SEROPREVALENCE AND FACTORS RELATED TO SCRUB TYPHUS AND MURINE TYPHUS INFECTIONS IN HILL TRIBE PATIENTS AT 4 HOSPITALS IN CHIANG MAI PROVINCE

URAIWON MUKTAREE

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URAIWON MUKTAREE : SEROPREVALENCE AND FACTORS RELATED TO SCRUB TYPHUS AND MURINE TYPHUS INFECTIONS IN HILL TRIBE PATIENTS AT 4 HOSPITALS IN CHIANG MAI PROVINCE. THESIS ADVISORS : CHARNCHUDHI CHANYASANHA, Ph.D., DUSIT SUJIRARAT, M.Sc., MONGKOL CHENCHITTIKUL, M.Sc. 201 p. ISBN 974-663-810-6.

Scrub typhus and murine typhus are a major public health problem. These rickettsial diseases are a major cause of pyrexia of unknown origin (PUO). The objectives of this study were to find the infection rates and prevalence rates of scrub typhus and murine typhus infections, to analyze the factors related to scrub typhus and murine typhus infections, and to find the geometric mean titer (GMT) to scrub typhus. The study population, a total of 393 patients, consisted of pyrexial hill tribe patients who came for treatment at 4 selected hospitals in Chiang Mai province. From June 1997 to February 1998, all of these patients were interviewed and blood samples were taken (single serum 194 cases, and paired sera 199 cases) to detect antibody levels to scrub typhus and murine typhus using and Immunofluorescent Antibody Assay (IFA). The results showed that the prevalence rates of scrub typhus and murine typhus infections were 30.3% (119/393) and 0.5% (2/393) respectively. The prevalence rates of scrub typhus and murine typhus antibody exposure levels were 37.4% (147/393) and 5.9% (23/393) respectively. The prevalence of scrub typhus infections was found to be higher in males (Odd ratio (OR)=1.9); age groups of >25 years old (OR=2.8), agricultural occupation (OR=3.1), a single marital status (OR=2.1), low income (OR=1.3) and low education (OR=2.3). The number of cases at Samoeng Hospital were higher than that other hospitals (OR=1.3). Environmental factors affecting the occurrence of scrub typhus were: having never cut grass (OR=2.1), having shrubs or grassed in living areas (OR=2.7), grassy areas used for break time (OR=3.7) and working areas in fields and forests (OR=2.5). All of the previous factors were found to be significant (p-value < 0.05). In murine typhus there were no significant factors related to antibody exposure. The highest IgM and IgG GMT was found in males, age group of >25 years, agricultural occupation, poor education, low income, a working environment of field or forest and a working time from 8.00 am – 20.00 pm. IgM and IgG antibodies were the main response to co-infection of Gilliam, Karp and Kato strains. The results of this study will be useful for planning and control both diseases.

3936037 PHPH/M : สาขาวิชาเอก : โรคติดเชื้อ ; วท.ม. (สาธารณสุขศาสตร์)

อุไรวรรณ มุคตารี : ความชุกโรคสครับไทฟัสและโรคมิวรีนไทฟัสและปัจจัยที่มีความสัมพันธ์กับการติดเชื้อในกลุ่มชาวเขาผู้มารับบริการที่โรงพยาบาล 4 แห่ง ในจังหวัดเชียงใหม่ (SEROPREVALENCE AND FACTORS RELATED TO SCRUB TYPHUS AND MURINE TYPHUS INFECTIONS IN HILL TRIBE PATIENTS AT 4 HOSPITALS IN CHIANG MAI PROVINCE) คณะกรรมการควบคุมวิทยานิพนธ์ : ชาญชุติ จรรยาสัณห์, Ph.D., ดุสิต สุจิรารัตน์, วท.ม., มงคล เจนจิตติกุล วท.ม., 201 หน้า, ISBN 974-663-810-6.

โรคสครับไทฟัสและมิวรีนไทฟัส เป็นโรคติดเชื้อริคเคตเซียที่สำคัญทางสาธารณสุข และเป็นสาเหตุที่สำคัญของไข้เฉียบพลันไม่ทราบสาเหตุ วัตถุประสงค์การศึกษาเพื่อหาอัตราความชุกของโรคสครับไทฟัสและโรคมิวรีนไทฟัส การตอบสนองของแอนติบอดีต่อสายพันธุ์ที่ติดเชื้อ, วิเคราะห์หาปัจจัยที่มีความสัมพันธ์กับการติดเชื้อดังกล่าว และหาค่าเฉลี่ยของระดับแอนติบอดีไตเตอร์ต่อโรคสครับไทฟัส โดยทำการศึกษาในกลุ่มชาวเขาที่มีไข้และมารับบริการการตรวจและรักษาที่โรงพยาบาลในจังหวัดเชียงใหม่ 4 แห่ง รวม 393 ราย ระหว่าง มิถุนายน 2540 ถึง กุมภาพันธ์ 2541 ผู้ป่วยทุกรายจะได้รับการสัมภาษณ์และเจาะโลหิต (เป็นซีรัมเดี่ยว 194 ราย และซีรัมคู่ 199 ราย) ตรวจหาระดับแอนติบอดีต่อแอนติเจนเชื้อสครับไทฟัสและมิวรีนไทฟัสโดยวิธี Immunofluorescent Antibody Assay (IFA) ผลการตรวจเลือดผู้ป่วยทางห้องปฏิบัติการพบค่าความชุกของการเป็นโรคสครับไทฟัสและมิวรีนไทฟัสเท่ากับ 37.4% (147/393ราย) และ 5.9% (23/393) ตามลำดับ อัตราความชุกโรคสครับไทฟัสพบสูงในเพศชายมากกว่าเพศหญิง (OR=1.9), พบมากในกลุ่มอายุมากกว่า 25 ปีขึ้นไป (OR=2.8), สถานภาพสมรสคู่ (OR=2.1), ส่วนใหญ่ประกอบอาชีพเกษตรกรรม (OR=3.1), การศึกษาต่ำ (OR=2.3), ผู้มีรายได้น้อย (OR=1.3), โรงพยาบาลสะเมิงพบผู้ป่วยมากที่สุด (OR=1.3), ผู้ป่วยส่วนใหญ่เคยถางหญ้า, ป่า (OR=2.1), สถานที่นั่งพักระหว่างทำงานอยู่บนพื้นหญ้า (OR=3.7), และสถานที่ทำงานอยู่ในบริเวณทุ่งหญ้าหรือป่า (OR=1.6) ปัจจัยเหล่านี้มีความสัมพันธ์กับการเป็นโรคสครับไทฟัสอย่างมีนัยสำคัญทางสถิติ (p-value < 0.05) การศึกษาครั้งนี้ไม่พบปัจจัยที่มีความสัมพันธ์กับการมีแอนติบอดีต่อการเคยสัมผัสโรคมิวรีนไทฟัส ค่าเฉลี่ยของระดับแอนติบอดีต่อทั้ง IgM และ IgG พบสูงในเพศชาย, กลุ่มอายุมากกว่า 25 ปี, อาชีพเกษตรกรรม, การศึกษาต่ำ, ผู้มีรายได้น้อย. สถานที่ทำงานอยู่ในบริเวณทุ่งหญ้าหรือป่า และช่วงเวลาทำงาน 8.00 น. – 20.00 น. การตอบสนองตอแอนติบอดีต่อทั้งชนิด IgM และ IgG พบเป็นการตอบสนองร่วมของสายพันธุ์ Gilliam, Karp และ Kato. การศึกษาครั้งนี้มีประโยชน์เพื่อเป็นแนวทางในการวางแผนป้องกันและควบคุมโรคทั้งสองต่อไป

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CHAPTER I

INTRODUCTION

The rickettsioses continue to constitute major health problems in many areas of the world. They occur in Asia, USA, Mexico, the north of South America, Canada, the island of Indian ocean, Papua New Guinea, and Queensland and northern New South Wales in Australia (1,2,3). The information that has accumulated in various reviews on the status of rickettsial diseases strongly supports this viewpoint (4,5,6). However, quantitative information about the distribution and prevalence of the rickettsioses on which health policies can be based is lacking many parts of the world, especially from developing countries where the problems are most likely to be the greatest. The three major groups of rickettsiae (mite-borne of scrub typhus group, flea -borne and louse-borne of typhus group and the tick-borne of spotted fever group) continue to be the cause of human health problems in many parts of the world. In Thailand, only two kinds of rickettsial diseases are commonly reported, scrub typhus and murine typhus.

Scrub typhus is a major rickettsial disease in Thailand. It is caused by *Orientia* (formerly *Rickettsia) tsutsugamushi* and was described more than 100 years ago in Japan. Scrub typhus is transmitted by an infected larval trombiculid mite bite. The mites may be encountered in grassy fields, along the banks of rivers, in neglected or abandoned rice fields, and plantation in forest or jungles in rural areas. The disease is recognized in many countries and now is known to be widespread in Europe, China,

Southeast Asia, India, Taiwan, Australia, and island of the South Pacific(2). This disease is best known from outbreaks occurring among groups of non-immune people entering into endemic areas such as military personnel, road builders, agricultural workers, land clearers, transmigrates and travelers (2). During World War II, scrub typhus was of considerable military importance in the Southwest Pacific area and in the Chinese, Burma and India areas. Approximately 18,000 cases were report among allied soldiers. In 1929, an epidemic of scrub typhus and malaria was reported in Thailand among soldiers in Ubonrajatanee province (7). Recently, in 1996 Lilarasami A. *et al.*, conducted sero-surveys to investigate the cause of pyrexia of unknown origin (PUO) cases at ten hospitals under the Ministry of Public Health (8). The results showed that rickettsial infection, especially scrub typhus was a major cause of PUO.

Murine typhus is caused by *Rickettsia typhi* (*R. mooseri*). The causative agent was isolated from oriental rat fleas (*Xenopsylla cheopis*), it living on *Rattus rattus* (the black rats) or *Rattus norvegicus* (the brown rat). Human case infected by close contact with rodents and their fleas in granaries, breweries, shops and food stores, and domestically in developing countries where there places in the urban areas close to areas of scrub typhus.

Scrub typhus is an infectious disease annually monitored in the surveillance program run by the Ministry of Public Health, Thailand, while cause of murine typhus have not yet been included. The number of reported cases of scrub typhus in Thailand are summarized in Table 1.

Table 1 Number of reported cases, incidence rate and fatality rate of scrub typhus in Thailand, during 1986 - 1996

Year	Number of reported (cases)	Incidence rate (per 100,000 population)	Number of death (cases)	Fatality rate (%)
1986	513	6.00	1	0.19
1987	659	1.20	1	0.15
1988	643	1.18	3	0.47
1989	797	1.44	0	0.00
1990	1,065	1.89	5	0.47
1991	1,196	2.10	6	0.52
1992	1,056	1.82	6	0.56
1993	1,367	2.34	1	0.07
1994	1,298	2.20	1	0.08
1995	1,157	1.94	8	0.69
1996	1,177	1.98	6	0.51

Source : Surveillance Report, Division of Epidemiology, Ministry of Public Health (9).

The incidence rate of scrub typhus in recent years have been increasing and in general this seems to be trend. More male than female cases were reported and the highest prevalence of disease found in northeastern Thailand. Most cases occur in the latter part of the rainy season and into the winter season September to December. Both scrub typhus and murine typhus is sometimes very difficulty to diagnosis without laboratory confirmation. PUO is often the final diagnosis. If these PUO cases are not treated with suitable antibiotics it may lead to multiple drugs resistant possibly resulting in large economic losses or increased fatalities. Presently, the "PUO

diagnosis" group is a major public health problems, the number of reported cases of PUO is summarized in Table 2.

Both scrub typhus and murine typhus infections are occurred throughout Thailand. The morbidity rates for both diseases have been increasing due to the lack of prevention and control programs by the government. Laboratory diagnosis by detecting rickettsia-specific antibodies are difficult to obtain in general hospitals, which may lead to misdiagnosis for both of these diseases. In the northern part of Thailand most of the scrub typhus cases were reported in 1987 and 1988 (9). Chiang Mai Province has been one in the top ten for prevalence of scrub typhus for many years up to the present in Table 3

Table 2 Number of reported cases, morbidity rate and case fatality rate of PUO in Thailand and Chiang Mai Province,1986-1996.

Year	Thailand			Chiang Mai		
	No. of cases	Morbidity rate (per100,000 Population)	Cases fatality rate(%)	No. of Cases	Morbidity rate (per 100,000 population)	Cases fatality Rate (%)
1986	223,895	425.30	0.08	13,286	1,051	0.11
1987	330,376	616.30	0.07	16,407	1,279	0.09
1988	246,685	452.35	0.07	10,959	841.77	0.09
1989	347,083	616.50	0.04	12,153	685.41	0.05
1990	347,083	616.50	0.05	12,153	888.40	0.12
1991	268,576	474.00	0.04	10,554	763.60	0.10
1992	227,171	393.10	0.05	7,534	492.17	0.24

Table 2 Number of reported cases, morbidity rate and case fatality rate of PUO in Thailand and Chiang Mai Province,1986-1996. (Continued)

Year	Thailand			Chiang Mai		
	No. of cases	Morbidity rate (per100,000 Population)	Cases fatality rate(%)	No. of Cases	Morbidity rate (per 100,000 population)	Cases fatality rate (%)
1993	236,721	405.80	0.06	7,729	503.82	0.22
1994	211,973	358.87	0.05	9,088	587.43	0.12
1995	218,977	368.27	0.05	7,014	451.73	0.20
1996	186,916	100.00	0.05	5,367	343.06	0.09

Source : Surveillance Report, Division of Epidemiology, Ministry of Public Health (9).

Table 3 Number of cases, incidence rate and case fatality rate of scrub typhus in Chiang Mai Province, 1986-1996.

Year	Number of reported (cases)	Incidence rate (per 100,000 population)	Number of deaths (cases)	Case fatality rate (%)
1986	34	3.69	1	2.94
1987	86	6.70	0	0.00
1988	73	5.60	1	1.37
1989	47	3.32	0	0.00
1990	55	4.02	0	0.00
1991	70	5.06	3	4.29
1992	16	1.05	1	6.25
1993	36	2.35	0	0.00
1994	70	4.52	0	0.00
1995	116	7.47	0	0.00
1996	71	4.57	4	5.63

Source : Surveillance Report, Division of Epidemiology, Ministry of Public Health (9).

The geographical feature of Chiang Mai Province includes waterfalls, mountains, plant fields and forests in the rural areas. In addition it has big communities and huge population with typical Thai culture in the urban areas, all which make this areas are very popular for travelers. The main occupations of the people are agriculture and the handicraft industry. Chiang Mai has many ethnic groups and the hill tribes are one of the largest. In 1996, the total hill tribe population in Chiang Mai was approximately 208,330. They were divided into 9 major groups: Karen, Meo (or Mong), Lahu, Lisu, Yao, Akha (or Eikao), Lua, Htin and Khamu. Their details are summarized in Table 4. However, there were other minor groups namely, Palong, Haow and Thaiyai. Hill tribe people lead traditional lifestyle that are different from the Thai lowland people in such areas as socio-economic, religion, language, occupation, education and their living environmental. Some of these may be the factors that lead to susceptibility to both rickettsial diseases present in Thailand. The hill tribe areas are now easier to travel in and around than before, so that many hill tribe patients go to the hospitals when they fell unwell. Chiang Mai Province has many reported cases of scrub typhus, while no reports of murine typhus, especially in the hill tribe groups. It would be of great interest to carry out research on the prevalence and incidence of both infections in these groups, as well as to find the factors that may be related to their infections. In order to promote the health status of hill tribe people in the government policy, this study focused on hill tribe people with pyrexia. The knowledge gained in this study will be important and valuable for health planning and controlling the transmission of both diseases in the hill tribe people of Chiang Mai Province.

Table 4 The number of village, household and hill tribe population in Chiang Mai, 1996.

Hill tribe group	No. of village.	No. of household.	No. of persons
Karen	803	23,649	123,630
Meo	69	2,354	20,228
Lahu	176	6,510	34,220
Lisu	70	2,473	14,464
Yao	9	230	1,631
Akha	23	760	4,001
Lua	31	1,989	9,774
Htin	1	62	380
Khamu	1	2	2
Total	1,183	38,029	208,330

Source: Tribal Research Institute, Chiang Mai, Thailand (10).

Objectives

1. General objectives.

To assay the antibody titers to scrub typhus and murine typhus, and to find the factors related to both infections in hill tribe patients with pyrexia at four selected hospitals in Chiang Mai Province.

2. Special objectives.

2.1 To determine prevalence rates and infection rates of scrub typhus and murine typhus among the hill tribe patients with pyrexia.

2.2 To differentiate the immunoglobulins of antibody response: Immunoglobulin M (IgM) and immunoglobulin G (IgG) to 3 scrub typhus serotypes (Gilliam, Karp, and Kato).

2.3 To analyze the factors related to scrub typhus and murine typhus infections,

Research Hypothesis

1. Prevalence rates and infection rates of scrub typhus and murine typhus in the hill tribe are different.
2. The geometric mean levels of IgM antibody titers and IgG antibody titers response to scrub typhus and murine typhus infections are different.
3. Some factors including general characteristics, personal behaviors, knowledge and environment are related to scrub typhus and murine typhus infections in hill tribe patients.

Definition of terms :

1. Paired sera.

   Blood samples were collected as early in the course of disease as possible (acute serum) and were collected again on days 10-14 (early convalescent) / later on days 21-28 after onset (convalescent).

2. Single sera.

   Blood samples were collected only as early in the course of disease as possible (acute serum).

3. Hill tribe patients.

Hill tribe patients refer to hill tribe people who have a fever of more than 38° C and visited one of the 4 selected hospitals for treatment.

4. Scrub typhus and murine typhus infections.

Scrub typhus and murine typhus infection referred to the incidence of infection (present infection or recent infection) or the prevalence of infection (past or present infection).

4.1 Prevalence rates of antibody exposured of both diseases is determined by IFA titers of IgM and / or IgG ≥ 1:50 in either acute or convalescence serum.

4.2 Infection rates of both diseases is determined by IFA titers of IgM ≥ 1:400 and / or IgG ≥ 1:400 in single serum or 4-fold rising titers to a level of ≥ 1:200 or either serum demonstrates a titer of ≥1:400 in paired sera.

4.3 Recent infection of both diseases are determined by IFA titers of IgM and / or IgG ≥ 1:50 but < 1:400 in single serum sample.

5. The infection rates of scrub typhus / murine typhus is calculated by :

$$= \frac{\text{number of patients with infection}}{\text{number of all patients in this study}} \times 100$$

6. The prevalence rates of antibdody exposure to scrub typhus / murine typhus is calculated by :

$$= \frac{\text{number of patients with antibodies exposured}}{\text{number of all patients in this study}} \times 100$$

7. Related factors

7.1 Control of mites, fleas and / or insects in working areas

Control of mites, fleas and / or insects in working areas referred to control using any methods for controlling or diminishing mites, fleas and / or insects.

7.2 Use of repellent to protect against mite, fleas and /or insect bite

Use of repellent to protect against mite, fleas and / or insect bites refered to a patient who has had previous experience in using chemical, herbs or other things to protect from bites by mites, fleas and/or insects.

7.3 Wearing clothing while working.

Wearing clothing while working mean the patients'clothing worn whilst as assessed by such indices as long sleeved shirts, trousers, and the inserting of trousers into boots.

7.4 History of clearing grass, shrubs, trees and forests.

History of clearing grass, shrubs, trees, forests referred to the patients who had previous exposure related to working on the clearing of grass, shrubs, trees and forests.

7.5 Working time.

Working time referred to the time from the beginning to the end of daily work.

. 7.6 Working areas and environment.

Working areas and environment referred to the patients' working areas and environment which can describe epidemiological and environmental areas such as plains, land clearing for plantations, sparse forests and evergreen forests, nearby house and rural areas.

7.7 Break time area at work.

Break time area at work referred to the area and environment use during work breaks especially sitting place such as shanty, grassy or cleared area.

7.8 History of working or living in an urban area.

History of working or living in an urban area referred to the occasions spent in the working or living place of patients in an urban area which present a chance to have contact with house rats or rat fleas.

7.9 Living environment.

Living environment refered to the style of patients' dwelling and the surrounding area of the house.

8. Knowledge.

8.1 Knowledge of concerning the disease vector.

Knowledge of concerning the disease vector mean the patient's knowledge of, trombiculid mites or rat flea as the vectors for both diseases.

8.2 Knowledge of disease transmission.

Knowledge on disease transmission is knowledge that the diseases are vector-borne and transmitted by biting, the patients' knowledge about the clinical development of these two diseases such as initial signs, symptoms and specific therapies.

8.3 Knowledge of the prevention and control disease.

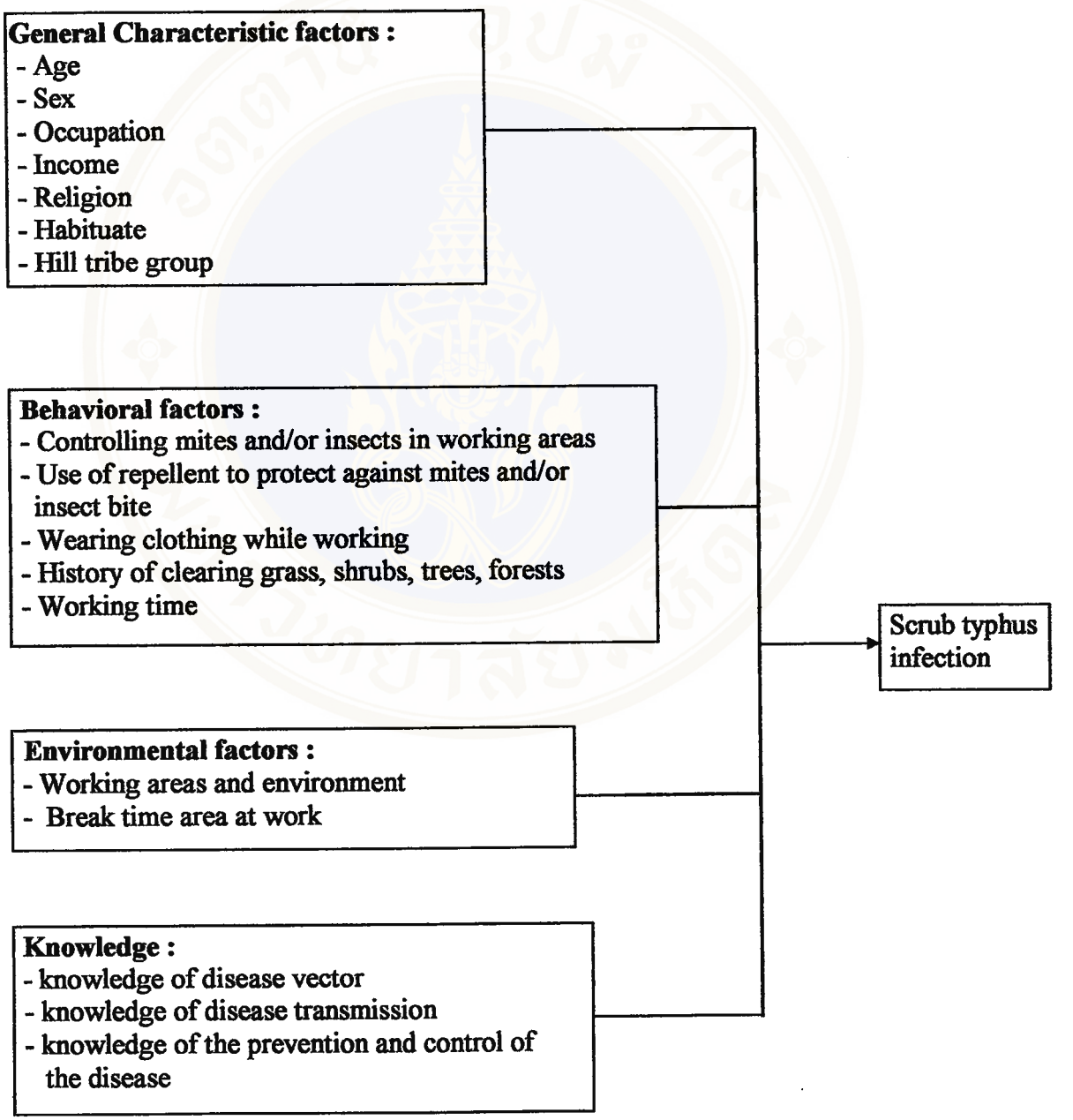
Knowledge on the prevention and control disease is the patients' knowledge about preventing vector bites and eliminating rodents.

Scope of Research :

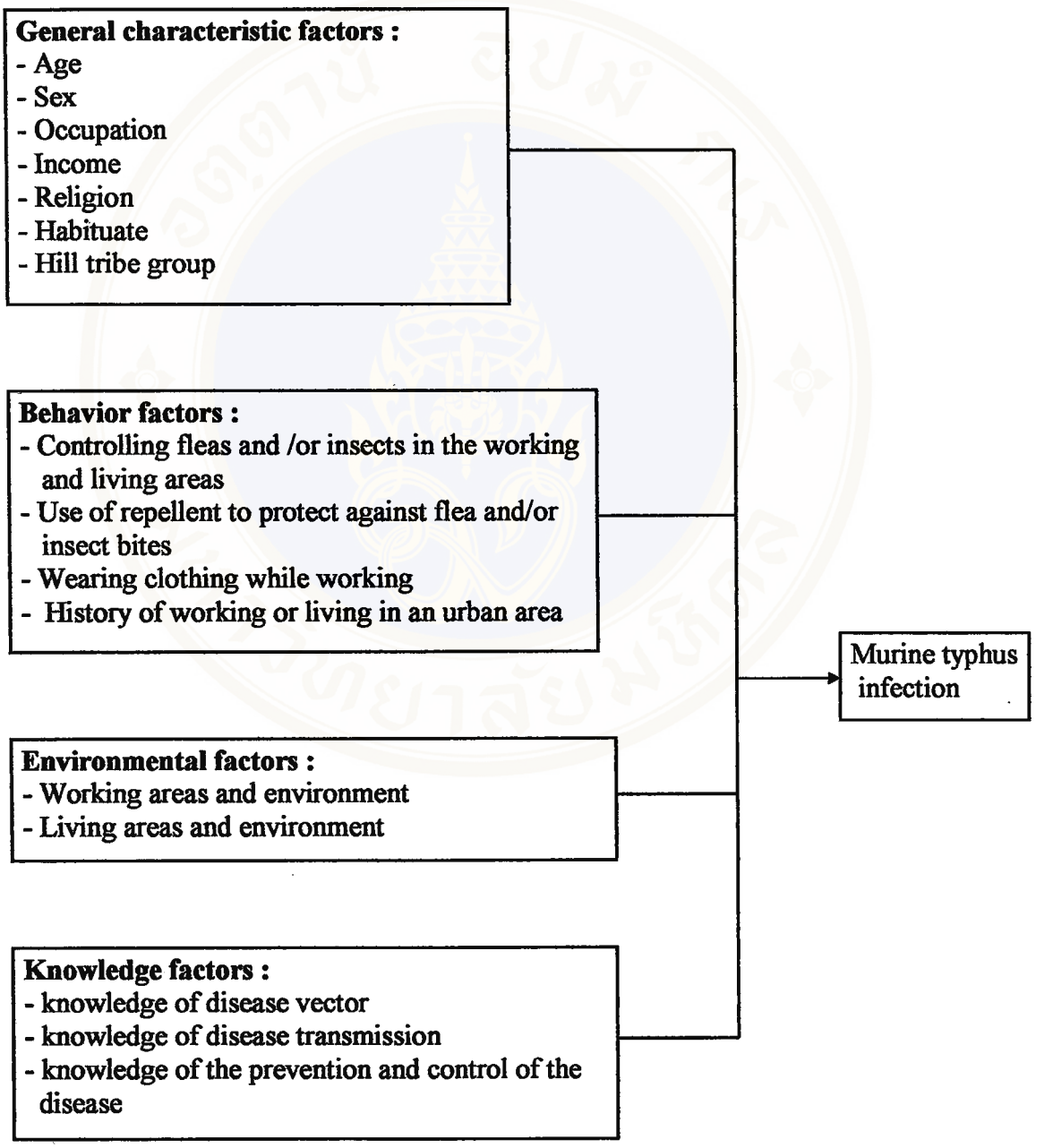
The antibodies titers of scrub typhus and murine typhus infections were assayed in hill tribe patients. The age of the hill tribe patients selected for this

study will be greater than one year and they must display symptoms of fever at any of 4 selected hospitals: Samoeng Hospital, Mae Taeng Hospital, Phrao Hospital and Chiang Dao Hospital, (all located in Chiang Mai Province). The patients were interviewed with a structured questionnaire that addressed point such as general characteristics, previous illnesses, personal behavior, working and living environment, and knowledge. All information was compared and analyzed to find factors related to both diseases infections.

