub typhus at the National Institute of Communicable Diseases, New Delhi, India, will be kept for a maximum of six months at – 20^0C at the sub-divisional hospital, Kurseong prior to the sending of the samples for testing. You will experience only a slight pain during collection of the blood sample. Any leftover blood following the test would be discarded after treating it with 1% sodium hypochlorite solution for one hour as per the Bio-Medical Waste (Management and Handling) Rules 1998. Taking part in this survey is voluntary. No compensation will be paid to you for participating in this study. You can choose not to participate. You can choose not to answer a specific question. You can also stop answering these questions at any time without having to provide a reason. This will not affect your rights to health care in the sub-divisional hospital, Kurseong, or any other rights. There is no specific benefit for you if you take part in the survey. However, taking part in the survey may be of benefit to the community, as it may help us to understand the problem, its causes and potential solutions. When the results will have been analyzed, a report will be shared with all the participants and the local health officials concerned with public health, so that appropriate steps can be taken to prevent and control scrub typhus and typhoid in the peri-urban and rural areas of Kurseong.

The information we will collect in this survey will be completely confidential. We may ask questions about various specific behaviors or practices. This

does not necessarily mean that we think that these behaviors or practices put you at risk for scrub typhus and typhoid. It does not necessarily mean either that we think that these behaviors or practices would protect you from scrub typhus and typhoid. We will not write your name on this form. We will only use a code instead. The key to this code will only be known only to the principal investigator of this survey. It will be kept under lock and key. It will be destroyed after the project.

If you wish to find out more about this survey before taking part, you can ask me all the questions you want. You can also contact Dr.P.K.Sharma MAE-FETP Scholar (Vth Cohort) and principal investigator of this survey attached to the National Institute of Epidemiology, Chennai, at the sub-divisional hospital, Kurseong or Dr.A.K.Barui , physician attached to the sub-divisional hospital at Kurseong, who will be happy to give you more details. If you are OK to take part, we will go ahead now.

I have read the above/the above has been read to me. I have had an opportunity to ask questions and the questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand that I have the right to withdraw from the study at any time without it in any way affecting my further medical care.

Name of the study participant or guardian (if minor)	Signature/thumb impression of the study participant or guardian
Name of the witness	Signature of the witness
Name of the interviewer	Signature of the interviewer

Data collection instrument for the study of risk factors for scrub typhus and typhoid in Kurseong in the district of Darjeeling, West Bengal, India

Case control study No._____

Interviewer's name..... **Date of interview (dd/mm/yyyy)**

Outcome variable:

1. **Scrub typhus case**
2. **Healthy control for scrub typhus matched for neighbourhood and age**
3. **Typhoid case**
4. **Healthy control for typhoid matched for neighbourhood and age.**

Match set No._____

DEMOGRAPHIC INFORMATION

As you know, we are conducting a study to understand why the people in Kurseong are getting sick with scrub typhus and typhoid. We would like to interview you. Do you have some time now?

If **YES**, Thank you. I will be asking you certain general questions. You do not have to respond to any questions that you do not wish to answer.

If **NO,** Would you be available at another time? 1) Yes 2) No
If YES, When and at what time? _____

If NO, Thank you very much for your time.

1. What is your age? _____ (years)

2. What is the patient's sex? 1) Male 2) Female

3. What is your religion? 1) Hinduism 2) Buddhism 3) Christianity 4) Islam

4. What is your caste? 1. General 2) Scheduled tribe 3) Scheduled caste
4) Other Backward Class

5. Do you reside in an urban or rural area? 1) Urban (municipal area)
2) Rural (non-municipal or block area)

6. In which block do you reside 1) Kurseong 2) Mirik

7. Is your house kuccha or pucca? – 1) Kuccha (made of wood)
2) Pucca (made of brick and cement)

8. What is your occupation? 1) Tea plucking 2) Farming 3) Armed forces 4) Trade
5) Housewife 6) Teaching 7) Office work 8) Student
8) Unemployed 9) Other, specify_____

9. Could you tell me what your monthly household income (i.e. average family income) is?
1) Nil 2) < Rs.500.00 3) Rs 501 to Rs1499
4) Rs 1500 to Rs 2999.00 5) > Rs 3000.00

10. How many members are there in your household (i.e. family members living in the house)?

1) one 2) two 3) three 4) four 5) five 6) six 7) seven 8) eight ☐

CLINICAL INFORMATION (**hospital patients**): These will be obtained from the patients' case records through the attending physician/physicians.