Conceptual framework of scrub typhus



Conceptual framework of murine typhus



CHAPTER 2

LITERATURE REVIEW

Etiology agent

Scrub typhus (Tsutsugamushi disease, mite-borne typhus, Japanese river fever, tropical typhus, rural typhus) was well known a long time ago. The disease is caused by *Orientia tsutsugamushi* (formerly *Rickettsia tsutsugamushi or R. orientalis*) and is transmitted by trombiculid mites of the genus *Leptotrombiduim* (2,3,11).

Murine typhus (endemic typhus or flea-borne typhus) is a natural infection of rats and mice transmitted sporadically to man by the oriental rat flea, *Xenopsylla cheopis,* collected from rats, and from the brains of *Rattus rattus.* The etiologic agent is *Rickettsia mooseri* in honor of Dr. Mooser H. although the currently accepted name is *Rickettsia typhi* (12,13,14).

The genus *Rickettsia* is classified into 3 major groups (Bergey's Manual of Systamatic Bacteriology, 1984) (15):

Firstly, the scrub typhus group. *Rickettsia tsutsugamushi* is the causative agent and is the only species in this group. There are multiple serotypes and the disease is transmitted by larvae of trombiculid mites.

Secondly, the typhus group containing 3 species. *R. prowazekii,* the caused of epidemic typhus, transmitted by the human body louse. *R. typhi* (*R. mooseri*), the cause of murine typhus (endemic typhus, urban typhus), carried by the rat flea. Lastly, *R. Canada,* carried by tick.

Thirdly, the spotted fever group, containing of 8 species, *R. rickettsii, R. siberica, R. conorii, R. parkeri, R. australis, R. akari, R. montana and R. rhipicephali,* Almost all of these species are transmitted by the ticks of rodents and other animals.

Recently, the genus *Rickettsia* was newly classified according to their group. Organisms in the scrub typhus group containing *R. tsutsugamushi* are now classified in the genus *Orientia* due to the structure of the outer leaflet of the cell wall. *O. tsutsugamushi* has a considerably thicker outer than inner leaflet, whilst the opposite is true of the other *Rickettsia* species. In addition, chemically, *O. tsutsugamushi* lacks two constitutional components; peptidoglycan and lipopolysaccharide. *O. tsutsugamushi* is very soft and fragile which reflects the lack of peptidoglycan, and the its'growth is more resistant to penicillin than the growth of other rickettsia. Therefore, the only species of the scrub typhus group has been renamed *Orientia tsutsugamushi* (16).

Morphology and chemistry

The rickettsiae are obligate intracellular parasites. They resemble bacteria morphologically; occurring as short rods, cocci in chains or filaments, and as plemorphic coccobacillary forms. They are non-motile and do not form spores. They have a length of 0.8 to 2.0 microns and a width of 0.3 to 0.5 microns and are seen in

the cytoplasm of host cells. They stain weakly with aniline dyes and are gram negative. The standard Macchiavello technique stain *O. tsutsugamushi* poorly. For many years, rickettsiae were thought to be biologically intermediate between bacteria and viruses, but since they resemble bacteria in many more ways than they do viruses. They are now considered to be bacteria according to the following criteria (17):

(a) Rickettsiae divide by transverse binary fission.

(b) Rickettsiae contained both ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) as found in bacteria.

(c) Rickettsial cell wals contained muramic acid, an essential component of mucopeptide, characteristically found in the cell walls of all bacteria studied.

(d) Rickettsiae are destroyed by antibiotics such as tetracycline, chloramphenicol and para-aminobenzoic acid.

The morphological demonstration that *R. prowazekii, R. rickettsii,* and *O. tsutsugamushi* have substantial slime layers which was demonstrated by the specific-antibody stabilization procedure on organisms gently liberated form their host cells. The slime layers stain with ruthenium red and silver methenamine, which is consistent with a polysaccharide nature (19,20).

O. tsutsugamushi, in contrast to other rickettsiae, adheres tenaciously to host cell components and is not purified without substantial loss of activity. This difference in adherence properties is reflected in the structure of the outer membranes (Figure 1). Although all rickettsia have two unit membranes, the cytoplasmic membrane and the cell wall, in the typhus group and spotted fever group the inner leaflet of the cell wall is

the thicker of the two, whereas the reverse is true in the scrub typhus rickettsia (18). Apparently, physico-chemical factors that remove host cell components from *O. tsutsugamushi* damage the cell wall.

The base composition of the DNA of rickettsiae was studied by acid hydrolysis and chromatography at the time when the information on the constant ratios of adenine (A) to thymine (T) and guanine (G) to cytosine (C) was being developed.

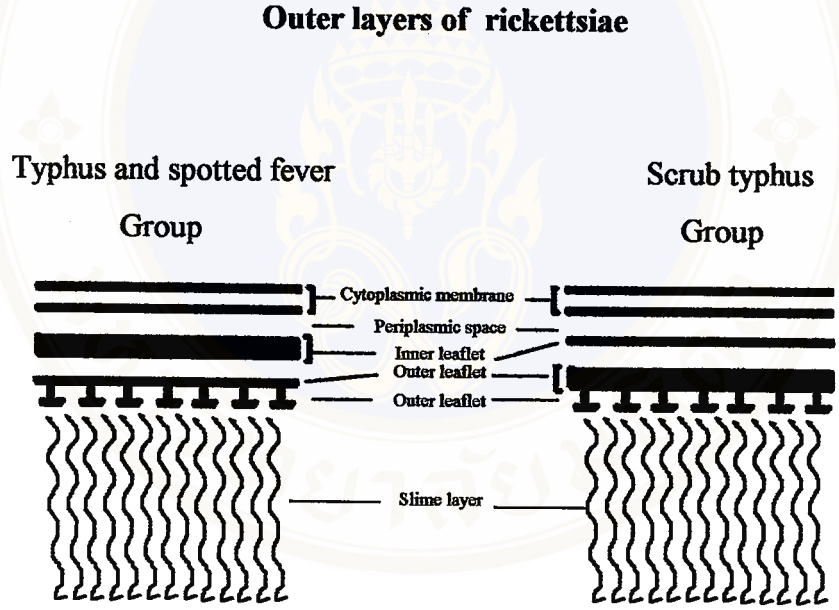


Figure 1 Diagrammatic representation of the outer layers of rickettsiae.

Source : Reproduced from Emilio Wesis from Naval Medical Research Institute, Bethesda, Maryland (19,20).

Effects of physical and chemical agents

Rickettsiae are relatively labile and readily inactivated by; heat (incubation at 56° C for 30 min), ultraviolet irradiation and various chemical agents such as 2 - 8 %

formaldehyde, 1 % lysol, 70 % ethanol and sodium hypochloride (21). However, organisms remain viable for 12 months after storage at -70° C. Undiluted sample of rickettsiae did not decline even being held at 4° C for eight hours. However, a similar sample held 37° C showed a 2-log (100 fold) decline after only four hours (22). Even, when subjected to freeze-drying (lyophilization) viable organisms are stable for at least seven months. If serum is to be retained for an extended time it should be stored frozen at -20° C or lower. Evaluation of the stability of the different immunoglobulin classes of rickettsial antibody in blood stored on filter-paper disks in the dried state showed that the IgM antibody activity declined rapidly and IgG activity was relatively constant over a 4 months period at -20° C (23).

Biological characteristics

The application of tissue culture techniques to study of the interaction of host cells and rickettsiae has provided information on the mechanisms and the course of cellular infection. *O. tsutsugamushi* has a relatively slow rate of multiplication in comparison with viruses and most bacteria. Approximately 3-fold increases in numbers occurred in 24 hours when the rickettsiae were grown in the MB cells (mouse lymphoblast) and the L929 cells (mouse fibroblasts). The penetration of scrub typhus rickettsiae into tissue culture cells was affected by factors concerned with the maintenance of viability of the microorganisms. Substances such as glutamic acid related metabolites, and protein, known to have a protective effect on infectivity in *vitro*, enhanced the invasiveness of rickettsiae into MB III cells maintained in a simple balanced salt solution devoid of these materials. Penetration was also dependent on

divalent cations in the fluid environment which had no known effect on the metabolism of either the cell or the rickettsiae. Chloramphenicol has a rickettsiostatic effect due to interference with protein synthesis by some, as yet unknown, mechanism. This drug had no effect on penetration in concentrations of 250 μg/ml, whereas 50 μg/ml of chlortetracycline, which has rickettsiocidal properties, markedly reduced penetration (3).

Maximum intracellular growth of the rickettsiae occurred only in the presence of complex media capable of supporting proliferation of host cells. In protein-deficient media which maintained cell viability, rickettsiae were inactivated and eliminated from infected mouse fibroblasts at a rate which roughly paralleled the effect of low therapeutic doses of specific antibotics in a complete growth medium. However, under both conditions, rickettsial infection of the cells still persisted at low levels at the end of 3 days. Continuous treatment of the cell culture with chloramphenicol for 3 to 4 weeks was necessary for eradication of the rickettsial infection (24).

Growth and metabolism

Although it is tempting to compare rickettsiae to each other and to use one as a model for another, this can be done profitably only when the great diversity of these organisms is taken into consideration. The most obvious one is the location of the intracellular rickettsiae. Rickettsiae are primarily found in the cytoplasm and sometimes in the nucleus. The optimal pH for metabolic activity reflects their respective environments. Rickettsiae appear to be free in the cytoplasm and not to be surrounded

by a membrane of host origin, a property shared with some of the insect endosymbionts. The few rickettsiae seen in an intracellular location appear to be degenerating. Another major difference is the absence of glycolytic enzymes in rickettsiae. Some rickettsiae are free in the cytoplasm, whereas others are still in the phagosome and some of these are degenerating. Escape from the phagosome is dependent on the virulence of the strain. A phospholipase appears to be involved in the mechanism of escape; *O. tsutsugamushi* achieves a very high density especially in the perinuclear region and often products from the surface of the cell. A curious cycle was described in mouse peritoneal mesothelium cells, which is common in *O. tsutsugamushi* infections. The organisms acquire a host membrane coat from host cell plasma membrane and bud from the surface. Rickettsiae enveloped by this membrane enter other cells by phagocytosis and shed their coat as they escape from the phagosome (19).

The nutrition requirements of rickettsiae, as distinct from those of the host cell, have not been extensively studied. Metabolism studies of the rickettsiae separated from host cells has been limited to the typhus rickettsiae.

Pathogenesis and pathology of scrub typhus

The basic pathologic lesions in scrub typhus, as in other rickettsial diseases, are found in the small blood vessels. Changes observed at necropsy are not striking. Usually an eschar is found, but no rash is seen. The body cavities contain a moderate amount of serofibrinous fluid. Congestion and cloudy swelling of the parenchymatous tissues are observed consistently. The lungs usually show evidence of hemorrhagic

pneumonia with a superimposed, secondary bronchopneumonia. The spleen and the lymph nodes are enlarged.

Microscopic examination a disseminated focal vasculitis and a perivasculitis of the smaller vessels consisting of accumulations of monocytes, plasma cells and lymphocytes. Vascular changes with resultant lesions in adjacent parenchymatous tissue are most conspicuous in heart, lung, brain and kidney. Thus, an acute, nonsuppurative myocarditis of focal and diffuse distribution and of varying intensity is characteristically present. Interstitial pneumonitis occurs in practically all fatal cases. The lesions in the brain may consist of a few vascular and perivascular reactions such as are found throughout the body. The spleen and the lymph nodes display similar changes, with infiltrations of cells of the mononuclear series into the pulp and the sinuses, and necrosis of the follicles. The kidneys characteristically show focal interstitial lesions which occasionally are associated with damage to adjacent nephrons (3).

Clinical finding of scrub typhus

Following an incubation period of 6 to 21 days, generally 10 to 12, illness begins suddenly with fever, chills, severe headache, conjunctival infection and moderate generalized lymphadenopathy. The primary lesion or eschar is found in the majority of Caucasians, but less frequently in Asians. It occurs at sites where skin surfaces meet or clothes bind, such as the axilla, the groin, the neck and the waist. At the onset of fever the eschar is an indurated erythematous lesion about 1 cm in diameter, surrounded by a multiloculated vesicle. Within a few days the vesicle ulcerates, the area is covered by a

black scab, and the regional lymph nodes become particularly prominent. Fever increases progressively during the first week of the disease, generally reaching 104° F or 105° F. The pulse rate during this period is relatively slow, being 70 to 100. A red macular rash appears on the trunk between the 5th and the 8th day and may extend to the arms and the legs. The macular eruption usually persists for several days and may become maculopapular, but it may disappear within a few hours of its' appearance.

During the first week of fever, cough is commonly present. Headache may abate somewhat after the 7th day, but apathy continues, and some degree of deafness is commonly present. Certain patients develop additional signs of involvement of the central nervous system, for example; delirium, stupor and muscular twitching. In the more severely affected patients, the pulse rate increases to 120 or 140, and the systolic blood pressure may fall below 100. Frank signs of pneumonia or of circulatory failure with accompanying oliguria and azotemia develop in some patients. Toward the end of the 2nd week or the beginning of the 3rd, the temperature of the untreated patient who is destined to recover falls by lysis over a period of several days. With the reduction in fever, the pulse rate and the blood pressure return to normal. At this stage the eschar is practically healed, and the spleen is no longer palpable if it had been felt during the febrile period. The clinical and laboratory features to scrub typhus are shown in Table 5.

Death, when it occurs, supervenes about the end of the 2nd week and is attributable in approximately equal numbers of cases to secondary bacterial pneumonia, encephalitis or circulatory failure. The mortality has varied as much as 1 -60% in

different geographic areas and different populations. The picture has changed radically since the introduction of specific antibiotic therapy in 1948, and now the mortality in appropriately treated patients approaches zero.

Second attacks of scrub typhus are not uncommon and may occur in exposed persons within a few years after the initial illness. Persons recovered from illness caused by one strain are resistant to this strain for a number of years and to heterologous strains for months (25).

Though specific antibodies develop during the disease, cell-mediated immunity seems to be the principle mechanism of acquired resistance *O. tsutsugamushi* strains are immunologically heterogenous. As a result, an attack of scrub typhus confers immunity for years against the homologous strain, but only partial immunity for several months against heterologous ones. Thus second and third attack of scrub typhus can occur the same locality (26).

Table 5. Clinical and laboratory features to scrub typhus .

Characteristic of patients	Determined the characteristic (compare to percentage)
Symptoms	
General	
Sudden onset	2^+
Fever	5^+
Chills	$1 - 4^+$
Myalgias	2^+

Table 5. Clinical and laboratory features to scrub typhus . (Continued)

Characteristic of patients	Determined the characteristic (compare to percentage)
Photophobia	0
Arthralgias	1^+
Lymphadenopathy	$1 - 5^+$
Conjunctivitis	$2 - 4^+$
Pharyngitis	2^+
Rash	
Eschar	$2 - 3^+$
Palm and soles	2^+
Face	2^+
Central nervous system	
Headache	$3 - 5^+$
Abnormal CSF	0
Meningismus	+
Seizures	0
Focal signs	0
Coma	0
Decreased hearing	$+ - 2^+$
Cardiovascular system	
Cough	$1 - 4^+$
Rales	0
Abnormal chest x-ray	$1 - 2^+$
Shock	+
Gastrointestinal system	
Nausea or vomiting	$2 - 3^+$
Constipation	0
Abdominal pain	2^+

Table 5 Clinical and laboratory features to scrub typhus. (Continued)

Characteristic of patients	Determined the characteristic (compare to percentage)
Splenomegaly	$1-3^+$
Hepatomegaly	1^+
Diarrhea	2^+
Jaundice	0
Routine laboratory tests	
Abnormal liver function	0
Azotenia	0
Thrombocytopenia	2^+

Source: Blake FG. *et al.*, American Journal Hygiene 1945; 41: 243-396 (27).

Information was complied from published reports as follow: Blake et al. (27) determined the percentage of patients with indicated characteristic : 0 = not reported to occur, + = < 1 - 5 %, 1^+ = 6 - 20 %, 2^+ = 21 - 40 %, 3^+ = 41 - 60 %, 4^+ = 61 - 80 %, 5^+ = 81 -100 %.

In Thailand, eschar was found 30 % of patients with scrub typhus infection (28). After fever 5 days, a macular rash develops in 10 % of patients.

Trishnananda M. et al. (29) classified scrub typhus as one of three types:

1. Classical type : presentation with high fever, chills, severe headache, muscular pain, conjunctival infection, the appearance of an eschar and moderate generalized lymphadenopathy. The history of patients always show that they worked in forested area.

2. Mild type : Mild clinical occurred without the eschar or a rash. Sometimes there is little or nothing to distinguish a scrub typhus patient from one with an other

tropical fever. These patients were infected at grassy or scrub areas associate with rodents.

3. Subclinical type : Clinically may be nothing or rarely fever, headache and myalgia. This type is often missed diagnosis.

Clinical of murine typhus

Murine typhus is often characterized as a mild disease because it is rarely fatal. However, it can cause severe illness. After an incubation period of 5 - 14 days, there is typically an abrupt onset of fever accompanied by persistent headache, chills, malaise, and myalgia. Several days after onset approximately 60 % of typhus patients develop a macular rash that is initially confined to the central aspects of the trunk without an eschar. Other symptoms may include nausea and vomiting, abdominal pain, pneumonitis, conjunctivitis, splenomegaly, and hepatomegaly. Rarely, stupor, delirium, and coma may occur. Prior to the advent of broad-spectrum antibiotics approximately 5 % of murine typhus cases were fatal. Since then fatality ratios have dropped to 1 % or less (30).

In 1993, Silpapogakul K et al. (31) studied the clinical features of murine typhus in Thailand. One hundred and thirty seven patients with murine typhus were reviewed, the result are showed in Table 6.

Table 6. Symptoms of patients with murine typhus

Symptoms	No. of patients (%)
Myalgia	61 (44.5)
Headache	57 (41.6)
Nausea and / or vomiting	42 (30.7)
Abdominal pain	15 (10.9)
Constipation	12 (8.8)
Diarrhea	7 (5.1)
Seizures	3 (2.2)

Source : Department of medicine, Songklanagarind Hospital, Prince of Songkla University and Department of pediatrics, Hadd-Yai Hospital, Songkla, Thailand (31).

Table 7. Physical findings in patients with murine typhus

Signs	No. of patients (%)
Fever	137 (100.0)
Hepatomegaly	33 (24.1)
Rash	28 (20.4)
Jaundice	15 (10.9)
Splenomegaly	7 (5.2)
Subconjunctival haemorrhage	3 (2.2)
Altered consciousness	3 (2.2)
Seizure	3 (2.2)
Hypotension	3 (2.2)

Source : Department of medicine, Songklanagarind Hospital, Prince of Songkla University and Department of pediatrics, Hadd-yai Hospital, Songkla, Thailand (31).

Humoral and cell-mediated immunity

Antibodies have not direct killing effect on typhus rickettsiae and do not interfere with their infection of nonprofessional phagocytes. When the macrophages are first coated with cytophilic anti-rickettsial serum and then are exposed to rickettsiae, phagocytosis of rickettsiae is accelerated, but subsequent growth of rickettsiae is not impeded. Only when rickettsiae are first coated with antibodies and then are exposed to macrophage does the interaction lead to increased phagocytosis as well as to digestion of the rickettsiae. Even under these circumstances rickettsiae escape digestion in an occasional macrophage and multiply. Nonspecific opsonization by preincubation of the rickettsia with methylated bovine serum albumin enhances phagocytosis, but the rickettsiae are not prepared for intracellular destruction. Instead, they grow within the macrophages and eventually destroy these cells. Thus, immune serum and macrophages, neither of which is capable of killing these rickettsiae alone, act in concert to destroy the virulent organisms. In this system, immune serum appears to exert two distinct, possibly dissociable actions on the rickettsiae; enhancement of phagocytosis and preparation for intracellular destruction. Complement is not required for this action but, when present with immune serum, markedly enhances phagocytosis of the rickettsiae, often leading to rapid destruction of the macrophage.

Peritoneal macrophages from BALB / C mice can be infected with the Gilliam strain of *O. tsutsugamushi* in *vitro*. The number of infected cells can be reduced to 50 % by pretreating the macrophages with antiserum and to 25 % by pretreatment with lymphokines. When lymphokines are added after infection and are maintained in the

culture medium for 24 hours, the number of intracellular rickettsiae declines sharply. Thus, lymphokines affect the macrophages in two different ways; They cause an immediate killing of rickettsiae and a reduction in the number of infected cells, when their action continues, most of the intracellular rickettsiae are killed also (32).

Two distinc types of anti-rickettsial responese are encountered in the humeral and cellular immune system. Type 1 responders : the initial response generally appears within 8 days after onset of illness and rapidly increasing in titer is IgM. The IgG dose not appear until 12 days and increases in titer more slowly. Type 2 responders : the initial response, detectable within 6 days, is IgG, whilst the IgM response is lower: The IgA response is transient and occurss almost exclusively in the type 1 responders. In later convalescence (>40 days after onset), IgG is the predominant anti-rickettsial antibody class in both groups and persists for approximately 1 year. The specificity of serological reactions toward the Gilliam, Karp and Kato serotypes is more clearly shown in the IgM responses of both group. The cellular response is characterized by depressed levels of activated T-cell (A-T cells) during acute illness (0-7 days post onset) and the early phase of convalescence (8-15 days after onset) in the type 1 responders, and by elevated A-T cell levels in both groups during the later stages of recovery.

Type 1 and type 2 responders also differ in age and in the clinical manifestations of disease. Type 1 responders are younger, exhibited a higher incidence of rash and conjunctivitis, and a somewhat greater incidence of transient fever and relapse

following tetracycline or doxycycline therapy. Type 2 responders exhibit a higher incidence of generalized lymphoadenopathy. This results suggest that the type 1 responders reflects primary infection, while type 2 indicates reinfection (33).

Immunity in scrub typhus is shorted lived immunity to the homologous serotype (of several serotypes) following an attack of scrub typhus lasting for a year. Cross immunity to the other serotypes is considerably shorter, only one to two months, so that multiple attacks are quite common. Reinfection with *O. tsutsugamushi* was found to be common highly endemic areas. Antibody which persists from one to several years does not appear to be protective and control mediated mechanisms play a major role (34).

Laboratory finding

In scrub typhus, an early leukopenia (1000 to 5000 white blood cells/mm^3) gives way to slightly depressed or normal total white blood cell counts, which may become somewhat elevated late in the disease. Total serum proteins are usually normal or low, but the gamma globulin levels may be greater than the albumin level. Occasional clotting disturbances have been reported, including the disseminated intravascular coagulation syndrome. Jaundice is rare, but serum transaminase enzyme levels may be elevated. Albuminuria is common, oliguria and azotemia may occur.

In 1993, Silpapojakul et al.(31) studied 137 patients with murine typhus in Thailand. Laboratory findings on admission revealed leucocyte counts were usually normal, only four patients (3.9%) had white blood count $\leq$ 3000/mm^3.

Thrombocytopenia was reported in four patients. Liver function tests were performed in 52 patients (38 %) and abnormal aminotransferases and alkaline phosphatase levels were found in most of the patients tested, 28 % of whom had ≥ 5 folds elevation of aminotransferases levels.

The cerebrospinal fluid was examined in 14 patients. Only three showed the presence of meningitis. The spinal fluid white cell counts in these three patients ranged from 15 to 54 / mm^3 and were predominantly mononuclear cells. The protein levels were slightly elevated and the glucose levels were normal.

Differential diagnosis

Recognition of scrub typhus on the basis of clinical evidence is often difficult owing to variability of occurrence of disease manifestations, and their similarities to other infectious disease also present in scrub typhus areas. A history of exposure in an endemic area and the finding of the eschar, rash and generalized or regional lymphadenopathy are the cardinal features of the usual illness. However, in many patients, one or more of these findings may be absent. Additional signs and symtoms such as fever, headache, conjunctival injection, relative bradycardia and absence of leukocytosis are not sufficiently distinctive to permit differentiation of scrub typhus from other rickettsial infections, dengue, leptospirosis, malaria infections, hepatitis or typhoid fever.

Murine typhus cannot be distinguished solely on clinical grounds in individual cases, it may be emphasized that the rash of typhus appears on the body first, then spreads to the extremities. The rash of the latter disease has a greater tendency to be papular and to become petechial or hemorrhagic.

Laboratory diagnosis

There are four ways in which rickettsial disease can be diagnosed in the laboratory : (1) direct examination. (2) isolation and identification of the causative agent. (3) the appearance of specific antibodies and (4) by genome detection using molecular biological techniques.

1. Direct examination

The concentration of rickettsiae in the blood of patients during the acute phase of illness is relatively low. It is virtually impossible to identify the microorganisms with any degree of certainty in leukocytes or monocytes in blood films stained by standard aniline dyes or immunofluorescence techniques. Although fluorescent antibody (FA) assays have been used satisfactority to demonstrate rickettsial organisms in tissues of experimentally infected animals during the early stages of disease, it is futile to examine smears of spleen or other tissues from wild animals suspected of being vertebrate hosts. When preparations of the tissues of various non - infected arthropods are stained with aniline dyes, structures are found which morphologically are indistinguishable from pathogenic rickettsiae. A highly reproducible method for counting rickettsiae has proven useful in quantitating the organism content of seed suspensions, purified corpuscular antigens, and experimental vaccines (35).

2. Rickettsia isolation

Although certain strains of *R. akari* and some strains of *O. tsutsugamushi* will cause overt signs of illness in guinea pigs, the white mouse is the preferred laboratory host. A group of 4-6 mice are injected intraperitoneally with 0.2 - 0.5 ml of the specimen under study. Mice infected with virulent strains of rickettsiae develop signs of illness at the end of the first or during the second week. Pathologic changes are minimal in animals that die between the sixth and ninth day and are limited to slight accumulation of a serofibrinous exudate. If seriously ill animals survive for 14 days or longer the pathology is more striking. Smears prepared from the surface of the spleen and from the parietal peritoneum are stained and examined microscopically for presence of rickettsiae. A 10 to 20 % spleen suspension is used for inoculation of additional mice, or for adaptation to cultivation in eggs. This suspension is injected intraperitoneally into 15-20 additional mice. Animals becoming ill in the second passage are processed and the agent is passaged serially until it is established in the host. If none of the animals become ill by the end of the second week, 4 or 5 of the group are sacrificed, and the spleens harvested, frozen rapidly, and stored at -65° C. These specimens can be used to initiate additional passages if indicated. The surviving animals are challenged 28 - 35 days after inoculation : one – half with 1,000 LD_{50} of *O.tsutsugamushi* and the other half with a comparable dose of *R. akari*. Survival of the mice indicates prior infections (18).

Strains of *R. typhi* may produce minimal changes that are apparent at the time the animal is sacrificed for blind passage on day 14. Some strains of *R. typhi* have killed

mice at the time of primary isolation. It is important to be alert to the possible recovery of these agents in mice.

3. Serological diagnosis

Diagnosis of the causal agent of rickettsial diseases can be accomplished most easily and rapidly by demonstrating a significant increase in specific antibodies in the serum of the patient during the course of acute infection and convalescence. There are several methods developed for serodiagnosis.

Weil-Felix reaction

The Weil-Felix reaction is the first serological procedure developed for rickettsial infection. It depends upon the development of antibodies that agglutinate certain strains of nonmotile *Proteus* organisms. Serial 2-fold dilutions of serum are mixed with equal portions of suspensions of *P. vulgares* OX19 (for murine typhus), OX2 (for spotted fever group or tick typhus), and *P. mirabilis* OXK (for scrub typhus). The test is considered positive if the antibody titer of the second specimen is ≥ 4 folds than that of the first specimen. The classic reactions obtained are summarized in Table 8.

Weil-Felix agglutinins, if they do develop, may appear as early as day 5 or 6 after the onset of disease, but they are usually present by day 12. Peak titers are evident during early convalescence, and the antibody levels then decline rapidly over the next several months. In murine typhus the *Proteus* agglutinins have been identified as IgM immunoglobulin. The test is nonspecific and provides only presumptive serologic evidence for the occurrence of rickettsial infection. The absence of a Weil-

Felix response does not exclude a rickettsial etilogy because patients with rickettsial pox, Q fever, and trench fever infections never develop *Proteus* agglutinins. There have been patients with other typhus group and spotted fever group infections who did not develop OX19 or OX2 agglutinins. Pseudo-outbreaks of scrub typhus have occurred, patients have presented with *Proteus* urinary tract infections, leptospirosis, *Borrelia* infections or severe liver disease due to a variety of causes (18).

Table 8. Interpretation of Weil - Felix test results.

	Magnitude of antibody response with *Proteus spp.* antigens		
Rickettsial diseases	OX19	OX2	OXK
Epidemic typhus (primary)	++++	+	0
Murine typhus	++++	+	0
Rocky mountain spotted fever	++++	+	0
Other tick borne SFG infection	++++	+	0
Rickettsial pox	0	0	0
Scrub typhus	0	0	++++
Q fever	0	0	0
Trench fever	0	0	0

Source : Bennett L, Elisberg BL and Bozeman FM, Diagnostic for viral, rickettsial and chamydial infection Book. 1979: 1061-1109 (18).

Complement fixation test (CF)

Highly purified suspensions of rickettsiae for use as species or type specific CF or agglutinating antigens have been obtained from infected yolk sac tissues by many different methods (36,37,38). No single procedure is applicable to all rickettsiae, and the methods that have been used do not give uniformly satisfactory preparations.

Scrub typhus, CF tests utilizing soluble antigens obtained by ether extraction of suspensions of infected yolk sacs have not been satisfactory for routine diagnostic use. Although positive findings in tests with soluble antigens provide an etiologic diagnosis, failure to demonstrate antibodies in convalescent phase serum does not exclude scrub typhus infection because of the strain specific reactivity displayed by these preparations and the marked antigenic diversity that exists among strains. Partially purified antigens of whole rickettsiae prepared from yolk sacs infected with Karp, Kato, and Gilliam prototype strains have been used for the laboratory diagnosis of acute disease and for seroepidemiological surveys for past and inapparent infections in Japan. However, since these antigens tend to be anticomplementary and to react nonspecifically with human serum, it is difficult to detect low levels of antibody (38). Furthermore, antigens derived from BS-C1 cell cultures infected with the prototype strains have given satisfactory results, but these reagents must be evaluated further before they can be accepted for routine diagnostic use. At the present time, indirect immunofluorescent staining is the preferred method for serological diagnosis of scrub typhus infection.

Indirect fluorescent antibody test (IFA)

The IFA test is currently the most widely used for diagnostic and epidemiological purposes. It can be made group-specific or reasonably species-specific. Additional, IgG, IgM and IgA antibody titers can be determined directly. The IgM response is good in primary infections and is suppressed in reinfections. Currently, the IFA test is most reliable and sensitive test available for the serodiagnosis of scrub typhus (39).

IFA staining employing smears of rickettsial organisms as antigens can be used for the serological diagnosis of rickettsial infections of humans. A micro IFA test was developed which has been shown to be a highly specific and sensitive method (40). The IFA test has its greatest value in the specific diagnosis of *O. tsutsugamushi* infection (41,42). At present, the Karp, the Kato and the Gilliam strains of the scrub typhus group are used as antigens. On the basis of information from a particular study, approximately 60 % of 65 patients experiencing their first scrub typhus infection had antibodies detected with one or more of the antigens as early as day 5 or 6 after onset. The number of positive reactions increased during the succeeding days, and between days 18 and 20, patients had significant levels of antibody. Peak titers were observed during the fourth week and declined slowly over a period of months. Significant titers ($\geq$1 : 40) were present after 6 months, and 73 % of the patients still had positive titers 12-19 months after onset of the disease. In patients who had experienced more than one scrub typhus infection, significant levels of antibodies persisted for as long as 12.5 years after the last illness (17).

Diagnosis depends on the demonstration of an $\geq$ 8-folds increase in antibody titer when 4-folds serial dilutions of the specimen are tested. This should be possible in all

instances if the acute phase serum is drawn on or before the fourth day of disease and the convalescent phase is collected during the fourth week. However, as in the serological diagnosis of other rickettsial diseases, it is possible in many instances to show significant increases in antibody levels when the first specimen is obtained at the end of the first week and the second specimen at the end of the second week. Second and third attacks of scrub typhus occur, and antibodies persisting from a previous infection may be detected at significant levels with one of the antigens early in the course of the current disease. Nevertheless, it should be possible to demonstrate diagnostically significant increases in antibody titer in later specimens with the other antigens.

However, the IFA test, for serodiagnosis is not practical in less than ideal situations. It has not been widely used within the edemic regions due to a general lack of fluorescence microscopes. Also, the results must be read quickly before fluorescence fades (18).

In 1988, Kelly DJ et al. (43) evaluated an indirect immunoperoxidase test (IIP) and compared it with an IFA test and the Weil-Felix OXK test for serodiagnosis of scrub typhus by measuring the rickettsial antigen specific activity of IgG, IgM, and whole globulin. The receiver operating characteristic for each test showed that IIP and IFA tests were more sensitive and specific than the Weil-Felix test using convalescent and acute, as well as paired sera. The IIP test showed no cross reactivity when *O. tstusugamushi* antigen was tested against sera collected from patients living outside the scrub typhus endemic area with diseases other than scrub typhus. The IIP and IFA

tests were comparable in measured response to *O. tsutsugamushi*, *R. typhi*, and TT-118 (spotted fever group) antigens. Thus the IIP test represents a sensitive, specific, reproducible, and practical semiquantitative test for rickettsial diseases diagnosis.

Indirect Immunoperoxidase Technique (IIP)

The IIP technique, in which fluorochrome is replaced by peroxidase, has several advantages over the IFA technique and has been widely applied in diagnostic by Suto T. in 1980 (44).

In 1982, Yamamoto S. and Minamishima Y. (45) investigated the IIP technique as an alternative to the IFA technique for detecting and measuring antibodies to *O. tsutsugamushi* as follows. The IIP technique was assessed for the serodiagnosis of tsutsugamushi fever. The antigens were peritoneal smears prepared from mice infected intraperitoneally with the Karp, the Kato, and the Gilliam strains of *O. tsutsugamushi* by the direct immunoperoxidase technique. Sera from all of the patients (49 samples from 30 patients) were positive for *O. tsutsugamushi* antibody. The antibody titers of immunoglobulin (IgG and IgM) were determined by the IIP technique. Thus, the IIP technique was useful for quantifying both IgG and IgM antibodies to *O. tsutsugamushi*. The IIP technique shares the same advantages over the complement fixation test with that of the IFA technique in that any rickettsial strain can be used as the antigen, and either IgG or IgM antibodies can be titrated individually. Additionally, the IIP technique has the following advantages over the IFA technique in that: (1) it gives permanent preparations for reexamination, (2) it does not require a fluorescence

microscope, (3) it makes it possible to observe all cells, infected and uninfected, (4) it is relatively easy to determine antibody end points, and (5) the reagent (the labeled antibody) is also applicable for use in electron microscopy and ELISA. However, the fact remains that in the IIP is inferior to the ELISA, by which antibodies can be objectively quantified. One limitation common to the IIP, IFA, and ELISA techniques is the unavailability of the rickettsial antigens from commercial sources.

In 1990, Kelly DJ et al.(46) evaluated the multi-laboratory use of a scrub typhus diagnostic kit. The IIP technique, configured into a test kit, was provided to technicians who were trained in its use. They used the kit during a 2 years field trial in their respective clinical hospital laboratories throughout Malaysia. In an evaluation using 1,722 consecutive sera tested in those laboratories, the kit was found to have a median sensitivity for IgG detection of 0.85 (range 0.33-0.95), a median specificity of 0.64 (range 0.88-1.00), reproducibility of 0.86, and efficiency of 0.92 when compared to the reference laboratory. In a proficiency survey in with 10 laboratories received 3 coded test samples, all but 2 laboratories had results within 1 dilution of the reference laboratory in quantitating specific IgG, whereas 7 laboratories were within 1 dilution in quantitating IgM. The shelf life of the kit was at least 1 year at 4° C.

Agglutination tests

The rickettsial agglutination test has not been adopted for routine serologic diagnosis in clinical laboratories.

A variety of methods of performing agglutination tests has been developed (47, 48,49). In this test, the presence of agglutination after incubation is evaluated by microscopic examination of the antigen serum dilution mixture on the slide after it has dried and has been stained. In this procedure it is often difficult to differentiate specific agglutination from artifacts produced by drying. The presence of agglutination is determined by observing the state of the antigen when the pellet is resuspended.

The agglutination test is more sensitive than the CF test and somewhat less so than the micro IF test. Rickettsial agglutinins generally can be detected earlier in the course of disease and persist longer after convalescence than do CF antibodies. In primary epidemic typhus, the agglutinins demonstrated from day 11 through day 58 were identified as IgM immunoglobulin. Similarly, in murine typhus the agglutinating activity was associated with the same class of immunoglobulin.

Indirect hemagglutination test. (IHA)

Antibodies are first detected during the second week of illness, attain peak titers during the third week, and disappear in 3-6 months. In murine typhus the erythrocyte-sensitizing substance (ESS) agglutinin is associated with the IgM immunoglobulin. Comparison of the relative sensitivity and specificity of the IHA test with the CF and Weil Felix tests for the diagnosis of rocky mountain spotted fever and typhus group infections, as well as with the CF, micro-IFA, and micro agglutination tests for the diagnosis of rocky mountain spotted fever have been carried out (50). Positive results can indicate only for the group identity of the infecting organism. Although the test is

considered more sensitive, economical, technically simpler, and as reliable as CF, the procedure has not been utilized as a diagnostic tool.

Neutralization test. (NT)

This test, in which antibody is added to a suspension of organisms and the mixture injected into susceptible animals, has not generally given as clear cut results with rickettsiae as has been obtained with certain viral agents. The procedure has been used as an extremely sensitive means of detecting serological evidence in epidemiological surveys for antigenic characterization of strains of the scrub typhus group. The early experience indicated that the technique would not be suitable for diagnostic purposes (51,52). Typhus immune serum, with or without complement, has no direct rickettsiocidal action on *R. typhi* and does not prevent infection of chicken embryo cells in culture and subsequent rickettsial growth.

Other serologic procedures

Microplate Enzyme linked Immunosorbent Assay (Microplate ELISA)

A microtiter enzyme linked immunosorbent assay (Microplate ELISA) has been developed for the titration of antibodies against scrub typhus in human and animal sera (53). The scrub typhus ELISA antigens were obtained from the purified viable rickettsiae by French pressure cell disruption and addition of 0.2 % formalin to the soluble extract. Antisera prepared in rabbits against the prototype Karp, Kato and Gilliam strains of scrub typhus were used to standardize the ELISA and to compare its

sensitivity and specificity to that of the IFA. The ELISA titers were measured as the greatest serum dilution showing an optical density of 0.25 as compare with control well or by the optical density achieved at a fixed serum dilution. The IFA and ELISA end point titers were quite similar, and all three measures of titer had comparable specificity for the strain of scrub typhus. No cross reactions between the typhus and scrub typhus sera were observed by ELISA. Both the immunoglobulin M (IgM) and IgG antibody titers of 12 sequential sera from four patients with scrub typhus were obtained by IFA and ELISA. The IFA and ELISA end point titers for IgM and IgG had corrections coefficients of 0.91 and 0.97 respectively, whereas the ELISA optical density values at a serum dilution of 1:1000 had slightly lower correlation with the IFA titers (0.80 and 0.94). Early rising IgM titers followed by rising IgG titers were demonstrated by ELISA in three patients with primary scrub typhus infections, whereas the IgG response predominated in a patient with a reinfection. It is concluded that the ELISA for scrub typhus is a very satisfactory alternative to the IFA test.

New Paper Enzyme link Immunosorbent Technique (New paper ELISA)

In 1980, Crum JW et al.(54) developed a new paper enzyme linked immunosorbent assay (New paper ELISA) for the screening and titration of human serum antibodies against the scrub typhus rikettsia, *O. tsutsugamushi*. The objective was to provide a relatively simple method for antibody screening which required neither sophisticated laboratory equipment nor a high degree of technical skill. The technique develops an enzyme product from filter paper saturated with a 5-amino salicylic acid substrate, enzymatically reacted with a commercially available anti-human

immunoglobulin G peroxidase conjugate. The product of the enzymatic reaction can be interpreted visually. Comparison of 351 human sera tested by the IFA and paper enzyme linked immunosorbent assays against a three antigen pool of the Karp, Kato, and Gilliam strains of *O. tsutsugamushi* demonstrated an agreement of 96 %. The sensitivity of the paper enzyme linked immunosorbent assay as compared to IFA was 98.2 %, and the specificity was 94.4 % .

Pasive Haemagglutination Assay (PHA) using recombinant 56 kilodalton polypeptide

In 1993, Kim IS et al. (55) studied rapid diagnosis of scrub typhus by a passive haemagglutination assay using recombinant 56 kilodalton (kDa) polypeptide. The genes encoding the 56 kDa polypeptides were amplified by polymerase chain reaction from the genomic DNA of three strains of *Orientia tsutsugamushi*: Gilliam, Karp, and Boryong. The amplified products were cloned into expression vector pIH821, and the recombinant antigens were expressed in *Escherichia coli (E. Coli)* as fusion proteins with maltose binding protein. The recombinant 56 Kda polypeptides were purified by affinity chromatography for the sensitization of sheep erythrocytes. The recombinant 56 Kda polypeptides were evaluated with 89 serum specimens from healthy blood donors, 94 serum specimens from scrub typhus patients and 31 serum specimens from patients with other febrile diseases by a passive hemagglutination assay (PHA). Among the scrub typhus patients diagnosed by IFA testing, antibodies to *O. tsutsugamushi* were detected in 93 patients (99 %). One serum specimen from a healthy person gave

a false positive reaction by this method. The recombinant PHA showed no cross reactions with sera obtained from other febrile patients with diseases such as murine typhus, haemorrhagic fever with renal syndrome or leptospirosis. This recombinant PHA could be substituted for the conventional IFA test and the IIP test.

IgM dot immunobinding assay (IgM DIA)

In 1995, Koay AS et al.(56) developed and evaluated an IgM dot immunobinding assay (IgM DIA) for rapid serodiagnosis of scrub typhus infection. The whole cell antigens of Karp, Kato and Gilliam strains of *O. tsutsugamushi* were immobilized on to nitrocellulose paper and reacted with patients'sera. The presence of IgM *O. tsutsugamushi* specific antibody in the patient sera could be detected by the observation of a visible brown dot on the nitrocellulose paper. The IgM-DIA has a sensitivity of 90.4 % and specificity of 81.4 % as compared to the IIP test. The IgM-DIA is rapid, simple, cost-effective and does not require microscope or incubator. It is recommended as a rapid screening test for the diagnosis of scrub typhus infection in the field or rural area within the hyper-endemic regions.

Also in 1995, Weddle JR et al. (57) studied an effectiveness of a dot blot immunoassay of anti *O. tsutsugamushi* antibodies for serological diagnosis of scrub typhus (57). Using a panel of 100 sera from patients with various rickettsial and non-rickettsial infection, they observed that the IFA was 99 % specific and the dipstick assay was 98 % specific. In tests of 91 sera (30 negative and 61 positive for scrub typhus antibodies from a study of febrile patients in Malaysia, using the standard of an IFA titer < 1:64 as negative, an IFA titer > 1:128 as positive, and an IFA titer = 1:64 as

either positive or negative as supported by clinical records) Dipsticks were 83 % specific and 90 % sensitive. The quantitative correlation of the dipsticks to IFA titers was confirmed by significant differences in geometric means of inverse IFA titers corresponding to the number of positive dipstick spots (no dots = 8.5, one dot = 43.3, two dots = 206.7, and three dots = 676.9). The assay would enable physicians and public health workers who deal with patients to quickly diagnose and appropriately treat most cases of the disease, especially in areas of high prevalence where the proportion of false positive results to true positive results would be low.

Dot blot enzyme linked immunosorbent assay (dot ELISA)

In 1995, Silpapojakul K et al. (58) studied the dot-blot enzyme-linked immunosorbent assay (dot ELISA) and compared it with the latex agglutination (LA) and the Weil Felix OX-19 test for the diagnosis of murine typhus using the IFA test as the gold standard. With a panel of 74 positive and 47 negative sera, the dot ELISA was 98 % specific and 74 % sensitive at a cut off value of the second dot. With acute sera, latex agglutination was 100 % specific and 74 % sensitive at a cut off titer of $\geq$ 1 : 64. Both tests were more sensitive than the OX-19 test, which was 98 % specific and 56% sensitive at a titer of $\geq$1:320. Both dot ELISA and latex agglutination were comparable and the results were available within one hour of testing. The rapidity, ease of performance and minimal requirement for electrical instruments made these two tests suitable for the diagnosis of murine typhus in countries where sophisticated laboratory facilities are lacking.

In 1997, Suwanabun N et al. (59) improved ELISA and showed that the single serum dilution, ELISA was as effective as the titration in determining presence of specific antibodies. The *O. tsutsugamushi* ELISA is a rapid and stable technique for accurately testing the large numbers of sera often obtained in seroepidemiological investigations.

Again in 1997, Pradutkanchana J et al. (60) compared and evaluated four serodiagnosis tests. The commercial dot-bot enzyme-linked immunosorbent assay Dip-S-Ticks™ dipstick test were compared with the IIP and Weil-Felix (WF) tests for the diagnosis of scrub typhus in Thailand using the IFA as the reference standard. With a panel of 117 positive and 75 negative sera, the dipstick test was 94% sensitive and 98.7% specific at a cut-off value of one or more positive dots. The IIP was 90.6% sensitive and 100% specific at a cut-off value of titer of 1:400 and was more sensitive than the IFA with acute sera (79.6% and 68.5% at a titer of $\geq$1:400). All 3 were superior to the WF test which lacked sensitivity. The dipstick assay was easy to perform, did not require sophisticated electrical equipment and the results were available within one hour. It is therefore suitable for use in rural Thailand, where scrub typhus is common.

4. Genome detection

Polymerase chain reaction (PCR)

In 1996, Tay et al. (61) employed the nested PCR as a rapid diagnostic system for scrub typhus and applied the technique to clinical samples from Malaysian aborigines.

Whole blood from 24 patients suspected having scrub typhus infections was tested by nested PCR, and sera were evaluated by the indirect immunoperoxidase test. Antibody responses towards *O. tsutsugamushi* were observed in 17 patients with the majority having high titers of IgG antibodies. Seven patients were seronegative. The nested PCR amplified *O.tsutsugamushi* DNA from 6 patients, of which 2 were serologically negative and 4 had high titers of IgG antibodies. Second samples collected seven days after treatment were negative by PCR testing. Nested PCR is highly sensitive and specific and may be used to provide rapid confirmation of scrub typhus cases in endemic regions.

Again in 1996, Tselentis Y et al. (62) identified murine typhus rickettsia in rats and their fleas in an endemic area of Greece by PCR and restriction fragment length polymorphism. Forty nine cases of murine typhus were diagnosed in recent years in residents of several communities around the city of Chalkis, the capital of the Prefecture of Evia. (Euboea). Evia is an island connected to central mainland Greece by a bridge. To investigate the endemicity of murine typhus in this area, 226 fleas (*Xenopsylla cheopis*) and blood samples were collected from 53 rats (*Rattus norvegicus*) trapped in this area. PCR followed by restriction fragment length polymorphism analysis (PCR-RFLP) was used to detect and identify *R. typhi*, the etiologic agent murine typhus, in the rat blood samples (buffy coat cells) as well as in their fleas. An IFA assay was performed to detect antibodies against *R. typhi* in rat serum samples. The presence of *R. typhi* in both fleas and rat blood samples was demonstrated. The frequency of infection for *X. cheopis* was 4 % , while 18 % of the

rats had infected buffy coat cells infected, and 92 % of the rat sera tested by IFA were positive for anti- *R. typhi* antibodies. This work in the first successful application of PCR-RFLP in a field study of naturally infected rats and their fleas in Europe.

Also in 1996, Horinouchi H et al. (63) combined the nested PCR and restriction fragment length polymorphism (PCR-RFLP) for genotypic identification of *O. tsutsugamushi*. Four primers were selected from the DNA sequence of the gene encoding a 56-KDa serotype-specific antigen of the Karp strain. Nested PCR produced rickettsia specific products of approximately 0.6 kb in the amplification of DNA prepared from three reference strains (Gillam, Karp and Kato) and two prototype strains (Irie and Hirano) prevalent in the Miyazaki prefecture of Japan. When the nested PCR products obtained from these five strains were digested with *Hha I*, profiles specific to each strain were generated. Fourteen of 17 DNA samples of peripheral blood mononuclear cells from patients with scrub typhus tested positive in the nested PCR, providing a rickettsia-specific band. The serotype of infected rickettsia isolated from 10 patients were identified as Irie and those of 4 patients identified as Hirano by indirect immunofluorescence methods. The fragment profiles of the PCR products of these 14 patients after digestion with *Hha I* corresponded closely with those serotypes. However, the PCR products from two of four samples, which were similar to Hirano strain by a serologic method and by the pattern of digestion with *Hinf I* and *Alu I*. These results may suggest that genetic variation exists within serotypes. Genotypic identification of *O. tsutsugamushi* by PCR-RFLP using three restriction enzymes is apparently useful.

Treatment

The response following administration of chloramphenicol or one of the tetracycline during the early stages of the illness is somewhat more rapid in scrub typhus than in other rickettsial infections. Patients are rendered afebrile and almost asymptomatic within 24 to 48 hours after initiation of therapy. In the usual case, an oral daily dose of chloramphenicol is calculated on the basis of 50 mg/kg of body weight, and of tetracycline 25 mg/kg. When chloramphenicol is given, therapy is begun with a loading dose equivalent to the daily dose. Due to restrictive alimentary absorption of single large doses of the tetracyclines, a primary loading dose is not indicated. Furthermore, with the tetracyclines, treatment is more effective if individual doses are given at intervals of 3 to 4 hours than when larger doses are administered on a 6 to 8 hour schedule. Antibiotic therapy is continued until the patient has been afebrile for at least 24 hours. Usually 5 gm of chloramphenicol given over a period of 24 hours (a loading dose of 3 gm, followed by 0.5 gm, every 6 hours is adequate). In severely ill patients who do not receive specific therapy until late in the disease, relapses respond promptly course of 3 to 5 gm of antibiotic, and no instances of rickettsiae developing drug resistance have been recorded. Moreover, relapses can be prevented in those patients in whom it might be expected, by administering a single 3 gm. Dose on the 6^{th} day after termination of the original course of therapy (3).

In 1995, Song JH et al. (64) to assess the clinical efficacy of short-course doxycycline in the treatment of scrub typhus, they compared conventional 7 days tetracycline therapy with 3 days doxycycline therapy in 116 patients. Patients were

randomized to receive either tetracycline (500 mg four times daily; n=50) or doxycycline (100mg twice daily; n = 66) and were followed for 4 weeks after the completion of treatment. The cure rate was 100% in the tetracycline group and 93.9% in the doxycycline group (p-value > 0.05). The two group did not differ significantly in terms of the interval required for defervescence or for the alleviation of symptoms. There were no relapses in either group. Those data suggest that 3 days doxycycline therapy is as effective as conventional 7 days tetracycline therapy for the cure of scrub typhus and prevention of relapses.

Those patients whose disease has been successfully treated by therapy during the first week after onset enjoy a rapid convalescence and may return to secondary occupations within 10 days to 2 weeks after becoming afebrile. Those with a more protracted illness should be permitted a longer convalescence.

For murine typhus, the recommended antibiotics for the treatment are tetracycline and chloramphenicol. Aminoglycosides and sulfonamides are ineffective. Therapy should be continued for 14 days even though patients usually become afebrile much sooner. These antibiotics are rickettsiostatic; and they only prevent organisms from multiplying within infected cells while the patient' immune system combats the infection. Therefore, if therapy is discontinued prematurely a relapse may occur. Newer antibiotics, such as ciprofloxacin, also may be effective for the treatment of this infection (14).

In 1996, Watt G et al. (65) collected the reports from local physicians in Chiang Rai, in the northern part of Thailand, and found that patients with scrub typhus responded badly to appropriate antibiotic therapy which prompted a prospective clinical evaluation and antibiotic susceptibility testing of human rickettsial isolates. The clinical response to doxycycline treatment in patients with early, mild scrub typhus in northern Thailand was compared with the results of treatment in Amphoe MaeSod, western Thailand. The result showed that Chloramphenical resistant and doxycycline resistant strains of *O. tsutsugamushi* occur in Chaing Rai. This is the first evidence of naturally occurring antimicrobial resistance in the genus *Rickettsia.*

Methods of control

In hyper-endemic areas where risk of infection is high, the following prophylactic measures have been used; intentional infection with live rickettsiae combined with antibiotic therapy thus producing the same degree of immunity as would result from a natural infection but preventing development of clinical symptoms, and chemoprophylaxis with chloramphenicol in dose of 3 to 4 gm every 4 to 7 days and continued for 4 to 6 weeks after the last exposure. For personal prophylaxis, miticides and repellents have been used successfully although they are not considered suitable for civilian populations because they are expensive and difficult to apply. Dimethyl phthalate, dibutyl phthalate, and benzyl benzoate applied to clothing gave a high degree of protection during World War II military operations in highly endemic areas. Exposed skin areas were also treated with repellents containing phthalate or diethyltoluamide.

To clear areas of scrub typhus requires a variety of control measures aimed at the life cycle and habits of the mite vectors and their host animals. These measures consist of (i) application of insecticides to the ground, (ii) rodent control, and (iii) removal of ground vegetation and surface organic matter. Chlorinated hydrocarbons have been shown to be effective residual chemical in killing chiggers on the ground. The WHO Expert Committee on Insecticides recommends the use of dieldrin which when applied as a spray, mist or fog produces a more than 90 percent reduction of chiggers for at least 2 years. Where toxicity to wildlife is to be avoided, organophosphorous compounds or carbamates may be used, although these require reapplication every 2 to 3 weeks.

Along with insecticides, antirodent measures through application of rodenticides, periodic trapping and hunting, and thorough control of camp refuse have been suggested.

Effective area control has also been achieved by removal and burning of all ground cover followed by scraping and plowing the top soil. This will render the areas unsuitable for mites and their rodent hosts. In tropical areas, the ground will dry sufficiently in 2 to 3 weeks to kill the chigger population (66).

Prevention and control of murine typhus measures directed against the vector fleas and their host animals include (i) application of residual insecticides (10 % DDT or other compounds) to runways, burrows and rat harborages, and (ii) control of rat populations by (a) environmental sanitation to eliminate food and harborage, (b)

effective rat proofing of buildings, and (c) efficient killing of rats by means of rodenticides. Application of insecticides should precede any rat-killing programs, otherwise the abundance of fleas that have left their dead hosts may lead to additional cases of murine typhus (67).

Vaccination

A vaccine against scrub typhus has not yet been successful because formalin or other inactivating agents reduce immunogenicity and in part because strains of scrub typhus are antigenically quite heterogeneous. Eisenberg and Osterman found that inactivation by gamma irradiation preserves the immunogenicity of the rickettsiae better than formalin treatment. A judicious combination of strains confers resonably good protection to mice challenged with a variety a strains (22).

For murine typhus, the immunization of large populations is impractical in view of the low attack rate, mildness of the disease and availability of effective antibiotics. Also, effective vaccines made from killed *R. typhi* for high-risk individuals are no longer commercially available. They afford protection in laboratory tests, but it is debatable whether vaccination of man is advisable as a control measure, except for persons who are frequently exposed to the infection (14).

Epidemiology and related factors of scrub typhus.

Scrub typhus is a significant and widespread disease in Asia. It is due to *O. tsutsugamushi*, also known as *R. tsutsugamushi* or *R. orientalis*, of which at least six distinct serological strains (Gilliam, Karp, Kato, Shimokoshi, Kawasaki, Kuroki) can be

detected by immunoperoxidase reaction. It occurs in Japan (some 900 cases annually), South Korea, Taiwan, the Philippines, Southern China (including Hong Kong and Hainan). East and West Malaysia, Thailand, Cambodia, Vietnam, Laos, Myanmar, Sri Lanka, India, Nepal, northern Pakistan, the islands of the Indian Ocean, Indonesia, Papua New Guinea and its neighboring islands, Queensland and Northern New South Wales (Figure 2).

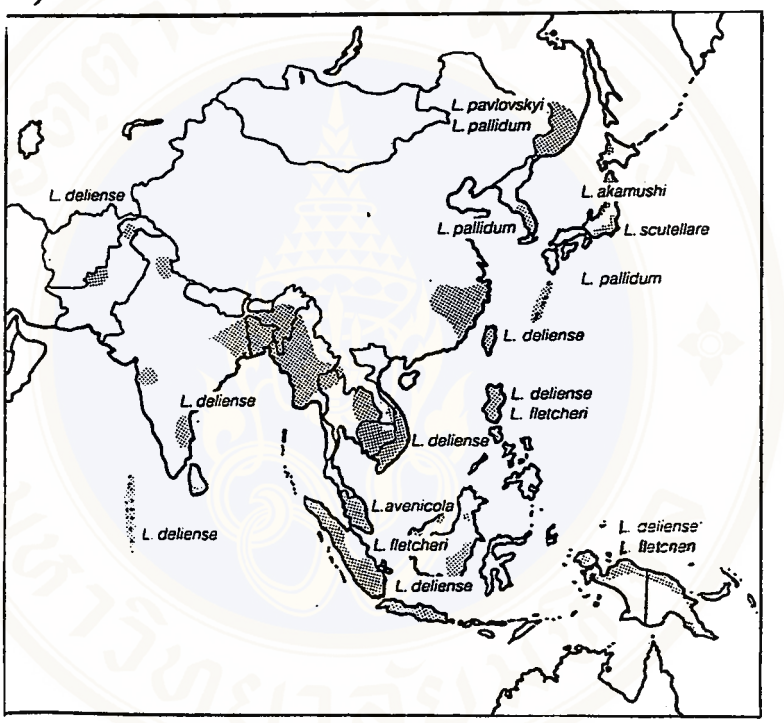


Figure 2. Geographical distribution of scrub typhus, mite-borne (*O. tsutsugamushi*). The black color in the figure represented the areas of epidemic.