---

11. Are any of the following symptoms/signs present?

---

a) Fever[1]:	1) Yes 2) No	☐
b) Rash[2]:	1) Yes 2) No	
c) Eschar: (black scab mark at the site of mite bite)	1) Yes 2) No	☐ ☐
d) Headache:	1) Yes 2) No	☐
e) Malaise :(uneasiness)	1) Yes 2) No	☐
f) Swelling of the glands near the eschar:	1) Yes 2) No	☐
g) Swelling of the glands at different places[3]:	1) Yes 2) No	☐
h) Severe muscle pain:	1) Yes 2) No	☐
i) Cough:	1) Yes 2) No	☐
j) Deafness:	1) Yes 2) No	☐
k) Tinnitus :(Ringing sound in the ears)	1) Yes 2) No	☐
j) Jaundice:	1) Yes 2) No	☐
k) Bleeding:	1) Yes 2) No	☐
l) Coated tongue	1) Yes 2) No	☐
m) Relative bradycardia[4]	1) Yes 2) No	☐
n) Splenomegaly	1) Yes 2) No	☐

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[1] Fever would mean temperature above 37.2^{0}C or above 99^{0}F, when the thermometer is placed in the mouth, axilla or groin (in case of young children) for one minute.

[2] Rashes would mean skin eruptions or changes in skin colour, their sizes ranging from a few millimeters to a more than one centimeter.

[3] This would mean generalised swelling of the major lymph glands like the inguinal, axillary and cervical.

[4] When with per degree Fahrenheit rise in body temperature the pulse rate increases less than 10 beats per minute, the condition is called relative bradycardia and is found in the first week of typhoid fever.

Now, I will ask you some questions regarding the illness and treatment you received, if any, prior to your hopitalisation.

12. When did the first symptoms occur? _____

13. Did you seek medical attention prior to coming to the hospital? If yes, when _____

14. Did you receive any antibiotics prior to hopitalisation? ☐
    1) Doxycycline 2) Tetracycline 3) Chloramphenicol
    4) Ciprofloxacin 5) Others, specify_____

EXPOSURE INFORMATION

Now, I would like to ask you regarding certain factors which might be related to the occurrence of scrub typhus.

RISK FACTORS FOR SCRUB TYPHUS (Cases and Controls)

15. Do you rear animals in the yard[1] of your house? 1) Yes 2) No ☐

IF YES,

17a. How many cows do you rear? ☐

17b. How many goats do you rear? ☐

17c. How many pigs do you rear? ☐

17d. How many fowls do you rear? ☐

17e. How many dogs do you rear? ☐

18. Are there shrubs/bushes/bamboo groves within (plants smaller than trees with many woody stems which arise from the same root 4-6 inches above the ground level) five metres radius of your house? ☐
    1. none 2) few (2-6 bushes) 3) many (>6)

19. How many piles (one pile is equal to 5 cubic feet) of wood for fuel you store in the yard your house? ☐
    1) none 2) few(1 to 2 piles 3) many(>2 piles)

Now, I would like to ask you about certain events which may have occurred during the 21 days starting from _____ through _____.

20. How frequently did you see rodents in / around your house within 21 days prior to the onset of symptoms/21days before? ☐
    1) never 2)Rarely (2-3 times a week) 3) often (>3 times a week) 4) daily

21. How frequently did you see rodents in your areas of activity within 21 days prior to the onset of symptoms/21days before? ☐
    1) none 2) few(2-3 times a week) 3) many(>3 times a week) 4) daily

[1] A yard would be an enclosure, usually small and near a residence or building. On an average, it would be an area of around 25 – 50 feet around the residence.

22. Were you involved in plucking of tea leaves within 21 days prior to the onset of symptoms/21days before?

1) yes 2) no ☐

IF YES,

22a. In the last 21 days, how many days have you spent doing it?

1) <3 days 2) 3-6 days 3) >6 days ☐

22b. On an average, how many hours did you spend doing it daily?

1) <3 hours 2) 3-6 hours 3) >6 hours ☐

23. Were you involved in farming within 21 days prior to the onset of symptoms/21days before?

1) yes 2) no ☐

IF YES,

23a. In the last 21 days, how many days have you spent in it?

1) <3 days 2) 3-6 days 3) >6days ☐

23b. On an average, how many hours did you spend in it daily?