Source: Reproduced from the Department of Entomology, London School of Hygiene and Tropical Medicine (2).

The vector to humans is the larva of a number of trombiculid mites by which transovarial transmission maintains the infection in nature. There is also a wild rodent reservoir, and the infection characteristically occurs in discrete foci (mite island) where

infected mites live on the jungle grass *Imperata cylindrica*, known as lalang (Malaysia and Indonesia), *illuk* (Philippines) or *kunai* (Papua New Guinea and Australia), which grows only where primary jungle has been cleared for cultivation or to build villages. Human cases occur when workers in oil palm and rubber estates, and policemen and soldiers traverse this habitat, brushing against the sharp stiff blades of waist-high *Imperata* grass, allowing the larval mites access. It is an important military disease, many thousands of case having occurred in the Far East theater in the Second Word War (2).

In 1975, Robinson DM et al. (68) reported that among resident of west Malaysia, the prevalence rate were significantly higher in people who worked in forest area and significantly lower in people with urban occupation. The geometric mean titers showed a progressive increase from the younger to the older age group, which was probably a reinfection of higher antibody titers in older people following repeat infection.

In 1983, Brown WG et al. (69) studied 1,629 febrile patients from a rural area of Malaysia, and made a laboratory diagnosis in 1,025 (62.9%) cases. Scrub typhus was the most frequent diagnosis (19.3% of all illness), followed by typhoid and paratyphoid (7.4%), flavivirus infection (7%), leptospirosis (6.8%), and malaria (6.2%). The high prevalence of scrub typhus in oil palm laborers (46.8% of all febrile illnesses in that group) were confirmed. In rural Malaysia, therapy with chloramphenicol or a tetracycline would be appropriate for undiagnosed patients in whom malaria has

been excluded. Failure to respond to tetracycline within 48 hours would usually suggest a diagnosis of typhoid, and indicate the need for a change in therapy.

The first case of scrub typhus was reported in a Thai male, in Banpong, Ratchaburi Province, 78 Kilometers west of Bangkok, Thailand in 1952. Serological test found that, the Wiel-felix test gave positive result against *Proteus* OXK with a titer of 1: 190 in the second week and 1: 250 in the third week (70).

The trombiculid mite vectors were found in the form of *Trombicular deliensis* is the main vector of scrub typhus among the high-grass areas of Nakhon Pathom, and Ratchaburi provinces. The major host of the vectors were *Rattus rattus* (71).

In 1955, the first isolation of scrub typhus in Thailand was reported from Ban Pong, Ratchaburi province by Traub R et al. (of the Walter Reed Army Institute of Research) (72).

After field training in the jungle of Ubonrajatanee Province for 15 days in September 1956, 247 soldiers of the 6th Regiment developed high fever and were admitted into military hospital. Blood examination showed that 115 cases were positive for malaria infection. Additionally, 11 cases gave positive reaction for Weil-Felix OXK agglutination test and were diagnosed as scrub typhus (7).

In 1964, Trishnananda M. et al. (73) reported that an investigation of scrub typhus in Thailand was carried out in Ratchaburi and Nakhon Pathom Province, where the first clinical case was reported 12 previous. *O. tsutsugamushi* was successfully isolated from both field rodents and patients. Three species of rodents namely, *Bandicota bengalensis*, *B. indica* and *R. rattus* were the main reservoir hosts. This suggested that the disease probably persisted in the area.

During 1963 and 1964, Sangkasuvana V et al. (74) reported that four strains of presumed scrub typhus rickettsiae were also recovered from humans with a relatively mild febrile disease in Chiang Mai area. The serological tested of 194 human sera from Thailand by means of the fluorescent antibody technique found that 13 % were positive for scrub typhus at a titer of 1:40 level and 22 % were positive at a titer of 1:10. Following studies during 1964 and 1965 in Chiang Mai, Udon Thani, Nong Khai, Nakhon Phanom and Ubon Ratchathani provinces, found that *Leptotrombidium* chiggers were suspected to be vectors of scrub typhus in Thailand. The rate of isolation of scrub typhus rickettsiae was correlated with the predominant of the species *Leptrotrombidium deliensis*, except in one area, Khao-Yai, where they were mostly identified as *L. akamushi*.

In June 1965, a classical outbreak of scrub typhus at Pak Thong Chai in Nakhon Ratchasima province was investigated in 185 Thai soldiers who carried out a training exercise in forest and grass areas (75). Subsequently, after an incubation period of 7-12 days, 41(20.8 %) of them were admitted to hospital presenting with fever and

headache. Eschar with regional adenopathy was seen in about one half of the cases. All patients responded promptly to chloramphenicol. *O. tsutsugamushi* was isolated from 10 of the blood specimens which were a vailable for study. Whilst only one of thirty-four sera tested had a significant titer of OXK agglutinins (1:160), 33 demonstrated specific rickettsial antibody in an immunofluorescent test.

Isolation of *O. tsutsugamushi* was attempted from blood obtained from 62 patients with pyrexia of unknown origin (PUO), the patients had no obvious clinical origin for their fever, generally suspected of having malaria or typhoid. These attempts at isolation were positive for scrub typhus in 6 of 35 PUO in-patients from Chiang Mai, Samut Sakhon and Nakhon Ratchasima hospitals and in 4 of 27 PUO out patients seen in Chiang Rai and Arran Yaprathet (76).

In1967, Sankasuwan V et al. (77) reported that *L. deliense* chigger population was highest during the rainy season which corresponds to the prevalence of scrub typhus infection in trapped animals. At Nan, Loei and Chiang Rai where *L. scutellaris* were present, an attempt to isolate scrub typhus rickettsiae from this chigger and the small mammals trapped in the same areas were unsuccessful. This chigger was known to be a main vector in Japan.

In 1982, a sero-epidemiological survey of a rural Thai village found a 77 % prevalence of antibody against *O. tsutsugamushi* in adults. Acquisition of antibody occurred very early in life, especially in females, but the prevalence of the antibody in the adult population showed no statistically significant sexual distinction. Antibody

against all three prototype strains was present in Thailand but antibody titers did not vary by strain type or the age of the individual (78).

Between June 1977 to August 1978, 2,251 an unengorged chiggers collected in Thailand by the black plate method, were identified and sent to USAMRU-M in Kuala Lumpur for rickettsia isolation (79). These specimens were collected in the following provinces: Chiang Mai, Kanchanaburi, Nakhon Ratchasima, Prachin Buri, Surin and Ubon Ratchatani. A total of 13 species of chiggers were isolated and *O. tsutsugamushi* strains found in them were antigenically characterized. Monotypic infections were observed in 76.7 % of them. The Karp strain was the most predominant.

The further seroepidemological survey of *O. tsutsugamushi* antibody was performed in Mae Chan district, Chiang Rai province in 1984. It was reported that IgG positive antibody was 59 %, while IgM positive antibody was 24 % by IFA method (cut-off titer 1:10) (80).

Leelarasamee A. and Aswapokee P. (81) reported two cases of scrub typhus in 1984. Both were male and employed to serve medication at hospitals in Bangkok. Both cases were initially diagnosed as enteric fever and treated with co-trimoxazole. A correct diagnosis was made when eschar was found in each patient; one on the scortal sac and the other on the back. They responded promptly to intravenous chloramphenical (3gm daily).

In 1972, Cardigan Fc Jr et al. (82) studied the effect of habitat on the prevalence of human scrub typhus in Malaysia. The result showed that 18% of persons under the age of 20 and 48% of adults had antibodies to *O. tsutsugamushi* whereas, in *kampong* area none of those under 20 and only 8% of those over 20 had antibody. In deep jungle, on the other hand, 56% of those under 20 and 73% of those over 20 had antibody. Arranged in order of *Kampong*, fringe and deep jungle, the percentages in children were 0,18 and 56 respectively. For adults the percentages were 8,48 and 56 respectively. One of the interesting findings of the study was the close agreement of prevalence ratios in groups within each type of habitat compared to their habitat, despite the fact that the groups were located in widely separated areas of west Malaysia. Not only were the ratios in close agreement but there was no overlap of the data from one habitat to another. When arranged by age groups, the under 20 year group ratios were 19, 10 and 16% for the fringe areas, whereas in *Kampong* areas they were both zero and in deep jungle areas 44, 81 and 58%. For adults (over 20 years), the same close agreement within habitat was seen while wide differences were revealed between habitat. What is unknown in this type of study is how long ago the individuals had scrub typhus infection or the frequency of subsequent infections. Both of these influence serological titer.

In 1979, Olson JG. and Bourgeois AL. (83) found that long term changes in risk of *O. tsutsugamushi* infection among civilian residents of the Pescadores Islands of Taiwan were associated with changing social conditions. Age specific incidence rates of scrub typhus in the Pescadores before 1940 were highest among children under 5 years

of age. Rickettsiae can be demonstrated in vectors, wild animals continue to be infected and scrub typhus occur in military personnel. A serologic survey of antibody to *O. tsutsugamushi* was conducted during 1975 and 1977 and fail to show evidence of previous infection among children. Two events appear to be associated with the decreased incidence of rickettsial infection in the young: increase urbanization and increased enrollment in schools. Both changes accompanied socio-economic development which took place in the islands during the past 50 years. Prevalence of antibody to *O. tsutsugamushi* continues to be equal in the sexes in fields and farms where vector are numerous. The absence of apparent morbidity due to scrub typhus among the civilian populace was attributed to the mild nature of the disease caused by Pescardores strains, misdiagnosis and a lack of obligatory reporting.

In 1987, Sirisanthana V. and Poneprasert B.(84) studied scrub typhus in children at Chiang Mai University Hospital and found that there were 25 pediatric patients with the diagnosis of scrub typhus. All were patients who came in with prolonged fever that had defervesced within 72 hours after tetracycline or chloramphenicol therapy. Their age ranged from 1.5 to 14 years (mean 9.9 years). The male to female ratio was 2.6:1. There were no case between January and April. An average of 9.4 days elapsed between onset of fever and hospitalization. The average peak temperature during the first 24 hours of admission was 39.7°C. Headache, nausea and vomiting were present in 52-60% of the patients. About one fourth had history of upper respiratory tract infection and non productive cough which subsided by the time of admission. There

were 5 cases with abdominal pain and tenderness. Four cases had history of convulsion before admission.

In1987, Ming-yuan F et al. (4) found that since 1949, information on rickettsial disease in the People's Republic of China has been virtually non existent in the west. This was the first comprehensive review of the ecology and epidemiology of Chinese rickettsial diseases to be published outside the People's Republic. At least five rickettsiosis exist in China: scrub typhus, murine typhus, epidemic typhus, Q fever, and one or more spotted fever-group (SFG) rickettsioses. Although epidemic typhus has been controlled and scrub typhus has abated in many areas, murine typhus, Q fever, and SFG rickettsiosis are important public health problems. Serologic surveys indicate a high prevalence of antibodies to *Coxiella burnetii* and *O. tsutsugamushi* in arthropods, and animals. Doxycycline has emerged as the best treatment for murine typhus, epidemic typhus, and scrub typhus. China offers both opportunities and challenges for the investigation and alleviation of the problems of rickettsial diseases.

In 1991, Sillapapojakul K et al. (85) conducted seroservey between 1985 and 1987. Serum sample from 320 children with obscure fever for 1 week or more were investigated using the weil-felix test. Twenty-eight (8.7%) had a confirmed diagnosis of typhus. Clinical records were available for study in 15 of 18 cases of scrub typhus and in all 10 patients with murine typhus. The mean age and gender ratio of patients with both rickettsioses were similar (8.7 years versus 7 years and 10 males and 5 females versus 7 males and 3 females, respectively). However, only one patient with scrub

typhus resided in the urban area compared with 7 of 10 with murine typhus. Scrub typhus was absent during the summer months (March through May). A history of mite bite was obtained in only one patient. Another child with murine typhus had been seen playing with a dead rat. The others did not recall a history of rat contact or flea bite even though rats are abundant in Hat-Yai.

In 1991, Nimlamal S. (86) reported a case scrub typhus infection at Yala hospital The patient presented, with acute febrile illness. On physical examination, he had an eschar and the result from laboratory investigation (Weil-felix and IIP) showed that he had *O. tsutsugamushi* infection and developed pneumonia, acute renal failure and severe hepatic dysfunction. However, he responded to doxycycline and other symptomatic and supportive treatment and he had complete recovery after 27 days of hospitalization.

In 1992, Dupon M et al. (87) reported a case of scrub typhus due to *O. tsutsugamushi*. This imported rickettsial disease was contracted by a 30 years old woman while traveling in Thailand, and was transmitted by an infected mite bite. Diagnosis was confirmed by specific serology and resolution was obtained by tetracycline therapy.

Recently in 1994, Watt G and Strickman D. (88) found that scrub typhus is not one of the more commonly encountered diseases in travelers returning from Asia, but it deserves more consideration in view of its severity and the abailability of specific

chemotherapy and chemoprophylaxis. They describe a case of scrub typhus that was associated with coma and multiorgan failure in a traveler returning to the United States from Thailand. The diagnosis was made only retrospectively despite a travel history and clinical signs that suggested infection with *O. tsutsugamushi*. No specific therapy was given, and marked neurological impairment persisted 6 months after the beginning of the illness. An increased awareness of scrub typhus is a prerequisite for recommending prophylaxis and instituting prompt therapy.

Kawamori F et al. (89) isolated in 1992. 59 strains of *O. tsutsugamushi* were isolated; from patients (24 isolated), *Apodemus speciosus* mice (30 isolated), unfed larvae of *Leptotrombiduim scutellare* (2 isolates) and *Leptotrombidium pallidum* (3 isolated). All were found in the Gotenba-Oyama District, Shizuoka Prefecture, Japan. All these isolates were classified into the three serotypes Karp, Kawasaki, and Kuroki based on reactivity with strain-specific monoclonal antibodies. Kawasaki and Karp-type rickettsiae were isolated from *L. scutellare* and *L.pallidum*, respectively ; From these results, they conclude that Kawasaki-type rickettsiae transmitted by *L. scutellare* and Karp-type ones are transmitted by *L. pallidum*. Kawasaki type rickettsial infections were prevalent in early autumn, and Karp-type infections showed a peak of occurrence in the late autumn, reflecting the seasonal fluctuations of *L. scutellare* and *L. pallidum*. Isolates of Kuroki-type rickettsiae were obtained only from four patients in October and November, and the relationship between this type of rickettsia and it's vector species could not be fully defined.

Tanskul P et al. (90) found *O. tsutsugamushi* in chiggers associated with rodents in central Thailand. The Chiggers were collected from rodents trapped at two military bases located 10 Km apart in central Thailand. One site was swampy and nearly treeless and the other site was well drained and partially wooded. Although 13 species of chiggers were collected, only three species of chiggers were found to be positive for *O. tsutsugamushi* : *Blankaartia acuscutellaris* (7.3 % infected), *Leptotrombidium delinese* (3.1 % infected) and an undescribed species of *Ascoschoengastia* (1.2 % infected). The *Ascoschoengastia species* occurred with equal frequency at the two study sites, *L. deliense* occurred more frequently at the well drained site, and *B. acuscutellaris* occurred more frequently at the swampy site. The results suggest that there are important foci of scrub typhus in central Thailand and *B. acuscutellaris* may be a vector in this area.

In1997, Crowin AL et al. (91) reported that Indonesian peacekeepers in Cambodia provide a unique study population to estimate the threat of rickettsial exposure to *R. typhi* (murine typhus), *O. tsutsugamushi* (scrub typhus), and *R. conorii* (spotted fever) for the region. Prescreening prevalence measure showed a large proportion(36%) of soldiers had antibodies to *R. typhi.* Predeployment prevalence for antibodies to *O. tsutsugamushi* was 8%, with no evidence of background *R. conorii* infections. Actual seroconversions of *R. typhi* (3 persons) and *O. tsutsugamushi* (1 persons), attributed to exposures in Cambodia, translated in to annualized incidence rates of 24 and 8 per 1,000 per year, respectively. Surveillance of rickettsial infections

and/or disease is particularly warrented in Cambodia with recent recognition of drug-resistant scrub typhus in neighboring Thailand.

Epidemiological and Related Factors of murine typhus.

Murine typus occurs in various geographic environments on every continent except Antarctica. Many cases have been associated with international seaports and large commensal rat populations (92,93,94,95). In nature, the *R. typhi* life cycle involves rodents and their ectoparasites, primarily fleas. Flea feces infected with rickettsiae contaminate the flea bite, which may be; scratched facilitating inoculation, inhaled when dried or rubbed into the conjunctival membrane. Rodents with rickettsaemia of this type do not suffer serious illness and so act as a reservoir of infection. Humans are infected by close contact with rodents and their fleas in granaries, breweries, shops and food stores, and domestically in developing countries. Garbage workers are at special risk. Human disease is known to occur in the USA, Mexico, the north of South America, Israel, Pakistan, India, South-East Asia, China and Australia (Figure 3). It is an important cause of fever in Khmer refugees in Thailand.

Figure 3 Geographical distribution of flea-borne (murine) typhus (*R.mooseri*). The gray color in the figure represented the areas of epidemic.

Source: Reproduced from The Department of Entomology, London School of Hygiene and Tropical Medicine (2).

In 1986, a serological survey of scrub, tick and endemic typhus in East Malaysia was reported by Taylor AC et al. (96) The antibody prevalence surveys were performed by the staff of the Vector Borne Diseases Control Program in conjunction with malaria and filariasis mass blood surveys. Random cross sectional surveys were made in a total of 837 individuals. Studies were conducted in seven different rural areas of Sabah-Tawau district (4 villages : Serundong Laut, Ulu Kalabakan Kalabakan Scheme, Luasong Camp; n=689) and on Banggi island (3 villages : Meliyu, Pangkalan

Darat, Kapitangan; n=148). The overall prevalence rate of antibody to spotted fever group (SFG) rickettsiae in the study population was 8.6 % where the rate of *O. tsutsugamushi* specific antibody was only 0.8 %. No serological evidence that an active case of scrub, tick or endemic typhus was a common cause of illness was found among the study population, the low prevalence of antibody to *O. tsutsugamushi* and typhus group rickettsiae suggest that scrub typhus and endemic typhus were an extremely uncommon cause of febrile illness. Assuming that the sub-populations studied reflect conditions within the general rural population, the results should have considerable relevance to public health planners providers in Sabah. Rickettsial diseases are a major cause of febrile illness in rural areas of Peninsular Malaysia demanding serological investigation and often presumptive treatment with a tetracycline.

McDonald JC et al. (97) reported in 1988 that the rickettsioses continue to constitute major health problems in many parts of the world. With increasing international travel, recognition of rickettsial diseases by physicians is becoming more important. The clinical features of four cases of rickettsial disease imported into Canada over a five-years period were presented; two patients with tick typhus (*R. conorii*), one patient with scrub typhus (*O. tsutsugamushi*), and one patient with murine typhus (*R. typhi*). They also present the North American data over the past 10 years from the Centers for Disease Control (CDC) at Atlanta. Result revealed that since 1983 in the United States there were three confirmed cases of imported scrub typhus, all following travel to India. In addition, there were six confirmed cases of murine typhus after travel to southeast Asia. Since 1967, CDC recorded 67 imported cases by

tick-borne which have been confirmed by IFA test. Most illnesses occurred after travel to Africa. In conclusion, rickettsial diseases are underrecognized by physicians, who should consider these diagnoses in travelers returning from endemic areas. Since effective treatment is available, prompt diagnosis and treatment are important in all cases, and specific serologic confirmation should be obtained.

In 1988, Kawamura A. and Tanaka H. (98) reported that the rickettsial diseases of man in Japan include tsutsugamushi disease (scrub typhus), murine typhus, which occurs sporadically, and one of spotted fever group diseases first, recognized a new entity in 1984. First starting from 1976, there has been a remarkable resurgence in the number of reported cases of tsutsugamushi disease in Japan after several years of virtual absence. Outbreaks are still continuing after reaching peak levels in 1984. It's yearly incidences from 1982 to 1986 were 538, 749, 971, 890 and 738 respectively. This resurgence is most likely related to an increase of vector mite colonies that carry *O. tsutsugamushi*. However, it cannot be explained as to how these foci of vector mites developed.

In Thailand, *R. typhi* was first isolated in 1964 from rodent tissue from the northern province of Chiang Rai (99). Since that time, extensive surveys for this disease have been carried out in 13 provinces of Thailand, and the findings indicate that murine typhus is widely endemic (100).

In 1969, Sankasuwan V et al. (100) investigated the murine typhus in 13 provinces in Thailand. The result showed that the organisms identified as *R. typhi* were

recovered from mammals in 9 of 13 provinces. The *R. typhi* strains were recovered from 35 of 1215 *Rattus exulans*, and from 1 of 209 *R. rattus*. No strains were recovered from *R. norvegicus* (297 specimens), *Suncus murinus* (38 specimens) or *Bandicota indica* (26 specimens). No marked seasonal trends were noted, since seasonal collections were carried out in only a few areas. In addition to these isolation attempts, acute and convalescent sera from 180 PUO patients at Korat provincial hospital were examined for a rise in diagnostic antibody to *R. typhi*, none of these had a 4 - fold or greater rise, but several had high level of detectable antibody. In Mae Chan district of Chiang Rai province, 14 of 68 persons (20.6 %) had evidence of prior murine typhus infection. In Ubon Rachatanee province, 10 residents (11.1 %) had detectable level of typhus antibody. The prevalence of these antibodies in Korat Province was 5.8 % overall (3.9 % for hospital PUO patients, and 7.6 % for normal residents).

In 1973, Sankasuwan V. (101) reported 15 cases of murine typhus cases from about 1,226 PUO patients admitted documented into Nakorn Rajasrima, Ubol Rajthani and Chiang Rai hospital.

The reported outbreak of murine typhus in Thailand was documented in 1983 by Silpapojakul K et al. (102) There were 11 cases of murine typhus which occurred from September 1983 through April 1985 from 166 patients with febrile illness at Songkhlanakarin hospital and Had Yai hospital.

Brown AE et al. (103) reported on 1988 an outbreak of febrile disease among 170 Khmer adults at an evacuation site in Thailand, which occurred during the dry season of 1986, eight months after the camp was constructed. The illnesses were characterized

by persistent fever, retro-orbital headache, myalgias, and clinical response to tetracycline within 2-3 days. The symptoms, effectiveness of tetracycline, and presence of a large rats population raised the suspicion of murine typhus. Fourteen of 19 patients (74 %) had elevated or rising antibody titers against *R. typhi*, confirming the clinical diagnosis. Rats were caught, and their fleas were identified. In agreement with the known Thai host and vector, 80 of 86 rats (93 %) were *Rattus exulans*, and all of 32 fleas were *Xenopsylla cheopis*. This first reported outbreak of murine typhus in Thailand is notable for its occurrence in a new human settlement only 8 months after construction.

A serological survey was perfomed in 1993 by Takada N, et al. (106) residents of Taiwan and Thailand were compared with Japanese resident to estimate the prevalence of spotted fever (SF) and murine typhus (MT) rickettsioses in South-East Asia. They performed the study by using the IIP test. The prevalence of antibodies (cut-off level of 1:80) to some SF/MT antigens was noticeable in Japanese SF endemic areas or in high-risk groups. From these studies it was determined that a cut off level to decrease false positives in IIP could provisionally be set at a titer of 1:80 dilution. In Tainan, Taiwan, SF antibodies were not so prevalent (3.5 to 4.4 % reactivity in dilution above 1:80), but MT antibodies were more so (23.9 %). In Chiang Rai, northern Thailand, SF antibodies were markedly prevalent (9.0 to 21.3 %); the reactivity with Thai tick 118 strain alone was 8.2 % but MT was less (2.5 %). These results suggested that these rickettsioses might be latently distributed in various parts of Asia.

In 1990, Duffy PE et al. (105) reported the scrub typhus and the murine typhus as causes of illness among the 238,000 displaced Khmer people residing in temporary settlements on the Thai side of the Thai-Cambodian border. Still, the true extent of the problem and the relative frequency of infection with scrub typhus as compared to murine typhus are unknown. They evaluated consecutive patients with unexplained pyrexia (documented fever, no exclusionary diagnosis, and constitutional symptoms) in one temporary settlement over a 1 month period. Laboratory studies included culture of blood and assay of paired sera for rickettsial IgM and IgG antibody, for dengue IgM and IgG antibody, and for leptospiral IgM and IgG antibody. Among 37 patients (27 adults and 10 children), 28 (75 %) had rickettsiosis (26 cases of murine typhus and 2 of scrub typhus). No case of enteric fever, dengue, or leptospirosis was diagnosed. The illnesses of 9 patients were not identified. Signs and symptoms did not distinguish confirmed rickettsial infections from undiagnosed illnesses. The 1 month attack rate of rickettsial infection was 29/100,000 for children and 185/ 100,000 for adults. The murine typhus was a major cause of febrile illness in this settlement.

Recently reported in 1994 by Strickman D et al. (106) was the prevalence of antibodies to rickettsiae in the human population of suburban Bangkok. They performed a serosurvey in the patient's communities, and both IgG and IgM antibodies were measured in an IIP assay. The spotted antigen used were from *O. tsutsugamushi* (for scrub typhus), *R. typhi* (for murine typhi), and TT-118 (for spotted fever group rickettsiae). In a total of 215 study cases, antibody levels indicative of most recent exposure to *O. tsutsugamushi* were the most in prevalent (21 %), followed by *R. typhi*

(8 %), and TT-118 (4 %). Seroprevalence found recent exposure to *O. tsutsugamushi* varied by location (range 13 - 31 %), gender (26 % of females and 13 % of males), and age (61-80 years old) the highest prevalence(38%), were among people in close contact with orchards and orchid farms (29 % of those had extensive contact, 38 % had occasional contact, and 10 % had no contact). These patterns indicated that exposure to *O. tsutsugamushi* was related to occupation and behavior, as has been observed in areas of rural transmission. Expansion of metropolitan Bangkok has created a situation in which people employed in agriculture live with people employed in the city, thus removing the boundary between rural and urban areas. Scrub and tick-born typhus are now being transmitted in urban areas as opposed to being confined to rural areas.

CHAPTER III

Materials and Methods

Study Design

The cross-sectional study was conducted to determine the prevalence of scrub typhus infection and murine typhus infection in 393 hill tribe patients with pyrexia of unknown origin (PUO) at 4 selected hospitals which there were many hill tribe peoples (Samoeng hospital, Mae Taeng hospital, Phrao hospital, Chiang Dao hospital) in Chiang Mai Province. All subject were interviewed for theirs general characteristic, personal behavior, knowledge, environmental factors, signs and symptoms using a structured questionnaire (see in Appendix A). Blood samples were collected as paired sera (acute and convalescent serum), and in some cases collected as single serum for assaying scrub typhus and murine typhus antibody titers.

All specimens were divided into two groups. The first consisted of those positive for scrub typhus and/or murine typhus antibody titers (IgM and/or IgG $\geq$ 1:50) and the second contained those negative for scrub typhus or murine typhus antibody titers (IgM and / or IgG < 1:50). Laboratory results indicated recent or active infection, and the prevalence of scrub typhus and murine typhus infection. Immunoglobulin G (IgG) and IgM geometric mean titers (GMT) to scrub typhus and murine typhus infection

were calculated. Factors were analyzed for any relationship to the recent / active scrub typhus and murine typhus infections.

All sera positive for scrub typhus and / or murine typhus antibody titers were determined to end point. The positive scrub typhus sera were determined for their end -point antibody titer to three strains, namely Karp, Kato and Gilliam.

Calculated sample size

All case studied were hill tribe patients with PUO. Only patients aged above one year were selected for study. 5 ml of blood was collected from each study patient.

Sample size was calculated by using the formula :

$$n = \frac{z^2 pq}{d^2}$$

n = Number of samples required

z = Normal standard deviation

p = Prevalence rate.

(Takada N. et al. Survey scrub typhus and chiggers in northern Thailand, Chiang Rai, 1984. found that the prevalence rate of scrub typhus was 58%) (80).

$q = 1 - p$

d = Allowable error = 0.05

Calculated sample size of scrub typhus.

$$n = \frac{(1.96)^2 (0.58) (0.42)}{(0.05)^2}$$

= 374

Sample size for scrub typhus was 374 persons

Calculated sample size of murine typhus.

(Sankasuwan V. et al. Study murine typhus in Thailand have the prevalence 20.6%) (100).

$$n = \frac{(1.96)^2 (0.206) (0.794)}{(0.05)^2}$$

= 251

Sample size for murine typhus was 251 persons

There for the sample size proposed for this study was approximately 380 persons

Number of samples collected

In this study, blood samples were collected from pyrexia hill tribe patients at four selected hospitals in Chiang Mai province. Blood samples and questionnaires were collected from a total of 393 cases, of which 199 cases provided paired sera and 194 cases provided single serum samples.

Method of the specimen and the data collection

Aseptic techniques were used to withdraw 5 ml of blood from patients during acute febrile illness. The cases were selected from either out - patients or admitted patients. Convalescent blood samples were collected one to two weeks later, except when patients failed to attend their prearranged appointment. Blood samples were aseptically transferred into sterile tubes and allow to stand for 30 minutes for blood

clotting. Tubes were then centrifuged at 2500 rpm for 15 minutes to separate serum from clotted blood. Each serum sample was transferred to the sterile eppendorf tube and stored at -20°C until use for antibody assay. All subjects were interviewed using the specially designed questionnaire.

The questionnaire in this study was designed and approved by the Thesis Supervisory Committee, the entomologist expert and the behavioral science and health education expert. The questionnaire was divided into separate sections which documented; general characteristics, previous illness, clinical features, personal behavior, environmental exposure and knowledge of both diseases.

Antibody Assays

The indirect immunofluorescent antibody assay (IFA) was performed to detect antibodies to scrub and murine typhus infections. The laboratory work was carried out at the Rickettsial Section, Health Science Research Institute, Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand.

In Principles, the IFA is a standard two-stage "sandwich" immunofluorescence technique. Firstly, an antigen is overlaid with dilutions of human serum, and the slides were incubated, washed and dried. In the second stage the antigen is overlaid with fluorescein isothiocyanate (FITC) labeled immunoglobulin. In this manner, antigens are only rendered fluorescent by positive sera.

Preparation of Rickettsial antigens for IFA test

The seed of the Gilliam, the Karp and the Kato strains of *O. tsutsugamushi* and the Wilmington strain of *R. typhi* were propagated in bottles of monolayered cell cultures of L-929 cells (see in Appendix B). The L-929 cells infected with each strain (Gilliam, Karp, Kato or Wilmington) in monolayers were removed by a cell scraper. The infected cell were resuspended in a small amount (5- 10 ml) of culture medium and centrifuged at 1500 rpm for 10 minutes. The sediment was resuspended in phosphate buffered saline (PBS, pH 7.4) and then used as the antigen for the test without further purification. The antigen were stored at -80°C and could be frozen and thawed up to three times. After the third thawing and remaining antigen is discarded. This is because antigen frozen and thawed more than three times have been found to be unsatisfactory for use; the organisms tend to break up and lose their characteristic morphology.

Preparation of antigens slide

The antigen suspensions were mixed prior use to ensure that they were evenly suspended. Both scrub typhus (mix types : Gilliam , Karp and Kato) and murine typhus antigen (Wilmington) were used to spot a slide for the same test. It was more convenient to dispense one drop of scrub typhus antigen and murine typhus antigen side by side on the respective wells of the slides. 1 µl of the *O. tsutsugamushi* mixed type antigens were spotted onto the left side wells, and 1µl of *R. typhi* antigen spotted onto the right side. L-929 uninfected cells were spotted onto the middle wells as controls. It is important to remember the order in which the antigens are applied. The

slide was allowed to air-dry for 30 minutes. Slides were then fixed in cold acetone for 10 minutes and then air-dry again at room temperature. The antigen slides were kept in a -20°C freezer until use.

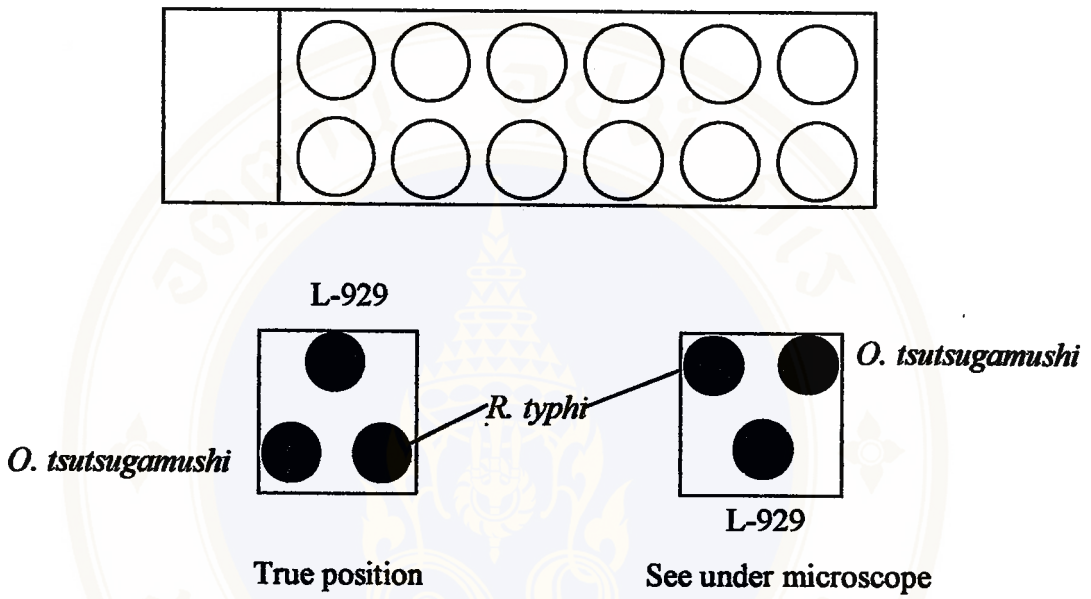


Figure 4 The 12 well antigen slide and position of each antigen spotted on the slide in each well (left), and the position see under light microscope (right).

Preparation of serum dilution for screening and for assay the end point titer

Each serum was diluted to 1:50 dilution. Briefly, 490 µl of PBS (pH 7.4) was added to an eppendorf tube as the diluent. 10 µl of test serum were add and mixed with diluent in the tube. This tube was labeled as 1:50 serum dilution. This dilution was used for antibody screening test and was stored at 4°C. Following a positive reaction with the screening dilution, 2-fold serial dilutions were made as for detecting antibody end-point titers. The dilutions were made as follows: 30 µl of PBS diluent was added to microplate and 30 µl of the 1:50 serum dilution was added to the first

well of microplate. After being thoroughly mixed, doubling dilutions were made, using a micropipette, up to the seventh well. The well were labeled; 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200 and 1:6400 respectively.

Materials and equipments required for the test

1. Rickettsia antigens and control antigen.

O. tsutsugamushi (Gilliam, Karp and Kato) antigen, either as mixed or individual strains, *R. typhi* (Wilmington) antigens and the control uninfected L-929 cell antigen, were spotted onto twelve-well slides and stored at -20°C until use.

2. Sera Samples.

Patients' sera use in this study were diluted to1: 50 dilution with PBS (pH 7.4) to screen for *O. tsutsugamushi* (mixed strains) and *R. typhi* antibodies. Positive sera were further diluted as described above to find the end-point titers for *O. tsutsugamushi* (each individual strain) and *R. typhi*.

3. Control sera.

Anti-rickettsial positive and negative control human sera were used to control for each assay.

4. Conjugate.

The FITC-conjugated rabbit anti-human IgG (specific γ chains) and IgM (spicific μ chain), Dako, Denmark (Cat No. F202 and F203 respectively) were diluted to 1:40 in PBS (pH 7.4), 1 % evans blue was added to gave a final concentration of 0.004%, and stored until depleted.

5. Slide mountant.

Buffered glycerol was used as mountant between the cover slip and the 12 well slides before the wells were examined under microscopically for fluorescence. This was prepared by using 0.5 M Carbonate buffer (pH 9.5) mixed with Fluorescence-free glycerol in the ratio 1: 9. The pH was adjusted to give a final pH of between 8.5 and 9.0.

6. Equipment use for antibody assay

(a) Auto micropipettes (volume 50 and 100 µl) and sterile fibro-fintips.

(b) 37°C incubator.

(c) Moist chamber.

(d) Timer.

(e) Staining jars or Coplin jars.

(f) Coverslips, size 24 X 60 mm.

(g) Microplate mixer.

(h) Vortex mixer.

(i) Fluorescence microscope. (Philips CS 100 W-2 lamp).

IFA test procedures

The prepared antigen slides were taken from a -20°C freezer and allowed to air-dry at room temperature before used. 10 µl of an initial 1:50 dilution of the test serum was placed over the antigen spot and the slide incubated at 37°C for 40 minutes in a moist chamber. The slide was then rinsed, washed with PBS (pH 7.4) for 5 minutes, 3 times, and allowed to air-dry. 10 µl of the diluted fluorescein conjugate (Rabbit anti-human IgM or Rabbit anti-human IgG) was then added to each well. After incubation

at 37°C for 40 minutes, the slide was again washed with PBS as described above and air-dried. The slide was mounted with buffered glycerol and cover slip. The slides were examined at ×200 magnification under a fluorescence microscope. All positive sera were future diluted 2-fold to determined the end point antibody titers in each antigen, which were expressed as the highest serum yielding detectable immunofluorescence. Positive and negative control sera were included in every batch tested.

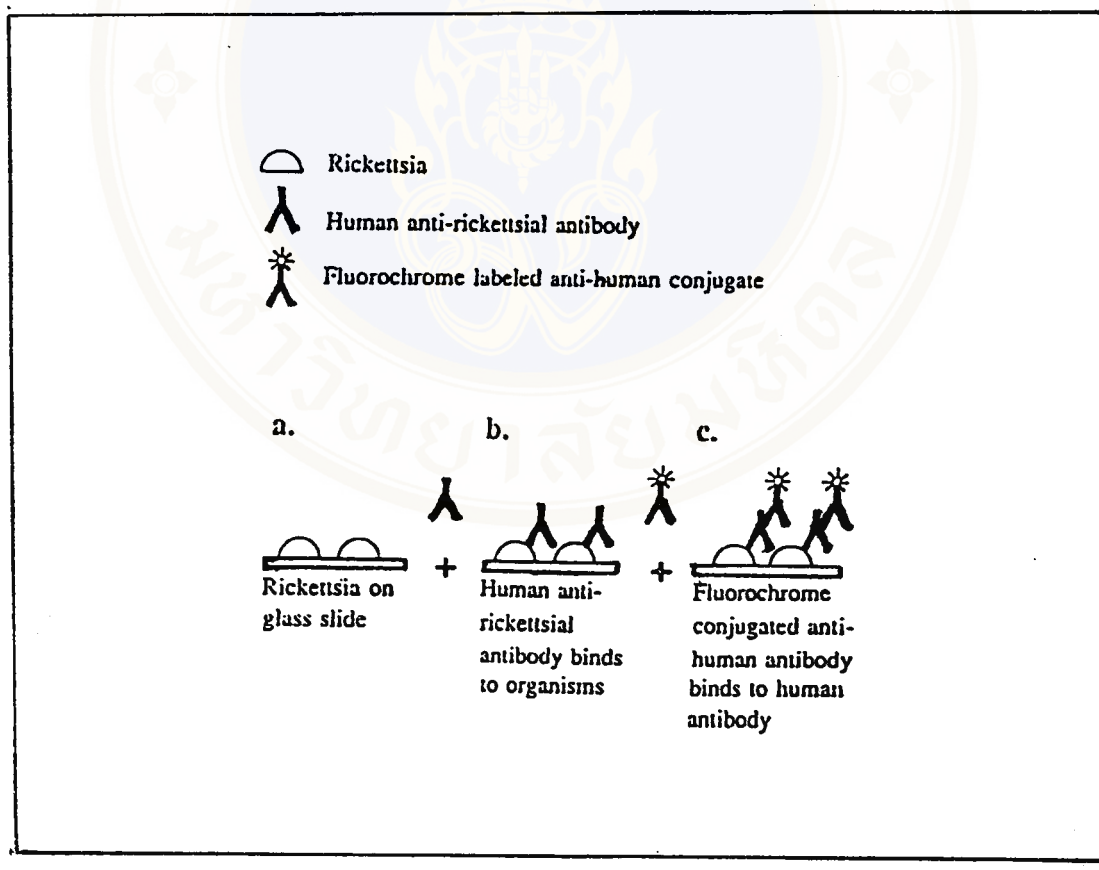


Figure 5 The principle of immunofluorescent antibody technique.

Interpretation of the IFA test for scrub typhus and murine typhus infection

The recommend phraseology which follows may be applied with regard to the reporting of IIP serological results. The current gold standard for antibody detection is the IFA but the IIP is a viable, practical substitute for the IFA (107).

a. Reports concerning a single serum sample, IgG : Request that second serum samples be drawn approximately 10-14 days after onset of signs / symptoms.

(1) If result is negative (< 1:50)

"No evidence of active infection. Please submit second serum sample."

(2) If result (titer) is ≥ 1: 50 but < 1: 400

"Consistent with either past or present infection. Please submit second serum sample for testing"

(3) If result (titer) is ≥ 1: 400

"Strongly suggestive of active infection. Please submit second serum sample"

b. Reports concerning paired serum samples, IgG

(1) If results show a four-fold rise in titer to a level of ≥ 1: 200

"Strongly suggestive of active infection"

(2) If either serum sample demonstrates a titer of ≥ 1: 400

"Strongly suggestive of active infection"

(3) If neither a four-fold rise to ≥ 1: 200, nor a result of ≥ 1: 400

" No serological evidence of active infection"

c. Reports concerning IgM results

this test has not as yet been fully validated in the field. The suggested interpretations below are conservative.

(1) IgM titer of ≥ 1: 50

"IgM titer suggestive of recent infection. Please submit second serum sample"

(2) IgM titer of ≥ 1: 400

"IgM titer strongly suggestive of active infection. Please submit second serum sample"

(3) IgM titer of < 1: 50

"No evidence of active infection. Submit second sample if clinically indicated"

Data analysis

1. The general characteristic data were described as percentage, mean and standard deviation.
2. The validity of test were used sensitivity, specificity, accuracy test, negative predict value (NPV), positive predict value (PPV).
3. Statistically significant difference were expressed as *p*-value calculated by the Chi-square test for some factors related to scrub typhus and murine typhus infection.
4. To differentiate the antibody response: IgM and IgG to 3 scrub typhus strains (Gillliam, Karp, Kato) calculated by Friedman test.

A p-value < 0.05 was used for determine statistical significance.

CHAPTER IV

RESULTS

The results in this studies are presented in to 9 parts as in the following :-

1. General characteristics of the total hill tribe patients.
2. Present illness of studied subjects.
3. Personal behaviors of the total hill tribe patients
4. The knowledge about scrub typhus of studied subject.
5. Validity of IFA test
6. Scrub typhus and murine typhus by laboratory confirmation
   - Prevalence of infection and prevalence of antibody exposure
   - Characteristics of scrub typhus infection and scrub typhus antibody exposure in studied subjects.
   - Clinical signs and symptoms of scrub typhus infection and scrub typhus antibody exposure in studied subjects.
   - Personal behaviors of hill tribe studied subjects.
7. Factors related to scrub typhus infection and factors related to murine typhus antibody exposure.
8. The level of IgM and IgG geometric mean titers in all hill tribe patients.
9. Percentage of positive IgM and IgG antibodies in all hill tribe patients in response to each strain of scrub typhus.

1. General characteristics of the total hill tribe patients

Three hundred and ninety three hill tribe patients who visited 4 selected hospital were used for these studies. Blood samples were collected and classified into 2 groups. The minor 194 patients gave single serum samples, while the major 199 patients gave in paired serum. According to their culture, these patients can be classified to 7 groups and came from one of four district hospital. On the total serum most of patients were Karen group (35.6%). The highest number of patients were treated at Samoeng Hospital (32.8%). Theirs cultural group and district hospitals are shown in Table 9 and 10

Table 9 The number of hill tribe patients visiting 4 hospitals classified by culture of hill tribe group.

Hill tribe groups	No. of cases in each hospital				Total (cases)
	Samoeng (S)	Mae Taeng (M)	Phrao (P)	Chiang Dao (C)	
Karen	105	6	21	8	140
Meo	13	31	7	11	62
Lahu	2	16	18	51	87
Lisu	6	16	20	37	79
Akha	-	-	2	7	9
Lua	3	1	1	4	9
Palong	-	-	-	7	7
Total	129	70	69	125	393

Table 10 The number of paired sera and single serum of hill tribe patients visiting 4 hospitals classified by cultural group.

Hill tribe group	Paired sera					Single serum				
	S	M	P	C	Total	S	M	P	C	Total
Karen	70	2	11	1	84	35	4	10	7	56
Meo	2	18	2	5	27	11	13	5	6	35
Lahu	2	8	9	18	37	-	8	9	33	50
Lisu	1	9	10	23	43	5	7	10	14	36
Akha	-	-	1	3	4	-	-	1	4	5
Lua	-	-	-	3	3	2	-	-	4	6
Palong	-	-	-	1	1	1	1	1	3	6
Total	75	37	33	54	199	54	33	36	71	194

Table 11 showed the demographic data of all patients categorized by sex, age, hill tribe group, marital status, occupation, religion, income, education, treatment hospital and migration. The results found that the number of male and female patients were not that different; a ratio of male to female of 1.07 to 1. Highest patients found in age group 10-19 years old (32.0%), and their major occupation was agriculture (55.7%). Most of the hill tribes in this studied had no migration history (81.9%), while those cases who had been migrated (18.1%) changed for reasons related to their agricultural occupation. Most of the religion is Buddhist (62.6%) and their income per month is mostly less than 1000 baht or no income at all.

Table 11 Number of hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their demographic data.

General demographics	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Sex						
Male	101	50.8	102	52.6	203	51.7
Female	98	49.2	92	47.4	190	48.3
Age group						
0-9	21	10.6	27	13.9	48	12.2
10-19	67	33.7	59	30.4	126	32.0
20-29	45	22.6	37	19.1	82	20.9
30-39	39	19.6	32	16.5	71	18.1
40-49	13	6.5	18	9.3	31	7.9
50-59	8	4.0	10	5.1	18	4.6
≥60	6	3.0	11	5.7	17	4.3
Mean ± SD	25.4 ± 15.0		26.6 ± 16.7		26.0±15.9	
Hill tribe group						
Karen	84	42.2	56	28.9	140	35.6
Meo	27	13.6	35	18.0	62	15.8
Lahu	37	18.6	50	25.8	87	22.1
Lisu	43	21.6	36	18.5	79	20.1
Akha	4	2.0	5	2.6	9	2.3
Lua	3	1.5	6	3.1	9	2.3
Palong	1	0.5	6	3.1	7	1.8
Marital status						
Single	98	49.3	87	44.8	185	47.1
Married	97	48.7	105	54.1	202	51.4
Widow	4	2.0	2	1.1	6	1.5

Table 11 Number of hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their demographic data. (Continued)

General demographics	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Education						
None	87	43.7	97	50.0	184	46.8
Prathom 1-6	97	48.8	82	42.3	179	45.5
Mathayom 1-3	11	5.5	10	5.2	21	5.3
Mathayom 4-6	4	2.0	3	1.5	7	1.8
Vocational education certificate	-	-	1	0.5	1	0.3
Bachelor's degree	-	-	1	0.5	1	0.3
Treatment hospital						
Samoeng	75	37.7	54	27.8	129	32.8
Mae taeng	37	18.6	33	17.0	70	17.8
Phrao	33	16.6	36	18.6	69	17.6
Chiang Dao	54	27.1	71	36.6	125	31.8
Migration						
No	168	84.4	154	79.4	322	81.9
Yes	31	15.6	40	20.6	71	18.1
Cause of migration						
To change place for agriculture	21	67.7	35	87.5	56	78.9
To be the employee	9	29.0	4	10.0	13	18.3
To escape from natural danger	1	3.3	1	2.5	2	2.8

The 11 Number of hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their demographic data. (Continued)

General demographics	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Occupation						
Agriculture	116	58.3	103	53.1	219	55.7
To look for something from the forest	-	-	1	0.5	1	0.3
Employee	25	12.6	20	10.3	45	11.4
Business	-	-	1	0.5	1	0.3
Student	51	25.6	58	29.9	109	27.7
Do not work	7	3.5	11	5.7	18	4.6
Religion						
Buddhist	131	65.8	115	59.3	246	62.6
Christian	67	33.7	77	39.7	144	36.6
Islam	-	-	1	0.5	1	0.3
Others	1	0.5	1	0.5	2	0.5
Income (baht / month)						
No income	61	30.7	68	35.1	129	32.8
1-1000	63	31.7	67	34.5	130	33.1
1001-2000	39	19.6	37	19.1	76	19.4
2001-3000	8	4.0	12	6.2	20	5.1
3001-4000	18	9.0	3	1.5	21	5.3
4001-5000	7	3.5	4	2.1	11	2.8
5001-6000	1	0.5	1	0.5	2	0.5
6001-7000	2	1.0	2	1.0	4	1.0
>7000	-	-	-	-	-	-

2. Present illness of studied subjects

The most common symptoms found in these patients were fever (100.0%), followed by anorexia (68.2%), headache (64.6%), muscle pain (42.2%), nausea (41.5%) and chills (37.7%).

Clinical signs were rarely found among cases in each category. In this study lymphadenopathy was not found in any patients, while an injected eye was reported very few cases (3.3%). The eschar and rash were rare (4.8% and 4.6% respectively) . One third of the hill tribe patients went to a primary health care service center when they had illness (31.5%). They bought the medicine by themselves but they didn't know what type of the medicine they bought and took.

The detail are summarized in Table 12 and 13.

Table 12 Prevalence of the present illness among the hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their clinical symptoms and signs.

Present illness	Paired sera		Single serum		Total	
	No.of cases (n=199)	Percentage (%)	No.of cases (n=194)	Percentage (%)	No.of cases (n=393)	Percentage (%)
Symptoms						
Fever	199	100.0	194	100.0	393	100.0
Headache	139	69.8	115	59.3	254	64.6
Chills	67	33.7	81	41.8	148	37.7
Orbital pain	14	7.0	13	6.7	27	6.9
Muscle pain	87	43.7	79	40.7	166	42.2
Cough	53	26.6	66	34.0	119	30.3
Anorexia	142	71.4	126	64.9	268	68.2

Table 12 Prevalence of the present illness among the hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their clinical symptoms and signs. (Continued)

Present illness	Paired sera		Single serum		Total	
	No.of cases (n=199)	Percentage (%)	No.of cases (n=194)	Percentage (%)	No.of cases (n=393)	Percentage (%)
Nausea	78	39.2	85	43.8	163	41.5
Vomiting	71	35.7	70	36.1	141	35.9
Abdominal pain	37	18.6	30	15.5	67	17.1
Diarrhea	41	20.6	29	14.9	70	17.8
Right costal margin pain	12	6.0	8	4.1	20	5.1
Signs						
Injected eye	5	2.5	8	4.1	13	3.3
Extrimities and/or body rash	11	5.5	7	3.6	18	4.6
Eschar	13	6.5	6	3.1	19	4.8
Lymphadenopathy	-	-	-	-	-	-
Jaundice	31	15.6	29	14.9	60	15.3

Note : each patient showed symptoms and signs in more than one category.

Table 13 Number of hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their health history.

Information of health history	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Activity after illness						
- Nothing	97	48.7	101	52.0	198	50.4
-Went to clinic	6	3.0	1	0.5	7	1.8
-Went to primary health care service center	59	29.7	65	33.5	124	31.5
- Bought some medicine from drug store	23	11.6	13	6.7	36	9.2
-Went to hospital	4	2.0	5	2.6	9	2.3
- Traditional treatment	5	2.5	5	2.6	10	2.5
- Others	5	2.5	4	2.1	9	2.3
	(n=23)		(n=13)		(n=36)	
Type of medicines bought						
- Antibiotic	1	4.4	4	30.8	5	13.9
- Analgesic	21	91.2	9	69.2	30	83.3
- Herbal medicines	1	4.4	-	-	1	2.8

3. Personal behaviors of the total hill tribe patients

It was found that almost three quarters of these patients had been clearing grass (71.0%). Their working time mostly was between 8.00 am and 16.00 pm (89.6%). They went to work on foot (93.6%) and the break times during their work were spent in the shanty area (52.0%). The majority of patients had not used repellent to prevent mites and / or insects bites (93.1%), and 77.1% had domestic animals. They did not get rid of neither the reservoirs host such as rats, rabbits and squirrels (97.5%) nor insects such as chiggers or mites (90.6%). The data are summarized in Table 14.

Table 14 Number of hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their personal behaviors.

Personal behaviors	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
To clear away the grass or shrubs or forest						
No	52	26.1	62	32.0	114	29.0
Yes	147	73.9	132	68.0	279	71.0
To get rid of garbage						
Burning	178	89.4	176	90.7	354	90.1
Burring	21	10.6	18	9.3	39	9.9
Having pets feeding						
No	45	22.6	45	23.2	90	22.9
Yes	154	77.4	149	76.8	303	77.1
Removal of insects						
No	185	93.0	171	88.1	356	90.6
Yes	14	7.0	23	11.9	37	9.4

Table 14 Number of hill tribe patients visiting 4 hospitals in Chiang Mai Province, classified by their personal behaviors. (Continued)

Personal behaviors	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Removal of reservoir hosts (rodents, rats and squirrels)						
No	194	97.5	189	97.4	383	97.5
Yes	5	2.5	5	2.6	10	2.5
Use repellent for prevention from insect bite						
No	186	93.5	180	92.8	366	93.1
Yes	13	6.5	14	7.2	27	6.9
	(n =192)		(n =183)		(n = 375)	
Travelling to work*						
On foot	179	93.2	172	94.0	351	93.6
By vehicle	13	6.8	11	6.0	24	6.4
The place during break time when working*						
Cleared area	19	9.9	26	14.2	45	12.0
Grassy area	76	39.6	59	32.2	135	36.0
Shanty	97	50.5	98	53.6	195	52.0
Dress when working*						
Covered cloth	75	39.1	86	47.0	161	42.9
Open cloth	117	60.9	97	53.0	214	57.1
Working time*						
8.00 –16.00	170	88.5	166	90.7	336	89.6
8.00 – 20.00	22	11.5	17	9.3	39	10.4

* Note : Does not include patients who did not work, which were 7 cases in paired sera and 11 cases in single serum.

More than half of studied hill tribe patients were living on the mountain, which has shrubs or grass (66.9%) and most of their working areas were in fields and forests (69.1%). The data in Table 15 shows the detail of their environment.

Table 15 Number of hill tribe patients visiting 4 hospitals classified by their related environment.

Environment	Paired sera		Single serum		Total	
	No.of case (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Having shrubs, grass in living area						
No	63	31.7	67	34.5	130	33.1
Yes	136	68.3	127	65.5	263	66.9
Frequency of seeing rats, rabbits, squirrels and chipmunks.						
Never	21	10.5	18	9.3	39	9.9
Every day	49	24.6	56	28.9	105	26.7
Very rare weekly	65	32.7	56	28.9	121	30.8
Very rare monthly	64	32.2	64	32.9	128	32.6
	(n=178)		(n=176)		(n=354)	
The number of reservoir hosts seen in each time.						
1-2 reservoir host/ time	163	91.6	145	82.4	308	87.0
> 2 reservoir hosts/ time	15	8.4	31	17.6	46	13.0
Environment of working area*						
Field and forest	130	67.7	129	70.5	259	69.1
In urban area	62	32.3	54	29.5	116	30.9

* Note : Does not include patients who did not work, which were 7 cases in paired sera and 11 cases in single serum.

4. The knowledge of scrub typhus (including murine typhus) in studied subjects.

There were very few cases who had knowledge of scrub typhus and murine typhus infections, in particular, they didn't know either chigger (85.2%) or mite (76.8%). Ninety-nine percent never received any knowledge and didn't know the etiology of scrub typhus and/or murine typhus infections. In addition, they didn't know the method for prevention (98.2%). The data are shown in Table 16.

Table 16 Number of hill tribe patients visiting 4 hospitals, classified by information of their knowledge of scrub typhus and murine typhus infections.

Information of knowledge	Paired sera		Single serum		Total	
	No.of cases (n = 199)	Percentage (%)	No.of cases (n = 194)	Percentage (%)	No.of cases (n = 393)	Percentage (%)
Know a chigger.						
No	166	83.4	169	87.1	335	85.2
Yes	33	16.6	25	12.9	58	14.8
Know a mite.						
No	151	75.9	151	77.8	302	76.8
Yes	48	24.1	43	22.2	91	23.2
Know about cause of scrub typhus and murine typhus infections.						
No	198	99.5	191	98.5	389	99.0
Yes	1	0.5	3	1.5	4	1.0
Having received knowledge of scrub typhus and murine typhus infections.						
No	198	99.5	191	98.5	389	99.0
Yes	1	0.5	3	1.5	4	1.0
Know about prevention of scrub typhus and murine typhus infections.						
No	195	98.0	191	98.5	386	98.2
Yes	4	2.0	3	1.5	7	1.8

5. Validity of IFA test

A total of 393 cases were classified into 2 groups, 194 cases had in single serum collected from themselves, and 199 gave paired sera. The paired sera samples were interpreted by the gold standard (a four fold rising titer of IgM and/or IgG to a level of ≥ 1:200). In single serum there was only acute sera; if the results were negative they may be positive in convalescence sera. So we need to calculate specificity and negative predictive value for reference to interpret the results of single serum with the gold standard (paired sera). The method for calculating sensitivity, specificity, accuracy of test, positive predictive value (PPV), and negative predictive value (NPV) are shown in Table 17.