1) <3 hours 2) 3-6 hours 3) >6 hours ☐

24. Were you involved in forest work (collection of edible plants/wood) within 21 days prior to the onset of symptoms/21days before?

1) yes 2) no ☐

IF YES,

24a. In the last 21 days, how many days have you spent in it?

1) <3 days 2) 3-6 days 3) >6days ☐

24b. On an average, how many hours did you spend in it daily?

1) <3 hours 2) 3-6 hours 3) >6 hours ☐

25. Were you involved in construction work (building/repair of roads passing through forests/dense vegetations within 21 days of onset of symptoms/21days before?

1) yes 2) no ☐

IF YES,

25a. In the last 21 days, how many days have you spent in it?

1) <3 days 2) 3-6 days 3) >6 days ☐

25b. On an average, how many hours did you spend in it?

☐

1) <3 hours 2) 3-6 hours 3) >6 hours

26. How frequently did you wear gumboots during your daily activities within 21 days prior to the onset of symptoms/21days before?

1) never 2) sometimes(1-2 times a week) 3) daily ☐

27. How frequently did you wear gloves during your daily activities within 21 days prior to the onset of symptoms/21days before?

1) never 2) sometimes(1-2 times a week) 3) daily ☐

28. How frequently did you wear aprons during your daily activities within 21 days prior to the onset of symptoms/21days before?

1) never 2) sometimes(1-2 times a week) 3) daily

29. Did you wash yourself/bathe after the daily activities within 21 days prior to the onset of symptoms/21days before?

1) no 2) sometimes (1-2 times a week) 3) daily

30. Do you sleep in the same clothes that you wear during your daily work?

1) Yes 2) No

31. How often did you change your clothes after the daily activities within 21 days prior to the onset of symptoms/21days before?

1) once a week 2) sometimes (1-2 times a week) 3) daily

32. Do you find lice and ticks on your body/clothes?

1) never 2) sometimes (1-2 times a week) 3) daily

33. Do you suffer from pruritus or itching over the body?

1) never 2) sometimes (1-2 times a week) 3) daily

34. Did you use insect/mite repellents on your body when going for your daily activities within 21 days of the onset of symptoms/21 days before?

1) none 2) sometimes(1-2 times a week) 3) daily

35. Did you use insect/mite repellents on your clothes when going for daily activities within 21days of onset of symptoms/21 days before?

1) none 2) sometimes(1-2 times a week) 3) daily

RISK FACTORS FOR TYPHOID

Now, I would like to ask you regarding certain events which may have occurred during the fourteen days/two weeks starting from _____ through _____.

36. Did you travel outside Kurseong two weeks before the illness?

[1] Yes [2] No

37. What is the **primary** source of drinking water for your home?
(choose one)

[1] Municipal piped tap water in your home
[2] Municipal piped tap water far from home
[3] Community tap water (close to home[1])
[4] Community tap water (far from home[2])
[5] Stream water
[6] Well (subsoil)
[7] Bottled water
[8] Other, Specify_____

[1] Close to home will mean within 2-5 minutes walking distance from the residence.
[2] Far from home will mean more than five minutes walking distance from the residence.

38. How many days per week is your primary drinking water source available?

[1] <1 day/week [2] 1-3 days/week [3] 4-6 days/week [4]-Everyday [9] DK [1] ☐

39. Is your primary source of drinking water free-flowing (i.e. water from pipes/taps or streams)?

[1] Yes [2] No [9] DK ☐

40. In the two weeks before you became ill/two weeks before, did you drink from any other of the following sources of water at home?

[1] Yes [2] No ☐

41 a. Municipal piped tap water in your home [1] Yes [2] No [9] DK ☐

IF YES,

41 a.1. How many glasses[2] a day? ☐

41 b. Municipal piped tap water far from home [1] Yes [2] No [9] DK ☐

IF YES,

41 b.1. How many glasses a day? ☐

41 c. Community tap water (close to home) [1] Yes [2] No [9] DK ☐

IF YES,

41 c.1. How many glasses a day? ☐

41d. Community tap water (far from home) [1] Yes [2] No [9] DK ☐

IF YES,

41 d.1. How many glasses a day? ☐

41 e. Stream water [1] Yes [2] No [9] DK ☐

IF YES,

41 e.1. How many glasses a day? ☐

41 f. Well (subsoil) [1] Yes [2] No [9] DK ☐

IF YES,

41 f.1. How many glasses a day? ☐

41 g. Bottled water [1] Yes [2] No [9] DK ☐

IF YES,

41 g.1. How many glasses a day? ☐

---

[1] D.K stands for 'don't know'.
[2] A glass of 250 milliliters capacity would be shown during the interview for assessment of water consumption.