T able 17 Calculation to show validity of IFA test.

Single serum	Paired sera		Total
	Positive scrub typhus (cases)	Negative scrub typhus (cases)	(cases)
Positive scrub typhus (cases)	50 (a)	0 (b)	50 (a + b)
Negative scrub typhus (cases)	26 (c)	123 (d)	149 (c + d)
Total (cases)	76 (a + c)	123 (b + d)	199 (a + b+ c+ d)

$$\text{Sensitivity} = (a) / (a + c) \times 100$$

$$= 50 / 76 \times 100$$

$$= 65.8\%$$

$$\text{Specificity} = (d) / (b + d) \times 100$$
$$= 123 / 123 \times 100$$
$$= 100\%$$

$$\text{Accuracy of test} = (a + d) / (a + b + c + d) \times 100$$
$$= 173 / 199 \times 100$$
$$= 86.9\%$$

$$\text{Positive Predictive Value (PPV)} = (a / a + b) \times 100$$
$$= 50 / 50 \times 100$$
$$= 100\%$$

$$\text{Negative Predictive Value (NPV)} = (d / c + d) \times 100$$
$$= 123 / 149 \times 100$$
$$= 82.6\%$$

In Table 17, a specificity of 100% refers to the result of single serum being negative in persons without diseases (100%) and to agree with NPV 82.6% refers to the probability of the results in single serum being negative in a person without disease 82.6%. Accuracy of test (86.9%) refers to this test giving a true value both positive in persons who have disease and negative in persons without diseases. For that reason is sufficiency for interpretation of negative results in single serum samples.

5. Scrub typhus and murine typhus by laboratory confirmation.

The results of laboratory confirmation by IFA test to scrub typhus and murine typhus among hill tribe patients found that 119 cases were IgM and/or IgG positive scrub typhus infection which composed of 34 cases of active infection (a four fold rise

a four fold rise titer of IgM and/or IgG ≥1:200), 3 cases of recent infection (a four fold rise but IgM and/or IgG antibody titer <1:200), 77 cases of presumptive infection (no four fold rise, but any sera show IgM and/or IgG antibody titer ≥1:400) and 5 cases of suggestive scrub typhus infection (IgM titer of = 1:200) . In addition, positive evidence of murine typhus infections were found in 2 cases.

These cases were used for the future study for the factors related to scrub typhus infection. The prevalence of infection shown in Table 18, of scrub typhus and murine typhus infection were 30.3 % (119/393) and 0.5% (2/393), respectively.

Table 18 Laboratory confirmation of hill tribe patients for rickettsia infections Assay by IFA test technique.

Laboratory diagnosis	Paired sera		Single serum		Total	
	No.of cases (n=199)	%	No.of cases (n=194)	%	No.of cases (n=393)	%
Positive						
Scrub typhus	76	38.2	43	22.2	119	30.3
-Active infection	34				34	
-Recent infection	3				3	
-Presumptive active infection	36		41		77	
-Suggestion infection	3		2		5	
Murine typhus	2	1.0	0	0.0	2	0.5
Negative	121	60.8	151	77.8	272	69.2

On the other hand, the prevalence rate of scrub typhus and murine typhus antibody exposure were 37.4% (147/393) and 5.9% (23/393) respectively, as shown in Table 19.

Table 19 Number of rickettsia antibodies exposure (antibody titer ≥ 1:50) in hill tribe patients assay by IFA technique.

Laboratory diagnosis	Paired sera		Single serum		Total	
	No.of cases (n=199)	%	No.of cases (n=194)	%	No.of cases (n=393)	%
Positive antibody exposure						
-Scrub typhus[a]	74	37.2	58	29.9	132	33.6
-Scrub typhus and murine typhus[b]	14	7.0	1	0.5	15	3.8
-Murine typhus[c]	6	3.0	2	1.0	8	2.0
Negative antibody exposure[d]	105	52.8	133	68.6	238	60.6

Note

Total positive cases of scrub typhus antibody exposure = a + b = 147 cases

Total positive cases of murine typhus antibody exposure = b + c = 23 cases

Characteristics of scrub typhus infection and scrub typhus antibody exposure in studied subjects.

Since positive murine typhus infection and murine typhus antibody exposure was rarely found, then analysis will only focus on scrub typhus. Of the total studied subjects of 393 cases, positive scrub typhus infection and positive scrub typhus antibody

exposure was found 119 and 147 cases, respectively, while negative scrub typhus infection and negative scrub typhus antibody exposure were 274 and 246 cases, respectively. The results of IFA test in studied subjects were classified by general demographic data. The results showed that more males, had scrub typhus infection cases (36.5%) than females (23.7%). The age group of more than 60 years old had the highest positive cases of scrub typhus infection (52.9%) and 50-59 years olds showed scrub typhus antibody exposure (61.1%). Most of the positive cases in both groups were associated with agricultural occupations. Theirs general demographics are summarized in Table 20.

The information about health care and treatment of studied subjects are shown in table 21. Most of the patients went to clinic before coming to the hospitals. In some cases who had bought medicine the type medicine bought was herbal drugs.

Table 20 Number of scrub typhus infections and scrub typhus antibody exposure in studied subjects, classified by their demographic data.

General demographics	Scrub typhus infections			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n= 393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n= 393)
Sex						
Male	74 (36.5)	129 (63.5)	203	84 (41.4)	119 (58.6)	203
Female	45 (23.7)	145 (76.3)	190	63 (33.2)	127 (66.8)	190
Age						
0-9	9 (18.8)	39 (81.2)	48	11 (22.9)	37 (77.1)	48
10-19	21 (16.7)	105 (83.3)	126	30 (23.8)	96 (76.2)	126
20-29	29 (35.4)	53 (64.6)	82	36 (43.9)	46 (56.1)	82
30-39	31 (43.7)	40 (56.3)	71	34 (47.9)	37 (52.1)	71
40-49	12 (38.7)	19 (61.3)	31	15 (48.4)	16 (51.6)	31
50-59	8 (44.4)	10 (55.6)	18	11 (61.1)	7 (38.9)	18
≥60	9 (52.9)	8 (47.1)	17	10 (58.8)	7 (41.2)	17
Hill tribe group						
Karen	47 (33.6)	93 (66.4)	140	62 (44.3)	78 (55.7)	140
Meo	14 (22.6)	48 (77.4)	62	15 (22.4)	47 (75.8)	62
Lahu	29 (33.3)	58 (66.7)	87	31 (35.6)	56 (64.4)	87
Lisu	25 (31.6)	54 (68.4)	79	31 (39.2)	48 (60.8)	79
Akha	1 (11.1)	8 (88.9)	9	2 (22.2)	7 (77.8)	9
Lua	3 (33.3)	6 (66.7)	9	4 (44.4)	5 (55.6)	9
Palong	0 (0.0)	7 (100)	7	2 (28.6)	5 (71.4)	7
Marital status						
Single	41 (22.2)	144 (77.8)	185	53 (28.6)	132 (71.4)	185
Married	74 (36.6)	128 (63.4)	202	90 (44.6)	112 (55.4)	202
Widow	4 (66.7)	2 (33.3)	6	4 (66.7)	2 (33.3)	6

Table 20 Number of scrub typhus infections and scrub typhus antibody exposure in studied subjects, classified by their demographic data. (Continued)

General demographics	Scrub typhus infection			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n=393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Occupation						
Agriculture	88 (40.2)	131 (59.8)	219	105 (47.9)	114 (52.1)	219
To look for something from the forest	0 (0.0)	1 (100)	1	0 (0.0)	1 (100)	1
Employee	9 (20.0)	36 (80.0)	45	11 (24.4)	34 (75.6)	45
Business	0 (0.0)	1 (100)	1	0 (0.0)	1 (100)	1
Students	16 (14.7)	93 (88.3)	109	24 (22.0)	85 (78.0)	109
Do not work	6 (33.3)	12 (66.7)	18	7 (38.9)	11 (61.1)	18
Religion						
Buddhist	73 (29.7)	173 (70.3)	246	90 (36.6)	156 (63.4)	246
Christian	44 (30.6)	100 (69.4)	144	55 (38.2)	89 (61.8)	144
Islam	0 (0.0)	1 (100)	1	0 (0.0)	1 (100)	1
Other	2 (100)	0 (0.0)	2	2 (100)	0 (0.0)	2
Education						
No study	73 (39.7)	111 (60.3)	184	86 (46.7)	98 (53.3)	184
Prathom 1-6	42 (23.5)	137 (76.5)	179	54 (30.2)	125 (69.8)	179
Mathayom 1-3	3 (14.3)	18 (85.7)	21	5 (23.8)	16 (76.2)	21
Mathayom 4-6	1 (14.3)	6 (85.7)	7	2 (28.6)	5 (71.4)	7
Vocational education certificate	0 (0.0)	1 (100)	1	0 (0.0)	1 (100)	1
Bachelor's degree	0 (0.0)	1 (100)	1	0 (0.0)	1 (100)	1

Table 20 Number of scrub typhus infections and scrub typhus antibody exposure in studied subjects, classified by their demographic data. (Continued)

General demographics	Scrub typhus infection			Scrub typhus antibody exposured		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n=393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Income (baht / month)						
No income	21 (16.3)	108 (83.7)	129	28 (21.7)	101 (78.3)	129
1-1000	49 (37.7)	81 (62.3)	130	61 (46.9)	69 (53.1)	130
1001-2000	34 (44.7)	42 (55.3)	76	42 (55.3)	34 (44.7)	76
2001-3000	6 (30.0)	14 (70.0)	20	6 (30.0)	14 (70.0)	20
3001-4000	3 (14.3)	18 (85.7)	21	4 (19.1)	17 (80.9)	21
4001-5000	4 (36.4)	7 (63.6)	11	4 (36.4)	7 (63.6)	11
5001-6000	1 (50.0)	1 (50.0)	2	1 (50.0)	1 (50.0)	2
6001-7000	1 (25.0)	3 (75.0)	4	1 (25.0)	3 (75.0)	4
Treatment hospital						
Samoeng	50 (38.8)	79 (61.2)	129	61 (47.3)	68 (52.7)	129
Mae taeng	6 (8.6)	64 (91.4)	70	8 (11.4)	62 (88.6)	70
Phrao	21 (30.4)	48 (69.6)	69	28 (40.6)	41 (59.4)	69
Chiang Dao	42 (33.6)	83 (66.4)	125	50 (40.0)	75 (60.0)	125
Migration						
No	94 (29.2)	228 (70.8)	322	119 (37.0)	203 (63.0)	322
Yes	25 (35.2)	46 (64.8)	71	28 (39.4)	43 (60.6)	71
Reason of migration						
To change place for agriculture	18 (32.1)	38 (67.9)	56	21 (37.5)	35 (62.5)	56
To employee	6 (46.2)	7 (53.8)	13	6 (46.2)	7 (53.8)	13
To escape natural danger	1 (50.0)	1 (50.0)	2	1 (50.0)	1 (50.0)	2

Table 21 Number of scrub typhus infections and scrub typhus antibody exposure in studied subjects, classified by their information health history.

Present illness	Scrub typhus infections			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n=393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Activity after illness						
Nothing	60 (30.3)	138 (69.7)	198	77 (38.9)	120 (61.1)	198
Went to clinic	3 (42.9)	4 (57.1)	7	3 (42.9)	4 (57.1)	7
Went to primary health care service center	39 (31.5)	85 (68.5)	124	46 (37.1)	78 (62.9)	124
Bought some medicine from drug store	9 (25.0)	27 (75.0)	36	12 (33.3)	24 (66.7)	36
Went to hospital	3 (33.3)	6 (66.7)	9	4 (44.4)	5 (55.6)	9
Traditional Treatment	3 (30.0)	7 (70.0)	10	3 (30.0)	7 (70.0)	10
Others	2 (22.2) (n=9)	7 (77.8) (n=27)	9 (n=36)	2 (22.2) (n=12)	7 (77.8) (n=24)	9 (n=36)
Type of medicine bought						
Antibiotic	0 (0.0)	5 (100)	5	1 (20.0)	4 (80.0)	5
Analgesic	8 (26.7)	22 (73.3)	30	10 (33.3)	20 (66.7)	30
Herbal medicines.	1 (100)	0 (0.0)	1	1 (100)	0 (0.0)	1

Clinical signs and symptoms of scrub typhus infection and scrub typhus antibody exposure in studied subjects.

Fever was found every case (100%) either in positive cases of scrub typhus infection or scrub typhus antibody exposure, followed by anorexia and headache. Jaundice, was found approximately 13% of patients, while eschar findings very rare. The details are shown on Table 22.

Table 22 Prevalence of clinical signs and symptoms of positive scrub typhus infections and scrub typhus antibody exposure in studied subjects.

Present illness	Scrub typhus infections		Scrub typhus antibody exposure	
	No. of cases (n=119)	Percentage (%)	No. of cases (n=147)	Percentage (%)
Symptoms				
Fever	119	100.0	147	100.0
Anorexia	90	75.6	108	73.5
Headache	87	73.1	102	69.4
Muscle pain	66	55.5	77	52.4
Nausia	52	43.7	68	46.3
Vomiting	45	37.8	57	38.8
Chills	43	36.1	61	41.5
Cough	31	26.1	40	27.2
Diarrhea	18	15.1	22	15.0
Abdominal pain	15	12.6	20	13.6
Orbital pain	14	11.8	17	11.6
Right costal margin pain	14	11.8	14	9.5

Table 22 Prevalence of clinical signs and symptoms of positive scrub typhus infections and scrub typhus antibody exposure in studied subjects. (Continued)

Present illness	Scrub typhus infections		Scrub typhus antibody exposure	
	No. of cases (n=119)	Percentage (%)	No. of cases (n=147)	Percentage (%)
Signs				
Jaundice	15	12.6	18	12.2
Extremities and/or body rash	12	10.1	12	8.1
Eschar	18	15.1	18	12.2
Infected eye	4	3.4	4	2.7
Lympha-denopathy	0	0.0	0	0.0

Note : each patient showed symptoms

Personal behaviors of hill tribe studied subjects.

The personal behaviors of hill tribe studied subjects are classified in scrub typhus infection and scrub typhus antibody exposure. These behaviors were their life style which may the causes, directly and indirectly of positive infection and positive antibody exposure. The details are shown in Table 23.

Table 23 Number of positive scrub typhus infection and scrub typhus antibody exposure of studied subjects who visited 4 hospitals in Chiang Mai Province, classified by theirs personal behaviors.

Personal Behaviors	Scrub typhus infection			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n=393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Clear away the grass or shrubs or forests						
No	23 (20.2)	91 (79.8)	114	27 (23.7)	87 (76.3)	114
Yes	96 (34.4)	183 (65.6)	279	120 (43.0)	159 (57.0)	279
Removal of garbage						
Burning	108 (30.5)	246 (69.5)	354	132 (37.3)	222 (62.7)	354
Burring	11 (28.2)	28 (71.8)	39	15 (38.5)	24 (61.5)	39
Having pets feeding						
No	25 (27.8)	65 (72.2)	90	33 (36.7)	57 (63.3)	90
Yes	94 (31.0)	209 (69.0)	303	114 (37.6)	189 (62.4)	303
Removal of insects						
No	113 (31.7)	243 (68.3)	356	140 (39.3)	216 (60.7)	356
Yes	6 (16.2)	31 (83.8)	37	7 (18.9)	30 (81.1)	37

Table 23 Number of positive scrub typhus infections and scrub typhus antibody exposure of studied subjects who visited 4 hospitals in Chiang Mai Province, classified by their personal behaviors. (Continued)

Personal behaviors	Scrub typhus infections			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n= 393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Removal of reservoir hosts						
No	117 (30.6)	266 (69.5)	383	144 (37.6)	239 (62.4)	383
Yes	2 (20.0)	8 (80.0)	10	3 (30.0)	7 (70.0)	10
Use repellent for prevention from insect bite						
No	111 (30.3)	255 (69.7)	366	137 (37.4)	229 (62.6)	366
Yes	8 (29.6)	19 (70.4)	27	10 (37.0)	17 (63.0)	27
Travelling to work*						
	(n =113)	(n = 262)	(n=375)	(n = 140)	(n = 235)	(n = 375)
On foot	109 (31.1)	242 (68.9)	351	135 (38.5)	216 (61.5)	351
By vehicle	4 (16.7)	20 (83.3)	24	5 (20.8)	19 (79.2)	24
The place during break time when working*						
Cleared area	12 (26.7)	33 (73.3)	45	15 (33.3)	30 (66.7)	45
Grassy area	65 (48.2)	70 (51.8)	135	75 (55.6)	60 (44.4)	135
Shanty	36 (18.5)	159 (81.5)	195	50 (25.6)	145 (74.4)	195
Dress when working*						
Covered cloth	49 (30.4)	112 (69.6)	161	60 (37.3)	101 (62.7)	161
Open cloth	64 (29.9)	150 (70.1)	214	80 (37.4)	134 (62.6)	214
Working time*						
8.00 -16.00	100 (29.8)	236 (70.2)	336	126 (37.5)	210 (62.5)	336
8.00- 20.00	13 (33.3)	26 (66.7)	39	14 (35.9)	25 (64.1)	39

*Note : Does not include patients who did not work (6 cases in positive and 12 cases in negative for infections; 7 cases in positive and 11 cases in negative for antibody exposure).

Information of theirs environment in scrub typhus infection and scrub typhus antibody exposure of studied subjects.

Most of these patients had shrubs and glass in their living area and worked in fields and forests as shown in Table 24.

Table 24 Number of scrub typhus infections and scrub typhus antibody exposure of studied subjects who visited 4 hospitals classified by their related environment.

Environment	Scrub typhus infections			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n= 393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Having shrubs, grass in living area						
No	23 (17.7)	107 (82.3)	130	30 (23.1)	100 (76.9)	130
Yes	96 (36.5)	167 (63.5)	263	117 (44.5)	146 (55.5)	263
Frequency of seeing rats, rabbits, squirrels and chipmunks						
Never	8 (20.5)	31 (79.5)	39	12 (30.8)	27 (69.2)	39
Every day	28 (26.7)	77 (73.3)	105	36 (34.3)	69 (65.7)	105
Very rare weekly	48 (39.7)	73 (60.3)	121	53 (43.8)	68 (56.2)	121
Very rare monthly	35 (27.3)	93 (72.7)	128	46 (35.9)	82 (64.1)	128

Table 24 Number of scrub typhus infections and scrub typhus antibody exposure of studied subjects who visited 4 hospitals classified by their related environment. (Continued)

Environment	Scrub typhus infections			Scrub typhus antibody exposure		
	positive cases (%) (n=111)	negative cases (%) (n=243)	Total cases (n= 219)	positive cases (%) (n=135)	negative cases (%) (n=219)	Total cases (n=354)
The number of reservoir hosts seen in each time						
1-2 reservoir host/ time	96 (31.2)	212 (68.8)	308	117 (38.0)	191 (62.0)	308
>2 reservoir hosts/ time	15 (32.6)	31 (67.4)	46	18 (39.1)	28 (60.9)	46
	(n=113)	(n=262)		(n=140)	(n=235)	(n=375)
Environment of working area						
Field and forest	92 (35.5)	167 (64.5)	259	115 (44.4)	144 (55.6)	259
In urban area	21 (18.1)	95 (81.9)	116	25 (21.6)	91 (78.4)	116

The knowledge of scrub typhus infections and scrub typhus antibody exposure.

The information of knowledge of scrub typhus infections were analyzed and found that most of them lacked of knowledge of this diseases. The detail are shown in Table 25.

Table 25 Number of hill tribe studied subjects, classified by information of their knowledge of scrub typhus infections and scrub typhus antibody exposure.

Information of knowledge	Scrub typhus infections			Scrub typhus antibody exposure		
	positive cases (%) (n=119)	negative cases (%) (n=274)	Total cases (n= 393)	positive cases (%) (n=147)	negative cases (%) (n=246)	Total cases (n=393)
Know a chigger.						
No	96 (28.7)	239 (71.3)	335	120 (35.8)	215 (64.2)	335
Yes	23 (39.7)	35 (60.3)	58	27 (46.4)	31 (53.4)	58
Know a mite.						
No	90 (29.8)	212 (70.2)	302	111 (36.8)	191 (63.2)	302
Yes	29 (31.9)	62 (68.1)	91	36 (39.6)	55 (60.4)	91
Know about cause of scrub typhus infection.						
No	118 (30.3)	271 (69.7)	389	145 (37.3)	244 (62.7)	389
Yes	1 (25.0)	3 (75.0)	4	2 (50.0)	2 (50.0)	4
Having received knowledge of scrub typhus infection.						
No	119 (30.6)	270 (69.4)	389	147 (37.8)	242 (62.2)	389
Yes	0 (0.0)	4 (100)	4	0 (0.0)	4 (100)	4
Know about prevention of scrub typhus infection.						
No	117 (30.3)	269 (69.7)	386	143 (37.0)	243 (63.0)	386
Yes	2 (28.6)	5 (71.4)	7	4 (57.1)	3 (42.9)	7

7. Factors related to scrub typhus infections, and murine typhus antibody exposure.

Various factors were studied for finding their relation to either scrub typhus infections or murine typhus antibody exposure as shown in Table 26 and 27. It was found that the prevalence of scrub typhus infections in males were higher than females and were significantly different (p-value < 0.05). The prevalence of scrub typhus infections in the age group > 25 years olds was higher than in ≤ 25 years olds, and differed significantly (p-value < 0.001). The prevalence of scrub typhus infections in agriculture was higher than in other occupation (p-value < 0.001). The prevalence of scrub typhus infections in low income patients was higher than in those with high income (p-value < 0.05). The prevalence of scrub typhus infections in those with poor education was higher than those educated patients (p-value < 0.001). The prevalence of scrub typhus infections was found significantly (p-value < 0.05) related to marital status, hospital treatment, clearing of grass or shrubs or forest, the place during break time at work, having shrubs or grass in the living area and the working environment.

There were only 2 cases of positive murine typhus infection found in this study, therefor factors related to theirs infections cannot be analyzed due to the low number of positive cases. However, antibody exposure to murine typhus were analyzed but no related factors were found to be statistically different.

Table 26 Factors related to scrub typhus infections in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province.

Factors	Scrub typhus infections		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 119)	Negative cases (%) (n = 274)				
Sex						
Male	74 (36.5)	129 (63.5)	7.580	0.006*	1.9	1.2-2.9
Female	45 (23.7)	145 (76.3)				
Age group						
≤25	47 (39.5)	176 (64.2)	20.685	< 0.001*		
>25	72 (60.5)	98 (35.8)			2.8	1.8-4.3
Hill tribe group						
Karen	47 (33.6)	93 (66.4)	5.751	0.089	2.7	0.9-8.2
Meo	14 (22.6)	48 (77.4)	df = 4**	0.495	1.5	0.5-5.2
Lahu	29 (33.3)	58 (66.7)		0.103	2.6	0.8-8.4
Lisu	25 (31.6)	54 (68.4)		0.137	2.4	0.8-7.8
Others (Akha, Lua, Palong)	4 (16.0)	21 (84.0)				
Marital status						
Single	41 (22.2)	144 (77.8)	10.911	0.001*	2.1	1.4-3.3
Maried and widow	78 (37.5)	130 (62.5)				
Occupation						
Agriculture	88 (40.0)	132 (60.0)	22.367	< 0.001*	3.1	1.9-4.9
Others	31 (17.9)	142 (82.1)				
Religion						
Buddhist	73 (29.7)	173 (70.3)	0.114	0.736		
Christian and others	46 (31.3)	101 (68.7)			1.1	0.7-1.7

Table 26 Factors related to scrub typhus infections in hill tribe patients studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Scrub typhus infections		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 119)	Negative cases (%) (n = 274)				
Education						
No	73 (39.7)	111 (60.3)	14.463	<0.001*	2.3	1.5-3.6
Yes	46 (22.0)	163 (78.0)				
Income (baht/month)						
≤ 2000	104 (31.0)	231 (69.0)	0.629	0.039*	1.3	0.7-2.4
> 2000	15 (25.9)	43 (74.1)				
Treatment hospital						
Samoeng	50 (38.8)	79 (61.2)	24.393	0.393	1.3	0.8-2.1
Mae taeng	6 (8.6)	64 (91.4)	df=3**	0.003*	0.2	0.1-0.5
Phrao	21 (30.4)	48 (69.6)		0.652	0.9	0.5-1.6
Chiang Dao	42 (33.6)	83 (66.4)				
Migration						
No	94 (29.2)	228 (70.8)	0.998	0.318		
Yes	25 (35.2)	46 (64.8)			1.3	0.8-2.3
Reason of migration						
To change place for agriculture	18 (66.7)	9 (33.3)	0.191	0.662	1.3	0.4-4.2
Others	7 (15.9)	37 (84.1)				
Activity after illness						
Nothing	60 (30.3)	138 (69.7)	0.000	0.992	1.0	0.7-1.5
Went to health care service and others	59 (30.3)	136 (69.7)				

Table 26 Factors related to scrub typhus infections in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Scrub typhus infection		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 119)	Negative cases (%) (n =247)				
Type of medicines bought.						
Antibiotic	0 (0.0)	5 (100)	F	0.302		
Analgesic and others	9 (29.0)	22 (71.0)			1.4	1.1-1.8
Clearing away the grass or shrubs or forests.						
No	23 (20.2)	91 (79.8)	7.766	0.005*		
Yes	96 (34.4)	183 (65.6)			2.1	1.2-3.4
Removal of garbage.						
Burning	108 (30.5)	246 (69.5)	0.088	0.766		
Burring	11 (28.2)	28 (71.8)			1.1	0.5-2.3
Having pets feeding.						
No	25 (27.8)	65 (72.2)	0.340	0.556		
Yes	94 (31.0)	209 (69.0)			1.2	0.7-1.9
Removal of insects.						
No	113 (31.7)	243 (68.3)	3.827	0.050	2.4	0.9-5.9
Yes	6 (16.2)	31 (83.8)				
Removal of reservoir hosts (rodents, rats and squirrels).						
No	117 (30.5)	266 (69.5)	F	0.730	1.8	0.4-8.4
Yes	2 (20.0)	8 (80.0)				
Used repellent for prevention of insects bite.						
No	111 (30.3)	255 (69.7)	0.006	0.939	1.0	0.5-2.4
Yes	8 (29.6)	19 (70.4)				

Table 26 Factors related to scrub typhus infections in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Scrub typhus infection		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 119)	Negative cases (%) (n =247)				
	(n = 113)	(n = 262)				
Travelling to work.						
On foot	109 (31.1)	242 (68.9)	2.209	0.137	2.3	0.8-6.8
By vehicle	4 (16.7)	20 (83.3)				
The place during break time at work.						
Cleared area and shanty	48 (20.0)	192 (80.0)	32.516	< 0.001*		
Grassy area	65 (48.2)	70 (29.8)			3.7	2.3-5.9
Dress when working.						
Covered cloth	49 (30.4)	112 (69.6)	1.956	0.162	1.0	0.4-2.3
Open cloth	64 (29.9)	150 (70.1)				
Working time.						
8.00-16.00	100 (29.8)	236 (70.2)	0.212	0.645		
8.00-20.00	13 (33.3)	26 (66.7)			1.2	0.6-2.4
Having shrubs, grass in living area.						
No	23 (17.7)	107 (82.3)	14.580	< 0.001*		
Yes	96 (36.5)	167 (63.5)			2.7	1.6-4.5
Seeing rats, rabbits, squirrels and chipmunks.						
No	8 (20.5)	31 (79.5)	1.956	0.162		
Yes	111 (31.4)	243 (68.6)			1.8	0.8-3.9
	(n = 111)	(n = 243)				
The number of reservoir hosts seen in each time.						
1-2 reservoir hosts / time	96 (31.2)	212 (67.4)	0.039	0.844		

Table 26 Factors related to scrub typhus infections in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Scrub typhus infection		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 119)	Negative cases (%) (n = 274)				
	(n = 111)	(n = 243)				
> reservoir hosts / time	15 (32.6)	31 (67.4)			1.1	0.5-1.8
Environment of working area.						
Field and forest	92 (35.5)	167 (64.5)	11.545	0.001*	2.5	1.5-4.3
In urban area	21 (18.1)	95 (81.9)				
	(n = 119)	(n = 274)				
Know a chigger						
No	96 (28.7)	239 (71.3)	2.833	0.092		
Yes	23 (39.7)	35 (60.3)			1.6	0.9-2.9
Know about cause of scrub typhus infection.						
No	118 (30.3)	271 (69.7)	F	1.000	1.3	0.1-12.7
Yes	1 (25.0)	3 (75.0)				
Having received the knowledge about scrub typhus infections.						
No	119 (30.7)	269 (69.3)	F	0.328	1.4	1.3-1.5
Yes	0 (0.0)	5 (100)				
Know about prevention of scrub typhus infection.						
No	117 (30.3)	269 (69.7)	F	1.000	1.1	0.2-5.7
Yes	2 (28.6)	5 (71.4)				

F = Fisher's exact test, * significant at *p*-value < 0.05, ** By Logistic Regression test

Table 27 Factors related to murine typhus antibody exposure in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province.

Factors	Murine typhus antibody exposure		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 23)	Negative cases (%) (n = 370)				
Sex						
Male	11 (5.4)	192 (94.6)	0.143	0.705		
Female	12 (6.3)	178 (93.7)			1.1	0.5-2.7
Age						
≤25	11 (4.9)	213 (95.1)	0.208	0.649		
>25	12 (7.0)	159 (93.0)			1.2	0.5-2.8
Hill tribe group						
Karen	12 (8.6)	128 (91.4)	6.088	0.446	2.3	0.3-18.1
Meo	1 (1.6)	61 (98.4)	df=4**	0.517	0.4	0.0-6.6
Lahu	3 (3.4)	84 (96.6)		0.896	0.9	0.1-8.6
Lisu	6 (7.6)	73 (19.7)		0.539	2.0	0.2-17.2
Others (Akha, Lua, Palong)	1 (4.0)	24 (96.0)				
Marital status						
Single	11 (5.9)	174 (94.1)	0.006	0.941	1.0	0.4-2.4
Maried and widow	12 (5.8)	196 (94.2)				
Occupation						
Agriculture	16 (7.3)	204 (92.7)	1.830	0.176	1.9	0.7-4.6
Others	7 (4.1)	166 (95.9)				
Religion						
Buddhist	18 (7.3)	228 (92.7)	2.561	0.110	2.4	0.8-6.2
Christian and others	5 (3.4)	142 (96.6)				

Table 27 Factors related to murine typhus antibody exposure in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Murine typhus antibody exposure		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 23)	Negative cases (%) (n = 370)				
Education						
No	9 (4.9)	175 (95.1)	0.580	0.446		
Yes	14 (6.7)	195 (93.3)			1.4	0.6-3.3
Income (baht/month)						
≤ 2000	18 (5.4)	317 (94.6)	F	0.359		
> 2000	5 (8.6)	53 (91.4)			1.7	0.6-4.7
Treatment hospital						
Samoeng	11 (8.5)	118 (91.5)	5.371	0.521	1.4	0.5-3.5
Mae taeng	1 (1.4)	69 (98.6)	df=3**	0.148	0.2	0.0-1.7
Phrao	3 (4.3)	66 (95.7)		0.557	0.7	0.2-2.6
Chiang Dao	8 (6.4)	117 (93.6)				
Migration						
No	20 (6.2)	302 (93.8)	F	0.780	1.5	0.4-5.0
Yes	3 (4.2)	68 (95.8)				
Reason of migration						
To change place for agriculture	2 (15.4)	11 (16.2)	F	0.111	8.5	0.7-100
Others	1 (1.7)	57 (98.3)				
Activity after illness						
Nothing	12 (6.1)	186 (93.9)	0.031	0.859	1.1	0.5-2.5
Went to health care service and others	11 (5.6)	184 (94.4)				

Table 27 Factors related to murine typhus antibody exposure in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Murine typhus antibody exposure		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 23)	Negative cases (%) (n = 370)				
	(n = 1)	(n = 35)				
Type of medicines bought						
Antibiotic	0 (0.0)	5 (100)	F	0.100		
Analgesic and others	1 (3.2)	30 (96.8)			1.0	0.9-1.1
Clearing away the grass or shrubs or forests.						
No	5 (4.4)	109 (95.6)	0 .627	0.429		
Yes	18 (6.5)	261 (93.5)			1.5	0.5-4.1
Removal the garbage						
Burning	19 (5.4)	335 (94.6)	F	0.268		
Burring	4 (10.3)	35 (89.7)			2.0	0.7-6.3
Having pets feeding						
No	9 (10.0)	81 (90.0)	3.645	0.056		
Yes	14 (4.6)	289 (95.4)			2.3	0.9-5.5
Removal of insects						
No	22 (6.2)	334 (93.8)	F	0.711	2.4	0.3-18.2
Yes	1 (4.3)	36 (97.3)				
Removal of reservoir hosts (rodents, rats and squirrels)						
No	22 (5.7)	361 (94.3)	F	0.457		
Yes	1 (1.0)	9 (90.0)			1.8	0.2-15.0
Use repellent for prevention from insects bite						
No	22 (6.0)	344 (93.0)	F	1.000		
Yes	1 (3.7)	26 (96.3)			1.7	0.2-12.8

Table 27 Factors related to murine typhus antibody exposure in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Murine typhus antibody exposure		χ^2	*p*-value	Odds Ratio	95% CI
	Positive cases (%) (n = 23)	Negative cases (%) (n = 370)				
	(n = 22)	(n = 353)				
Travelling to work						
On foot	20 (5.7)	331 (94.3)	F	0.643		
By vehicle	2 (8.3)	22 (91.7)			1.5	0.2-3.0
The place during break time at work						
Grassy area	11 (8.2)	124 (91.8)	1.988	0.159	1.9	0.8-4.4
Cleared area and shanty	11 (4.6)	229 (95.4)				
Dress when working						
Covered cloth	10 (6.2)	151 (93.8)	0.061	0.805	1.1	0.5-2.7
Open cloth	12 (7.0)	202 (94.4)				
Working time						
8.00-16.00	19 (5.7)	317 (94.3)	F	0.489		
8.00-20.00	3 (7.7)	36 (92.3)			1.4	0.4-4.9
Having shrubs, grass in living area						
No	6 (4.6)	124 (95.4)	0.540	0.463		
Yes	17 (6.5)	246 (93.5)			1.4	0.6-3.7
Seeing rats, rabbits, squirrels and chipmunks						
No	3 (7.7)	36 (92.3)	F	0.485		
Yes	20 (5.6)	334 (94.4)			1.4	0.2-2.5
	(n=20)	(n=334)				
The number of reservoir hosts seen in each time.						
1-2 reservoir host / time	18 (5.8)	290 (94.2)	F	1.000	1.4	0.3-6.1

Table 27 Factors related to murine typhus antibody exposure to scrub typhus in hill tribe studied subjects who visited 4 hospitals in Chiang Mai Province. (Continued)

Factors	Murine typhus antibody exposure		χ^2	*p*-value	Odds Ratio	95%CI
	Positive cases (%) (n = 23)	Negative cases (%) (n = 370)				
>2 reservoir hosts/ time	2 (4.3) (n=22)	44 (95.7) (n=353)				
Environment of working area.						
Field and forest	17 (6.6)	242 (93.4)	0.437	0.391	1.6	0.6-4.3
In urban area	5 (4.3)	111 (95.7)				
Know a mite.						
No	16 (5.3)	286 (94.7)	0.728	0.394		
Yes	7 (7.7)	84 (92.3)			1.5	0.6-3.7
Know about cause of murine typhus infections.						
No	23 (5.9)	366 (94.1)	F	1.000	1.1	1.0-1.1
Yes	0 (0.0)	4 (100)				
Having received knowledge about murine typhus infections.						
No	23 (5.9)	365 (94.1)	F	1.000	1.1	1.0-1.1
Yes	0 (0.0)	5 (100)				
Know about prevention of murine typhus infections.						
No	22 (5.7)	364 (94.3)	F	0.347		
Yes	1(14.3)	6 (85.7)			2.8	0.3-23.9

F = Fisher's exact test, * significant at *p*-value < 0.05, ** By Logistic Regression test

8. The level of IgM and IgG geometric mean titers in all hill tribe patients.

The IgM and IgG geometric mean titers (GMT) of 393 acute sera and 199 convalescence sera in each strain were classified by sex, age group, occupation, education, income, migration, working time, environment of working area, hill tribe group and treatment hospitals as shown in Table 28. The results found that IgM and IgG GMT were found higher in male than female, and the highest GMT were found in the age group >25 years, agriculture, haven't education, low income, working time in 8.00 am-20.00 pm. and environment of working area were in field and forest.

Table 28 Geometric mean titers (GMT) of IgM antibodies responsed to each strain of scrub typhus in acute and convalescence sera.

Factors	No. of cases (n=393)	Acute IgM GMT±SD			No. of cases (n=199)	Convalescence IgM GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Sex								
Male	203 (51.6)	36.2±2.7	31.8±2.4	31.9±2.4	101 (50.8)	38.3±2.5	34.3±2.3	33.3±2.3
Female	190 (48.4)	29.0±1.6	31.4±2.2	35.6±2.5	98 (49.2)	31.8±2.3	30.9±2.1	30.5±2.2
Age								
0-9	48 (12.2)	38.0±3.7	34.3±3.2	34.8±3.3	21 (10.6)	32.6±2.3	32.5±2.3	32.6±2.3
10-19	126 (32.1)	34.2±2.6	34.0±2.6	33.1±2.4	67 (33.7)	29.5±2.0	29.2±1.9	28.0±1.7
20-29	82 (20.9)	36.6±2.7	31.1±2.2	32.5±2.5	45 (22.6)	41.6±2.9	36.2±2.8	38.5±2.9
30-39	71 (18.0)	36.9±2.5	31.9±2.2	29.8±2.1	39 (19.6)	38.9±2.3	35.7±2.0	34.4±2.2
40-49	31 (17.9)	44.7±3.0	33.4±2.2	33.4±2.1	13 (6.5)	32.6±1.8	26.4±1.8	26.4±2.1
50-59	18 (4.6)	34.0±2.7	34.0±3.1	32.7±3.1	8 (4.0)	54.5±4.7	50.0±3.8	50.0±3.8
≥ 60	17 (4.3)	29.4±1.7	26.0±1.8	26.0±1.8	6 (3.0)	25.0±1.0	25.0±1.0	25.0±1.0
Education								
No	184(46.8)	39.6±2.9	33.1±2.7	33.3±2.6	87(63.3)	36.1±2.4	32.5±2.1	32.3±2.1
Yes	209(53.2)	33.4±2.6	31.8±2.3	31.3±2.3	112(36.7)	34.1±2.4	32.6±2.3	32.4±2.3

Table 28 Geometric mean titers (GMT) of IgM antibodies responsed to each strain of scrub typhus in acute and convalescence sera. (Continued)

Factors	No. of cases (n=148)	Acute IgM GMT±SD			No. of cases (n=199)	Convalescence IgM GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Income (bath / month)								
≤ 2000	335 (85.2)	36.6±2.8	32.9±2.5	32.5±2.5	163 (81.9)	35.1±2.5	33.4±2.3	33.0±2.3
> 2000	58 (14.8)	33.7±2.2	31.4±2.2	30.6±2.2	36 (18.1)	34.0±2.2	29.2±1.8	29.7±2.0
Migration								
No	322 (81.9)	35.7±2.7	32.8±2.5	32.5±2.5	168 (84.4)	34.9±2.5	32.8±2.3	32.6±2.3
Yes	71 (18.1)	38.0±2.9	30.5±2.6	31.0±2.2	31 (15.6)	35.0±2.1	31.3±1.9	31.3±1.9
Working time								
8.00-16.00	336 (89.6)	36.4±2.8	32.9±2.5	32.1±2.4	170 (88.5)	35.4±2.4	33.3±2.2	32.7±2.2
8.00-20.00	39 (10.4)	38.3±2.7	36.3±5.31	35.7±2.9	22 (11.5)	34.3±2.4	30.2±2.1	31.2±2.4
Environment of working area								
Field and forest	259 (69.1)	37.9±2.8	32.8±2.5	32.4±2.4	130 (67.7)	36.7±2.5	33.3±2.2	33.3±2.4
In urban area	25 (17.7)	33.9±2.8	32.8±2.5	32.5±2.6	62 (32.3)	32.3±2.3	31.9±2.2	30.9±2.1

Table 28 Geometric mean titer(GMT) of IgM antibodies responsed to each strain of scrub typhus in acute and convalescence sera. (Continued)

Factors	No. of cases (%) (n=393)	Acute sera IgM GMT±SD			No. of cases (%) (n=199)	Convalescence sera IgM GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Occupation								
Agriculture	219 (55.7)	40.1±3.0	34.5±2.6	33.7±2.6	116 (58.3)	37.9±2.6	34.5±2.3	34.5±2.5
To look for something from the forest	1 (0.3)	25.0±1.0	25.0±1.0	25.0±1.0	0 (0.0)	-	-	-
Employee	45 (11.4)	28.7±1.5	27.0±1.4	26.6±1.3	25 (12.6)	27.9±1.4	25.0±1.0	25.0±1.0
Business	1 (0.3)	25.0±1.0	25.0±1.0	25.0±1.0	0 (0.0)	-	-	-
Student	109 (27.7)	33.9±2.9	32.4±2.6	32.9±2.7	51 (25.6)	31.9±2.4	31.9±2.2	31.9±2.1
Do not work	18 (4.6)	28.1±1.6	28.1±1.6	28.1±1.6	7 (3.5)	37.0±2.2	37.2±2.9	37.2±2.2

Table 28 Geometric mean titers (GMT) of IgM antibodies responsed to each strain of scrub typhus in acute and convalescence sera. (Continued)

Factors	No. of cases (n=393)	Acute IgM GMT±SD			No. of cases (n=199)	Convalescence IgM GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Hill tribe group								
Karen	140 (35.6)	32.3±2.0	30.2±2.6	29.0±1.8	84 (42.2)	39.4±2.6	35.6±2.4	34.8±2.5
Meo	62 (15.8)	33.8±2.6	32.2±2.6	33.2±2.4	27 (13.6)	34.9±2.8	32.3±2.3	33.2±2.2
Lahu	87 (21.1)	42.0±3.5	36.4±3.2	35.5±3.0	37 (18.6)	30.7±1.8	28.5±1.6	28.5±1.6
Lisu	79 (20.1)	39.1±3.2	35.3±2.8	35.3±2.8	43 (21.6)	30.8±2.1	30.3±2.1	30.3±2.1
Akha	9 (2.3)	42.9±5.0	42.0±5.0	42.9±5.0	4 (2.0)	50.0±4.0	50.0±4.0	50.0±4.0
Lua	9 (2.3)	39.7±2.2	25.0±1.0	25.0±1.0	3 (1.5)	25.0±1.0	25.0±1.0	25.0±1.0
Palong	7 (1.8)	36.1±1.3	25.0±1.0	25.0±1.0	1 (0.5)	25.0±1.0	25.0±1.0	25.0±1.0
Treatment hospital								
Samoeng	129 (32.8)	37.4±2.6	33.8±2.4	32.1±2.3	75 (37.7)	38.6±2.6	35.2±2.3	34.2±2.3
Mae taeng	70 (17.8)	26.7±1.4	26.8±1.4	26.0±1.3	37 (18.6)	25.0±1.0	25.0±1.0	25.0±1.0
Phrao	69 (17.6)	36.6±3.0	31.8±2.3	33.1±2.5	33 (16.6)	32.8±2.0	30.2±1.7	30.8±1.9
Chiang Dao	125 (31.8)	41.2±3.4	35.6±3.1	35.9±3.1	54 (27.1)	39.7±2.9	36.8±2.8	36.7±2.6

Table 29 Geometric mean titers (GMT) of IgG antibodies responsed to each strain of scrub typhus in acute and convalescence sera.

Factors	No. of cases (n=393)	Acute IgG GMT±SD			No. of cases (n=199)	Convalescence IgG GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Sex								
Male	203 (51.6)	56.7±4.0	64.7±4.5	52.6±3.6	101 (50.8)	87.2±5.1	97.9±5.7	78.5±4.9
Female	190 (48.4)	53.0±3.9	53.4±4.3	47.7±3.6	98 (49.2)	58.4±4.6	55.6±4.6	51.4±3.9
Age								
0-9	48 (12.2)	37.5±2.6	36.4±2.6	33.9±2.2	21 (10.6)	41.0±3.7	38.4±2.9	39.7±3.3
10-19	126 (32.1)	43.8±3.3	47.1±3.8	40.8±3.0	67 (33.7)	33.7±2.4	34.8±2.5	30.7±1.8
20-29	82 (20.9)	26.7±1.8	67.8±4.9	52.1±3.7	45 (22.6)	87.0±5.5	91.2±6.3	78.6±6.3
30-39	71 (18.0)	64.4±4.0	73.1±5.0	63.8±4.2	39 (19.6)	123.8±5.3	130.5±6.2	109.3±4.6
40-49	31 (17.9)	107.2±5.8	87.4±5.5	73.1±4.6	13 (6.5)	261.1±5.7	222.5±6.0	161.6±4.5
50-59	18 (4.6)	89.1±5.8	103.9±6.4	79.4±5.1	8 (4.0)	168.2±8.1	218.1±8.2	183.4±7.7
≥ 60	17 (4.3)	78.3±4.6	69.3±4.5	66.5±4.1	6 (3.0)	282.9±7.4	400.0±5.5	282.8±4.5
Education								
No	184 (46.8)	73.5±4.4	75.9±4.9	51.6±3.7	87 (43.7)	117.3±6.0	116.3±6.1	62.6±4.3
Yes	209 (53.2)	44.7±3.4	47.3±3.9	47.9±3.5	112 (56.3)	48.8±3.6	52.2±4.2	45.1±3.6

Table 29 Geometric mean titer(GMT) of IgG antibodies responsed to each strain of scrub typhus in acute and convalescence sera. (Continued)

Factors	No. of cases (%) (n=393)	Acute sera IgG GMT±SD			No. of cases (%) (n=199)	Convalescence sera IgG GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Occupation								
Agriculture	219 (55.7)	74.8±4.6	79.9±5.4	64.6±4.4	116 (58.3)	106.8±5.7	110.0±6.2	93.3±5.4
To look for something from the forest	1 (0.3)	25.0±1.0	25.0±1.0	25.0±1.0	0 (0.0)	-	-	-
Employee	45 (11.4)	40.9±3.0	39.7±3.0	36.2±2.5	25 (12.6)	38.9±2.7	43.5±3.4	37.9±2.5
Business	1 (0.3)	25.0±1.0	25.0±1.0	25.0±1.0	0 (0.0)	-	-	-
Student	109 (27.7)	37.8±2.7	39.0±2.9	34.8±2.3	51 (25.6)	37.6±3.0	36.6±2.8	33.7±2.4
Do not work	18 (4.6)	50.0±3.6	54.0±3.8	52.0±3.4	7 (3.5)	90.6±5.3	121.9±4.9	90.6±5.3

Table 29 Geometric mean titers (GMT) of IgG antibodies responsed to each strain of scrub typhus in acute and convalescence sera. (Continued)

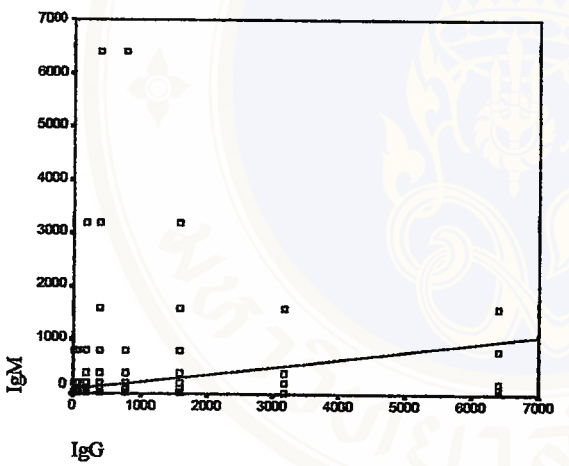
Factors	No. of cases (n=393)	Acute IgG GMT±SD			No. of cases (n=199)	Convalescence IgG GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Income (bath / month)								
≤ 2000	335 (85.2)	57.9±3.9	60.5±4.5	23.3±3.6	163 (81.9)	80.5±5.3	82.2±5.6	71.5±4.6
> 2000	58 (14.8)	48.2±3.8	51.2±4.2	43.8±3.6	36 (18.1)	42.1±2.8	46.3±3.4	39.2±3.5
Migration								
No	322 (81.9)	56.8±3.9	60.9±4.6	51.0±3.7	168 (84.4)	66.5±4.8	69.8±5.2	59.3±4.5
Yes	71 (18.1)	54.7±3.8	51.0±3.7	46.7±3.2	31 (15.6)	106.9±5.2	102.2±5.2	97.8±4.4
Working time								
8.00-16.00	336 (89.6)	57.0±3.9	58.7±4.4	50.1±3.6	170 (88.5)	71.6±5.1	71.3±5.2	63.9±4.4
8.00-20.00	39 (10.4)	53.8±3.8	64.1±5.2	50.0±3.6	22 (11.5)	58.5±3.5	82.8±5.8	60.4±3.7
Environment of working area								
Field and forest	259 (69.1)	67.3±4.4	71.4±5.1	59.4±4.1	130 (67.7)	94.3±5.5	97.9±6.1	83.6±5.2
In urban area	116 (30.9)	38.7±2.8	39.1±2.9	34.3±2.3	62 (32.3)	37.4±2.9	38.7±2.9	33.8±2.3

Table 29 Geometric mean titers (GMT) of IgG antibodies responsed to each strain of scrub typhus in acute and convalescence sera. (Continued)

Factors	No. of cases (n=393)	Acute IgG GMT±SD			No. of cases (n=199)	Convalescence IgG GMT±SD		
		Gilliam	Karp	Kato		Gilliam	Karp	Kato
Hill tribe group								
Karen	140 (35.6)	55.7±4.0	57.7±4.3	48.5±3.4	84 (42.2)	92.1±5.2	106.8±5.9	86.2±4.9
Meo	62 (15.8)	67.0±3.9	55.9±4.8	47.8±3.8	27 (13.6)	43.9±4.1	43.9±4.1	36.1±4.2
Lahu	87 (21.1)	64.5±4.3	71.0±5.2	61.5±4.5	37 (18.6)	66.2±4.9	68.7±5.5	61.4±4.3
Lisu	79 (20.1)	56.6±3.7	56.5±3.9	48.7±3.3	43 (21.6)	69.0±4.9	61.7±4.3	58.7±3.9
Akha	9 (2.3)	39.7±2.7	39.7±2.7	39.7±2.7	4 (2.0)	50.0±4.0	50.0±4.0	50.0±4.0
Lua	9 (2.3)	73.4±5.1	79.3±3.9	42.9±3.1	3 (1.5)	25.0±1.0	25.0±1.0	25.0±1.0
Palong	7 (1.8)	30.5±1.7	27.6±1.3	27.1±1.3	1 (0.5)	50.0±3.3	25.0±1.0	50.0±4.0
Treatment hospital								
Samoeng	129 (32.8)	64.4±4.2	74.8±5.3	55.7±3.8	75 (37.7)	114.9±5.9	130.7±6.7	96.4±5.4
Mae taeng	70 (17.8)	42.3±4.3	31.4±2.5	30.2±2.1	37 (18.6)	25.9±4.6	25.9±4.6	23.8±1.8
Phrao	69 (17.6)	53.1±3.5	53.6±3.7	48.0±3.4	33 (16.6)	56.7±3.6	56.7±3.8	64.3±3.8
Chiang Dao	125 (31.8)	67.1±4.3	62.3±4.8	61.4±4.2	54 (27.1)	85.7±5.4	81.4±5.3	71.6±4.3

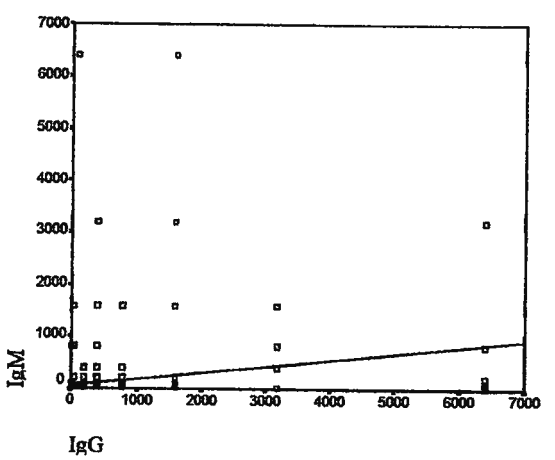
The IgM and IgG antibodies of each serum in the acute and convalescent phase of each strain were plotted on graph to see the correlation (r) for each strain. It was found that the correlation of three strains were not so much different. IgM and IgG of Karp strain was found the highest correlate more than the other two remaining strains. The details are shown in Figure 6.

Figure 6 The correlation of IgM and IgG antibodies response to scrub typhus classified by strains (Gilliam, Karp and Kato) and murine typhus. (A→D)



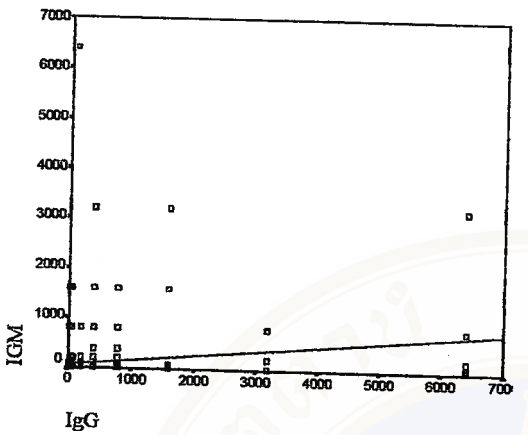
(A) Gilliam strain

r = 0.186



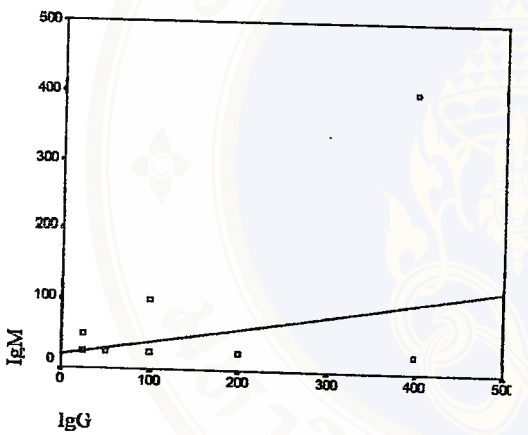
(B) Karp strain

r = 0.215



(C) Kato strain

r = 0.199



(D) Murine typhus

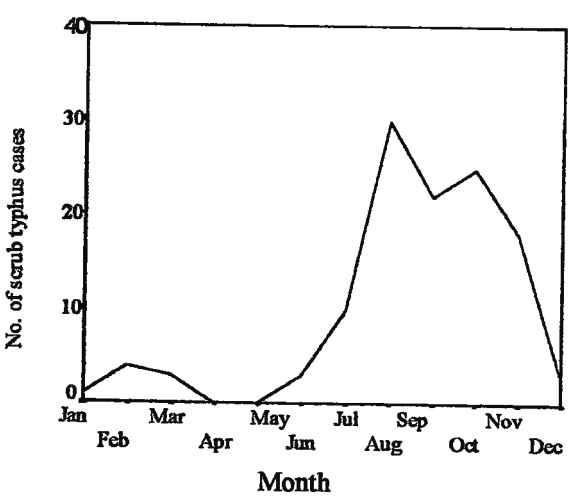
r = 0.435

Table 30 shows the number of positive scrub typhus infection cases classified by month. The results show that the highest positive scrub typhus infections are found in August (25.2%), October (21.0%), and September (18.5%), respectively. No positive cases were found during April to May. The highest positive rates were confirmed and found in August.

Table 30 Number of positive scrub typhus infections, confirmed by the laboratory classified by month.

Month	No. of examined cases (%)	No. of positive cases (%)	Positive rate %
January	6 (1.5)	1 (0.9)	16.7
February	10 (2.5)	4 (3.4)	40.0
March	16 (4.1)	3 (2.5)	18.8
April	2 (0.5)	0 (0.0)	0.0
May	1 (0.3)	0 (0.0)	0.0
June	15 (3.8)	3 (2.5)	20.0
July	32 (8.1)	10 (8.4)	31.3
August	71 (18.1)	30 (25.2)	42.3
September	63 (16.0)	22 (18.5)	34.9
October	90 (22.9)	25 (21.0)	27.8
November	63 (16.0)	18 (15.1)	28.6
December	24 (6.1)	3 (2.5)	12.5
Total	393(100.0)	119(100.0)	30.3

Figure 7 The number of positive cases scrub typhus classified by month.



On analysis of the number of IgM positive sera (titers ≥1:400), it was found that 38 sera from 29 cases of acute and convalescent phase could detected by IFA (4 cases or 5 sera showed IgM positive, and 25 cases, or 33 sera, showed IgM and IgG positive), as shown in Table 31. Almost all of these IgM positive sera (79.0% or 30/38 sera) were detected during day 1 up to day 17. In positive cases the number of days (between acute phase and convalescent phase) was detected by being IgM positive; 1 case on day 21 and IgG positive 1 case on day 129. Either positive IgM or IgG antibodies were commonly detected on day 3 to day 6 from the onset of the disease. The details are shown in Table 32.