41 h. Other [1] Yes [2] No [9] DK

Specify_____

IF YES,

41 h.1. How many glasses a day?

42. In the two weeks before you became ill/two weeks before, did you drink from any of the following sources of water outside the home, such as at work, when working in the field, or when visiting friends?

[1] Yes [2] No

42 a. Municipal piped tap water in your home [1] Yes [2] No [9] DK

IF YES,

42 a.1. How many glasses a day?

42 b. Municipal piped tap water far from home [1] Yes [2] No [9] DK

IF YES,

42 b.1. How many glasses a day?

42 c. Community tap water (close to home) [1] Yes [2] No [9] DK

IF YES,

42 c.1. How many glasses a day?

42 d. Community tap water (far from home) [1] Yes [2] No [9] DK

IF YES,

42 d.1. How many glasses a day?

42 e. Stream water [1] Yes [2] No [9] DK

IF YES,

42 e.i. How many glasses a day?

42 f. Well (subsoil) [1] Yes [2] No [9] DK

IF YES,

42 f.1. How many glasses a day?

42 g. Bottled water [1] Yes [2] No [9] DK

IF YES,

42 g.1. How many glasses a day?

42 h. Other [1] Yes [2] No [9] DK

IF YES,

Specify_____

42 h.1. How many glasses a day? ☐

43. In the two weeks before you became ill/two weeks before, was the water you drank at home **usually** treated with any of the following treatments?

43a. Boiled [1] Yes [2] No [9] DK ☐

43b.Treated at home with purification (Chlorine) tablets /liquids

[1] Yes [2] No [9] DK ☐

43c.Filtered [1] Yes [2] No [9] DK ☐

43d.No treatment [1] Yes [2] No [9] DK ☐

44. On average, how many glasses of water do you drink a day? _____ (glasses)

45. In the two weeks before you became ill/two weeks before, how many glasses of **unboiled** water (not tea or coffee) did you drink **outside** your home on a typical day?

_____ (glasses)

46. In the two weeks before you became ill/two weeks, how many glasses/handfuls of **unboiled** stream water did you drink **outside** your home on a typical day?

_____ (glasses)

47. Do you store your drinking water in a container? [1] Yes [2] No [9] DK ☐

IF YES,
47 a. What kind of a container do you store water in?

[1] Narrow mouthed [2] Wide mouthed ☐

47b. How often is it filled?
[1] More than once per day
[2] Once per day
[3] Every 2-3 days
[4] Every 4-6 days
[5] ≥ Once per week
[9] DK ☐

47c. Is the water in the container covered? [1] Yes [2] No [9] DK ☐

47d. How do you get drinking water out of this container?

[1] Tip the container
[2] Scoop out with a cup
[3] Use its spout or spigot
[4] Drink directly from container
[5] Other
Specify _____ [9] DK ☐

48. In the two weeks before you became ill/two weeks before, did you drink any **water** (not including that from a can or bottle) from a **street vendor**?

[1] Yes [2] No [9] DK ☐

49. In the two weeks before you became ill/two weeks before, did you drink any **juice** (not including that from a can or bottle) from a **street vendor**?

[1] Yes [2] No [9] DK ☐

50. In the two weeks before you became ill/two weeks before, did you eat any **ice** (not ice cream) or drink any beverages with ice?

[1] Yes [2] No [9] DK ☐

IF YES

50a. Where did you get the ice from?
[1] Home made [2] Street vendor [3] Restaurant [4] DK ☐

51. In the two weeks before you became ill/two weeks before, did you take bath in a nearby stream or river?
[1] Yes [2] No [9] DK ☐

52. In the two weeks before you became ill/two weeks before, did you eat any food

52a. In the field while working? [1] Yes [2] No [9] DK How many times/week?_____ ☐

52b. From a restaurant? [1] Yes [2] No [9] DK How many times/week?_____ ☐

52c. From a street vendor? [1] Yes [2] No [9] DK How many times/week?_____ ☐

52d. Was it cooked food from vendor?
[1] Yes [2] No [9] DK How many times/week? _____ ☐

52e. Was it uncooked food from vendor?
[1] Yes [2] No [9] DK How many times/week?_____ ☐

53. Do you attend school? [1] Yes [2] No [9] DK ☐
If YES,

53a.Where do you get your lunch from in school?
[1] Bring from home [2] Provided at school [3] At restaurant
[4] From street vendor [5] Do not eat at school [5] Other (specify) _____ ☐

54. In the two weeks before you became ill/two weeks before, did you eat any of the following raw vegetables and unwashed fruits? If yes, how many servings of these did you eat in a typical week?

54a. Carrots [1] Yes [2] No [9] DK How many servings/week? _____ ☐

54b. Tomato [1] Yes [2] No [9] DK How many servings/week? _____ ☐

54c. Cucumber [1] Yes [2] No [9] DK How many servings/week? _____ ☐

54d. Onions [1] Yes [2] No [9] DK How many servings/week? _____ ☐

54e. Leafy vegetables [1] Yes [2] No [9] DK How many servings/week? _____ ☐

54f. Lettuce [1] Yes [2] No [9] DK How many servings/week? _____ ☐

54g. Spinach [1] Yes [2] No [9] DK How many servings/week? _____

54h. Cabbage [1] Yes [2] No [9] DK How many servings/week? _____

54i. Other vegetable [1] Yes [2] No [9] DK How many servings/week? _____

Specify_____

54j. Peach [1] Yes [2] No [9] DK How many servings/week? _____

54k. Apricot [1] Yes [2] No [9] DK How many servings/week? _____

54l. Grapes [1] Yes [2] No [9] DK How many servings/week? _____

54m. Plums [1] Yes [2] No [9] DK How many servings/week? _____

54n. Papaya [1] Yes [2] No [9] DK How many servings/week? _____

54o. Oranges [1] Yes [2] No [9] DK How many servings/week? _____

54p. Mango [1] Yes [2] No [9] DK How many servings/week? _____

54q. Guava [1] Yes [2] No [9] DK How many servings/week? _____

54r. Other fruit [1] Yes [2] No [9] DK How many servings/week? _____

Specify_____

55. In the two weeks before you became ill/two weeks before, did you eat any of the following dairy products? How many servings of these did you eat in a typical week?

55a. Milk [1] Yes [2] No [9] DK How many servings/week? _____

55b. Butter [1] Yes [2] No [9] DK How many servings/week? _____

55c. Cheese [1] Yes [2] No [9] DK How many servings/week? _____

55d. Cream [1] Yes [2] No [9] DK How many servings/week? _____

55e. Ice cream [1] Yes [2] No [9] DK How many servings/week? _____

55f. Yoghurt [1] Yes [2] No [9] DK How many servings/week? _____

55g. Sweets 1] Yes [2] No [9] DK How many servings/week? _____

55g.Other [1] Yes [2] No [9] DK How many servings/week? _____

Specify_____

56. In the two weeks before you became ill/two weeks before, how often were vegetables and fruit washed in the home before eating?

[1] Always
[2] Usually
[3] Sometimes
[4] Never (***skip to question 53)***
[9] DK

57. Was the water you used for washing food frequently boiled, treated at home with chlorine, or not treated at all?

[1] Boiled
[2] Treated at home with chlorine
[3] No treatment DK

58. Are cooking and eating utensils washed in a stream? [1] Yes [2] No [9] DK

59. Is there a refrigerator in your home? [1] Yes [2] No

IF YES,

59 a. Do you store vegetables and food in it? [1] Yes [2] No

60. Do you wash clothes in a stream? [1] Yes [2] No [9] DK

61. Has anyone in your house other than you been diagnosed with typhoid fever in the past 3 months?

[1] Yes [2] No [9] DK

62. What sort of toilet facilities do you have at home?
[1] Flush toilet
[2] Pit latrine (emptied)
[3] Pit latrine (not emptied)
[4] Other
[9] DK

63. Where does the sewage from your home flow?

[1] nearby stream(jhora)
[2] septic tank
[3] sewage system
[4] Other _____
[9] DK

64. If you do farm work, where do you defecate while working in the field?

[1] Flush toilet
[2] Latrine
[3] Field
[4] Near the stream
[5] Never work in the field
[9] DK

65. Have you received a typhoid vaccine in the last three years?

[1] Yes [2] No [9] DK

66. In the two weeks before you became ill/two weeks before, did you receive any antibiotics?] Yes [2] No [9] DK