Table 31 Number of positive IgM and IgG (titers ≥ 1:400) scrub typhus cases and sera assay by IFA.

Group of serum	No. of IgM positive		No.of IgM & IgG positive		No.of IgG positive	
	cases	serum	cases	serum	cases	serum
Pair serum group						
A +, C -	2	2	3	3	6	6
A- , C+	1	1	3	3	18	18
A+, C+	1	2	8	16	29	58
Single serum group						
A+	0	0	11	11	26	26
Total	4	5	25	33	79	108

Note; A = acute sera. C = convalescent sera.
+ = positive cases. - = negative cases.

Table 32 Number of positive IgM and IgG (titers ≥ 1:400) scrub typhus sera according to the day after onset of the disease.

Duration of serum withdraw from onset (days)	No.of positive detection			Duration of serum withdraw from onset (days)	No.of positive detection		
	IgM sera	IgM & IgG sera	IgG sera		IgM sera	IgM & IgG sera	IgG sera
1	0	0	1	27	0	0	2
2	1	0	1	28	0	0	1
3	1	4	12	29	0	0	1
4	0	9	19	30	0	1	1
5	0	3	10	31	0	0	1
6	0	4	9	41	0	1	0
7	1	0	5	44	0	0	1
8	0	1	6	45	0	0	1
9	0	1	1	47	0	0	1
11	0	0	3	50	0	0	1
13	0	1	0	51	0	0	1
14	0	1	0	53	0	0	1
15	0	1	4	56	0	0	1
16	0	1	1	65	0	1	0
17	0	1	3	67	0	0	1
18	0	0	4	68	0	1	0
19	0	0	3	74	0	0	1
20	1	1	3	82	0	0	1
21	1	1	1	92	0	0	1
23	0	0	1	111	0	0	1
26	0	0	1	129	0	0	1
		Total			5	33	108

9. Percentage of positive IgM and IgG antibodies in all hill tribe patients response to each strain of scrub typhus.

The IgM and IgG antibodies response were found highest in co-infected of Gilliam, Karp and Kato strains either in acute or convalescent sera. Karp strain gave the highest monotypic response found in positive infection cases. The details are shown in Table 33.

Table 33 Number of positive IgM and IgG antibodies rickettsiosis infection cases in acute and convalescent sera of scrub typhus and murine typhus.

Positive rickettsiosis	Positive in acute sera			Positive in convalescent sera		
	IgM cases (%)	IgM & IgG cases (%)	IgG cases (%)	IgM cases (%)	IgM & IgG cases (%)	IgG cases (%)
Scrub typhus						
Gilliam	2 (25.0)	2 (11.8)	3 (4.7)	1 (25.0)	0 (0.0)	2 (4.2)
Karp	0 (0.0)	0 (0.0)	8 (12.3)	1 (25.0)	0 (0.0)	3 (6.2)
Kato	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	2 (4.2)
Gilliam+Karp	0 (0.0)	0 (0.0)	13 (20.0)	0 (0.0)	0 (0.0)	5(10.4)
Gilliam+Kato	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
Karp+Kato	0 (0.0)	0 (0.0)	2 (3.1)	0 (0.0)	0 (0.0)	2 (4.2)
Gilliam+Karp + Kato	5 (62.5)	15 (88.2)	37 (56.9)	2 (50.0)	10(100.0)	32(66.6)
Murine typhus	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	1 (2.1)
Total	8(100.0)	17(100.0)	65 (100.0)	4(100.0)	10(100.0)	48(100.0)

The IgM and IgG GMT were calculated according to each strain of scrub typhus infection and to murine typhus infection. Acute sera showed IgM GMT sera greater than convalescent sera, in contrast with IgG GMT which was showed greater in convalescent than acute sera. IgM GMT was the highest in Gilliam strains and IgG GMT was the highest in Karp strains. A statistical difference (p-value < 0.001) was found when comparing GMT in acute and convalescent phase serum for each antigen.

Table 34 The IgM GMT and IgG GMT sera to scrub typhus and murine typhus infection in acute and convalescent sera.

Strains	Acute sera		Convalescent sera	
	IgM	IgG	IgM	IgG
	GMT±SD	GMT±SD	GMT±SD	GMT±SD
Scrub typhus				
Gilliam	36.0±2.7	56.4±3.9	34.9±2.4	71.6±4.9
Karp	32.6±2.4	59.0±4.4	32.6±2.2	74.1±5.2
Kato	32.2±2.4	50.2±3.6	32.4±2.2	64.1±4.5
Murine typhus	25.4±1.0	26.8±1.4	25.5±1.0	27.5±1.5
p-value	< 0.001*		< 0.001*	

* Significant by Friedman test at p-value < 0.001.

CHAPTER V

DISCUSSION

The demographic data of hill tribe patients in Chiang Mai Province from this study indicates that this group of people had very low income and low education. These main problems lead to theirs poor health status and unhygienic life style, and contribute to their low immune status which may lead to infection with several infectious diseases as reported in many studies in hill tribe people (108,109,110,111,112,113). The general characteristic data reported in this study will be useful for the health personnel who are responsible in these areas, to improve their health status and life style, as well as to prevent and control scrub typhus and murine typhus in the future.

The common symptoms and signs found in this study were fever (100.0%), anorexia (75.6%), headache (73.1%) muscle pain (55.5%), jaundice (12.6%), rash (10.1%) and injected eye (3.4%). These findings were similar to others previous studies of scrub typhus and murine typhus (114,115,116,117). Eschar, which was the typical symptom of scrub typhus was found in 15.1%, these occurred and varied considerably depending on the patients location. However, eschar and rash are still generally regarded as typical of the infection (118).

In this study the prevalence of antibodies exposure (titers ≥ 1:50) to scrub typhus in Chiang Mai Province was 37.4 % (147/393). Several studies report this prevalence for scrub typhus. In the northern part of Thailand, where the temperature, climate and environment are similar to Chiang Mai Province, the prevalence of antibodies exposure reported using an initial screen of antibody titers 1:10 by IFA was 59.0 % (80). However, they can not be compared with each other due to the difference in cut off levels. In the rural village of Prajinburi Province, which is located in the central part of Thailand, it was found that the prevalence of antibodies exposure, using the same assay and same screening point level (1:50), was 77.0 % (78). Prajinburi Province is located in a moist and warm area, which was more suitable and preferable for growth of the chigger vectors and *O. tsutsugamushi* agents (119). In addition, people in rural village of this province had high chances of contact with deep forest, which may lead to exposure and infection with scrub typhus more than hill tribe patients in Chiang Mai Province who are located in a dry and cool (119). Many areas of forests and several trees have already been cleared away by hill tribe peoples, and are not a good environment for the life cycle of this disease. The other possibility may be that the sandal shoes and open clothing bares the arms and legs of rural people in Prajinburi Province, allowing a higher chance of chigger bite, while hill tribe people always wear boots and covered clothes. The same comparisons were done recently, when analyzing the prevalence of antibodies (59.5%) in pyrexial patients at malaria clinics in three western provinces along the Thai Myanmar border (120). However, it was less than when compared with people who stayed in urban areas according to several studies. A rate of 21% was found in suburban Bangkok (by IIP, initial screening at 1:50) (106), and 0.4 – 4% (by IFA, initial screening 1:50) by month in

Thai soldiers who lived and worked near the Thai Cambodia border in Srisaket Province at the north eastern part of Thailand (121).

When compared with the prevalence of antibodies exposure of scrub typhus in other countries within Asia, this study found a higher percentage. Prevalence was found to be 0.8% (by IFA, initial screening at 1:50) in the rural population of Sabah, east Malaysia (122), and 32% by the same assay at initial screening of 1:40 in Pescardares Islands, Taiwan (83). In addition, 1.3% were found in Malang, Indonesia by ELISA at initial screening 1:100 (123), and 8% by the same assay and initial screening point in the military personnel of Indonesia who worked on the peace keeping operations in Cambodia (91). There was one study reported in aborigines of Malaysia according to their habitat (by IFA, initial screening at 1:50), which found a prevalence rate of 25%, 3% and 64% in fringe areas, Kampong areas and deep jungle areas, respectively. It was possible that deep jungle dwellers acquired their infection in small clearings in which they had built their houses as opposed to fringe area and Kampong area people. The other possibility why the risk is higher in small clearing in the jungle than in the similar area in the fringe forest was the majority of infections were actually contracted in the jungle itself rather than in the clearings (82).

The prevalence of active scrub typhus infection assayed by IFA in this study was 30.3%. Since the blood samples were comprised of either single serum or paired sera, these prevalence rates may less than ideal. In cases where convalescent phase single serum can be collected, a positive four fold rising interpretation may find higher numbers, and especially for antibodies titers in acute phase serum between the range ≥ 1:50 to 1:200 which may be found to be positive. Furthermore, the positive infection

cases of the single serum group interpreted in this study were cut off at ≥ 1:400, which was the same as in several previous studies with the same assay (42,60). However, this prevalence was higher when compared to other reports. The reason may be explained by the predominant occupation and nearby habitat of hill tribe patients (68).

The study performed in 1988 among subjects in Thai Kampuchea border displaced persons camps found 11.9% of scrub typhus infection from febrile illness (116), while in 1989 a studyfound 5.4% in a camp for displaced Khmer people who had temporary settlements on the Thai side of the Thai Cambodian border (105). However, even though both of these investigations used IIP assay which was a different assay, but was interpreted the same as IFA defined positive; ≥ 4-fold rise in antibody titers to ≥ 1:200 or a four 4-fold rise antibody from ≥ 1:200 in either IgM or IgG antibodies. Statistically, there was no significant difference between the two methods (43). During 1975-1979, prevalence rates of 23.3% and 13.7% (interpreted by both the IFA and Weil Felix test) were reported in febrile illness patients in central west Malaysia and in rural of central Penisular Malaysia, respectively (114, 69). Both studies showed that scrub typhus infection was the most common cause of febrile illness. In the southern part of Thailand, scrub typhus rates of 4.9% were reported in Yala Hospital (124). Cases were confirmed by the Weil-Felix test using cut off point of ≥ 1:320. The sensitivity and specificity of this test was found 50% and 97%, respectively (42,125).

In this study, the prevalence of active scrub typhus infection increased according to the age group. This trend is in agreement with the reports of prevalence in each age

group, in the whole country, by the National epidemiological surveillance for scrub typhus (126). It seems that this disease is the disease of adults or working groups. Moreover, the highest prevalence rates were found in agricultural occupations. This result confirmed that scrub typhus still a rural disease problem. Lisu was one of the hill tribe groups which had the highest prevalence of scrub typhus infection. It would be interesting for further studies to see if their life style and culture is the reason why this group had highly positive infected cases, and whether it might be related to their infection.

The prevalence of murine typhus antibodies exposure found in this study was 5.9%. The rate was not high, confirming that murine typhus is still an urban disease rather than rural. In 1989, the prevalence of antibody exposure was assayed by CF test at an initial screening point of 1:10. The reported stated reported that in Chiang Rai Province, Ubon Ratchathani Province and Korat Province prevalence rates were 20.6%, 11.1% and 5.8% respectively (101). In 1994 a report from suburban Bangkok found 8 % by IIP at initial screening of 1:80 (106). Antibody exposure prevalence was compared in Tainan of Taiwan, Chiang Rai Province of Thailand in 1983-1989 by IIP at initial screening of 1:20, and found rates of 23.9%, 2.5%, respectively (104). Several studies of murine typhus antibodies exposure were also reported from Southeast Asia Countries. In Indonesia, 15% was reported from Java Island (determined at screening point titers of CF $\geq$ 1:8 and IFA $\geq$ 1:80) (127), 34.7% from Java, Sumatra and Malang (by ELISA screening at 1:100), (123) and 14.7% from Java Island (by IFA initial screening at 1:50) (116), while 37% was found in Indonesian military personnel (by ELISA screening at 1:100) who participated in peace keeping

operations in Cambodia (91). In addition high prevalence of murine typhus was found 45% in human and 35.0% in rodents populations of West Malaysia (128). All of those reported, including this study, showed that murine typhus is still emerging disease in tropical countries, especially in Asia and Southeast Asia.

The prevalence rates of active murine typhus infections of hill tribe patients by IFA was 0.5 %. In southern Thailand, during 1983 through 1985, 11 from 166 cases, or 6.6%, of murine typhus infection were seen at Songkhlanagarind and Hat Yai Hospitals (102), while during 1987 to 1988, 5.8% of prevalence was found in Yala Province. Both were detected by Weil Felix test at an initial screen of ≥ 1:320 or 4-fold rising titers (124). Murine typhus infection was also identified as a major cause of febrile illness in 1988 and 1989 by IFA assay on the Thai Kampuchean border, at displaced person camps in Thailand; 58.0% and 70.3% were found, respectively (116,105). This camp and surrounding areas were populated with a highly density of Khmers. Many residents from the camp had noted an increase in the rat population. Such settlements are unfortunately, found in tropical and subtropical regions worldwide, and are a cause of high numbers of murine typhus infection when compared with hill tribe people in rural areas.

In this study, both the prevalence of scrub typhus infection and scrub typhus antibodies exposured in hill tribe patients were higher than both prevalence of murine typhus infection and murine typhus antibodies exposure. This was due to these hill tribe patients living on environment is close to forest, trees and shrubs, which are the normal habitat for chiggers; transmission vectors of scrub typhus. Almost all of their

occupations was agriculture, which provied a high chance of contract with infected chiggers. Murine typhus was the urban disease transmitted from flea; a few infected cases were found possibly, because of less suitable mammal reservoirs surrounding their houses, or a lack of flea. It had been reported that murine typhus is particularly high during hot and dry season (105); this may possible be a reason for the low numbers in this study.

There are no reports on the factors related to scrub typhus infection, although several studies have reported scrub typhus antibody exposure which are in agreement with the factors related to scrub typhus infection in this study. A sero-epidemiological survey of scrub typhus antibody exposure in a rural Thai village in Prajinburi Province during three months in 1976 found that age of samples especially in males of 15 years and older was significantly different to the younger male population (p-value < 0.001). The rates in these young males were less than that of theirs female contemporaries (p-value < 0.05) (78). A related significance of scrub typhus antibody exposure in the human population of suburban Bangkok were found in gender, age group and occupation (106). This studied found antibodies more often in women than men, in older age groups than younger, and in agricultural workers who had contacted with orchards and orchid farms more often than reporting no contact. In the Pescadores Islands of Taiwan, a changing risk of scrub typhus exposure was associated with social economic development (83). The change in social condition and age in the rural residents was a higher than in the urban residents (p-value < 0.001). In addition, occupation, terrain and nearby habitat were predominant factors for scrub typhus antibodies found in residents of west Malaysia (68). Populations having the

predominant occupation, and spent a great deal of time in forested areas (rubber tappers, field workers) had an extremely higher risk than in urban type occupations (office workers, shop keepers, drivers, watchman, clerks).

No factors related to murine typhus antibodies exposure were found from this study. In some reports from Indonesia Javanese males who were more than 30 years old had a higher risk of risk in exposure than those who were younger (p-value < 0.005) (127). Moreover, wild rodents for murine typhus in Java Island of Indonesia had significantly higher positive antibodies exposure in the dry season than in the rainy season (p-value < 0.01) (129). One of the reports on sero-epidemiological evidence for murine typhus infection in civilian personnel who resided within three Malang neighbourhoods of Indonesia, representing urban, suburban and rural communities found prevalence of infection in the communities significantly higher according to the degree the of urbanization (p-value < 0.05) (113). Unfortunately, murine typhus infection in this study did not analyze related factors because 12 positive cases of active infection were only found, which could not be analyzed to find any statistical significant factors.

Actually, cross sectional sampling was the research design for this study, and sample size was calculated according to prevalence of previous reports. However, after laboratory assay was done, case findings were found either in the antibodies exposure or in the active infection of scrub typhus and murine typhus groups. The factors, which are related to scrub typhus antibodies exposure, scrub typhus infection and murine typhus antibodies exposure with statistical significant difference, were the

knowledge gain finding after analyzed with the data from interviewed questionnaires. Several equivalents were not found to be significant. This does not mean that it was not the measured factors, but that the sample size was not high enough to cover all risk factors. To find out these related factors, a case control study should be recommended, and sample size should be calculated from the odds ratio of previously studied factors.

The high GMT found in acute sera for IgM and in convalescent sera for IgG, could be explained by antibodies produced by immunological knowledges. The IgM is the first antibody produced after infection and could be detected in the early or acute stage of the disease, while the IgG antibodies were produced later which could be detected in the late or convalescent phase (130,131,132). All of positive sera from this study showed that the IgM antibodies were easily detected in the first month after the onset of the disease. This also expresses that the IgM antibody was is the first antibody detected, and that it can persist in the early stages up to one month after the onset of the disease.

The antibody responde of either in IgM or IgG was found almost in all trivalent strains (Gilliam, Karp and Kato). For IgM in acute and convalescence were 62.5% and 50.0%, while in IgG were 56.9% and 66.6% respectively. The results confirmed that these three strains are still predominant in Thailand. Furthermore, Karp and Gilliam were the bivalent strains common found in this study, this was in agreement with the previous reports where found that both strains were predominant co-infecting strains (120,133,134). In contrast, a monovalent responded was highest in Gilliam

strain for IgM antibodies and Karp strain for IgG antibodies. It would be very interesting for further studies to find out if it would be the same monovalent strain found in antibodies from the rodent trapping at the same location or not.

Co-infections of scrub typhus and murine typhus were found 2 cases in this study. Two groups of rickettsial diseases infection in the same person is a very rare finding. Although IFA is the gold standard method recommended for scrub typhus and murine typhus infections, this highlights the need for confirmation by an other technique which highly sensitivity and specificity, such as the polymerase chain reaction (PCR) (135,136), and to see whether IFA had some cross reaction between these two groups or not, even though it would be a high cost operation.

Several district hospitals always lack good laboratory facilities and sophisticated technicians. More often febrile patients are treated without benefit. At present, the regional hospitals have fluorescent microscopes, which can be used for laboratory confirmation for several infectious diseases, and possible could be supportive to rickettsioses diagnosis in the district hospitals. The results would support the clinical diagnosis if laboratory surveillance system networks were fully in operation. According to the this research data, scrub typhus was rather high in the hill tribe people in Thailand, and probably grossly under reported, while murine typhus was rather rare. This communication demonstrates that both diseases are also present in the northern part of Thailand. Recognition of both diseases may lead to more suitable use of antibiotics in febrile hill tribe patients.

CHAPTER VI

CONCLUSION

The prevalence rates of scrub typhus and murine typhus either in infection or antibody exposure and the factors related to scrub typhus infection and murine typhus antibody exposure were studied in 393 hill tribe patients who had pyrexia of unknown origin (PUO) in 4 selected hospitals namely : Samoeng hospital, Mae Tang hospital, Phrao hospital and Chiang Dao hospital of Chiang Mai Province.

Blood samples were collected and a questionnaire completed during June 1997 to February 1998. The samples were composed of 199 cases of paired serum and 194 cases of single serum. After separating serum from clotted blood, sera were stored at -20°C until use for antibody assay. The immunofluorescent antibody assay (IFA) was performed to detect antibodies to scrub typhus (using 3 strains; namely Gilliam, Karp and Kato) and murine typhus infections. The designed questionnaire in this study was divided into separate sections which included general characteristics, previous illness, clinical features, personal behaviors, environmental exposure and knowledge of both diseases.

The results show that the prevalence rate of scrub typhus infection (titers $\geq$ 1: 400) was 30.3% (119/393 cases) and murine typhus infection was 0.5% (2/393 cases).

The prevalence rate of scrub typhus antibody exposure (titers ≥ 1: 50) was 37.4% (147/ 393 cases), that and of murine typhus antibody exposure was 5.9% (23/393 cases). Positive scrub typhus infection cases were found mostly in males more than females and were found in the Karen group. The highest positive age group was > 60 years old, agriculture was the main occupation and their income per month was lower than 1,000 baht. Samoeng hospital had the highest number of scrub typhus infections.

The factors related to scrub typhus infection with a statistical difference (p-value < 0.05) analyzed by Chi-square, were sex, age, marital status, occupation, education, income, hospital treatment, grass or shrubs or forest clearing, the place during break time at work, having shrubs or grass in the living area and the working environment.

The factors related to murine typhus infection could not be calculated because of a few cases. There were no significant factors related to murine typhus antibody exposure.

The highest IgM and IgG GMT was found in males, age over 25 years old, occupation being agriculture, poor or no education, low income, working environment in fields or forests and a working time between 8.00 am - 20.00 pm. IgM and IgG antibody was the highest response to co-infection of Gilliam, Karp and Kato strains. The highest IgM GMT were found mainly in Gilliam strain but IgG GMT were found mainly in Karp strain. A statistical difference (p-value < 0.001) was found when comparing GMT in acute and convalescent phase serum for each antigen.

The knowledge gained in this study, especially the data regarding prevalence rates, either in infection or antibody exposure to scrub typhus and murine typhus, and the factors related to scrub typhus in these hill tribe patients will be useful for planing, prevention and control of both diseases.

Suggestion for the prevention and control of both diseases.

Scrub typhus infection among hill tribe patients in this study was found to be related to sex, age, occupation, income, and the environment of working and living areas. Moreover, their behavior including clearing the grass and shrubs, the placed for break time at work and dress when working also the main factors with a statistically significant difference.

Scrub typhus is often unrecognized and substantially under reported, although it is often a clinical presentation, and some cases a severe illness with fatalities. The authorities and public health personnel should concentrate on health education about this disease, including the prevention and the control in high risk groups whose infections were related to factors found in this study.

Although murine typhus is rare in these areas it is possible that in the future the environment and socioeconomic factors as well as changing human behavior, can contribution to the distribution and spread of disease from urban to rural areas. Health education about murine typhus can be given in parallel with scrub typhus as both diseases use the same mode of transmission.

Suggestion for future research

1. Ideally, convalescent serum should be collected on day 10-14 when antibodies may first be present (early convalescent) and may be collected later on day 21-28 after onset (late convalescent).
2. The distribution of study subjects should be equal in every group of sex, age, hill tribe group, occupation and hospital treatment.
3. Future study should include tick typhus (or spotted fever).
4. An interesting study should be done on the vectors and the reservoir hosts of both diseases.
5. The major cause of PUO among hill tribe patients should be determined by investigations for other diseases in parallel with rickettsioses.
6. More intensive studies in knowledge, attitudes and practices of scrub typhus and murine typhus infections among hill tribe patients.
7. In-depth interviews should be conducted in positive murine typhus cases because it was rare in this area.

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APPENDIX A

Questionnaire

Questionnaire for interviewing the hill tribe patients at 4 selected hospitals in Chiang Mai Province in the title "Scrub typhus and Murine typhus Infection and the factors related with infections".

Name of patient.....

Date of interview.....

Hospital number (HN).....

Part 1 Demographic Characteristic.

1. Age.....years old

2. Sex

   1) Male 2) Female

3. Religion.

   1) Buddhist 2) Christian 3) Islam 4) Other (specify.....)

4. Hill tribe group

   1) Karen 2) Meo 3) Lahu 4) Lisu

5) Yao 6) Akha 7) Lua 8) Other (specify.....)

5. Marital status

1) Single 2) Married 3) Divorced 4) Widow 5) Separated

6. Occupation

1) Agriculture (specify job.....)

2) Farmer

3) To look for something from the forest

4) Government official (specify job.....)

5) Employee (specify job.....)

6) Business

7) Other (specify job.....)

7. Average legitimate income per month.....Baht

8. Level of education

1) None

2) Prathom 1-6

3) Mathayom 1-3

4) Mathayom 4-6

5) Vocational education certificate

6) Bachelor's degree

7) Others

9. Present address.....

10. Previous address.....

11. Before admission, have you ever had any of the following signs or symptoms?

(the answer may be more than one)

1) Fever for.....days
2) Chills
3) Orbital pain
4) Muscle pain
5) Headache
6) Cough
7) Anorexia
8) Nausea
9) Vomiting
10) Abdominal pain
11) Diarrhea
12) Right costal margin pain

12. Clinic feature (the answer may be more than one)

1) Infected eye
2) Extremities and/or body rash
3) Eschar
4) Lymphadenopathy
5) Jaundice

13. Before visiting this hospital, what did you do?

1) Nothing
2) Went to clinic
3) Went to primary health service
4) Bought some medicine from drug store
5) Went to hospital
6) Traditional treatment
7) Other (specify.....)

14. If you bought some medicine, what was it?

1) Antibiotics

2) Analgesics

3) Natural medicine

4) Don't know

5) Others (specify.....)

Part 2 Behavior information.

15. Have you cleared away the grass, shrubs forest?

1) No 2) Yes

How often have you done this?.....

16. How you dispose of the garbage?.....

17. Do you have the pets?

1) No 2) Yes (specify.....)

18. Have you got rid of chiggers and/or insects by using chemicals at the working area?

1) No 2) Yes

How often have you done this?.....

19. Have you got rid of the reservoir (rat, rabbit, squirrel, chipmunk)

1) No 2) Yes

How often have you done this?.....

19. Have you used repellent or some herbs to prevent mite and/or insect bites?

1) No, why.....

2) Yes, why.....

3) Sometimes, why.....

21. How do you go to work?

1) Walk

2) By vehicle

3) Other (specify.....)

22. During break time where have you rested?

1) Cleared area

2) Grassy area

3) Shanty

4) Other (specify.....)

23. How do you dress yourself, when you have gone to work?

.....

24. When is your working time?

1) Morning to evening (8 am to 4 pm)

2) Morning to sun-set (8 am to 6 pm)

Part 3 Environmental factors.

25. In 100 metres around your house, do you have shrubs or grass in the area?

1) No

2) Yes

26. How often do you see rat, rabbit, squirrel, chipmunk?

1) None

2) Every day

3) More than once/week

4) Less than once/week

27. How many "reservoir" animals (rat, rabbit, squirrel, chipmunk) do you see each time?

.....

28. Please describe your working area

.....

Part 4 Scrub typhus and murine typhus information.

29. Do you know a chigger?

1) No

2) Yes

30. Do you know a mice?

1) No

2) Yes

31. Do you know about cause of scrub typhus or murine typhus infections?

1) No

2) Yes

32. Do you know the methods for prevention of scrub typhus and murine typhus infections?

1) No

2) Yes

33. Do you have received the knowledge about scrub typhus and murine typhus infection?

1) No

2) Yes

APPENDIX B

Antigen preparation

Preparation of rickettsial antigens for IFA test

The process requires 4 steps which are the following:

1. Recovering L-929 cells
2. Passaged L-929 cells
3. Inoculation of seed into L-929 cells
4. Storing infected cells

Materials required

1. Constant temperature water bath 37°C
2. Centrifuge tubes, volumes 15 and 50 ml
3. Cryogenic vials, size 2.0 ml
4. Tissue culture bottle, size 25 and 75 cm^3
5. Sterile bottle, size 500 ml
6. Plastic pipettes: 2 ml, 5 ml, 10 ml and 20 ml
7. Automatic pipettes
8. Centrifuge
9. 37° C incubator
10. Air-tech

Chemicals required

1. 1X MEM
2. PBS for cell culture
3. 7.5 % $NaHCO_3$
4. 0.25 % trypsin
5. 3 % L-glutamine
6. 1X PBS working solution
7. 2 % MEM
8. 10 % MEM
9. Synder I diluent

Notes

- Eagle's Minimal Essential Medium (MEM). MEM with kanamycin, sodium bicarbonate and L-glutamine. Nissui Pharmaceutical Co. Ltd. (Code 05900).
- Dulbecco's Phosphate Buffered Saline (PBS) for cell culture.Nissui Pharmaceutical Co., Ltd. (Code 05913).
- Fetal Calf Serum (mycloplasma screened). GibcoBRL Co., Ltd. (Cat. No.10270 – 023, Lot No. 40 F 7456 K).
- NaCl. Merck Co. Ltd., Germany. (Cat. No1.06404 1000 / K21552604 / 504).
- KCL. Merck Co. Ltd., Germany. (Cat. No. 4936.1000 023 TA 825236).
- $NaHCO_3.12H_2O$. Merck Co. Ltd, Germany (Cat. No. 6579.1000 / 033A510573).
- K_2HPO_4. Merck Co. Ltd., Germany.
- Trypsin. DIFCO Laboratories, Michegan, USA.
- Dimethyl Sulphoxide (DMSO). Merck Co. Ltd, Germany

- L-glutamine. Gibco Laboratories Co. Ltd, USA (Cat. No. 810-1051 IM)
- Sucrose. Merck Co. Ltd, Germany
- Kh_2PO_4. Merck Co. Ltd, Germany
- Na_2HPO_4. Merck Co. Ltd, Germany
- L-glutamic acid. Fluka Co. Ltd, Japan

Preparation of Plain MEM solution (1XMEM) pH 7.1 - 7.4

1. Plain MEM	4.7	gm
2. Distilled water	500	ml

Mix together and autoclave at 121°C for 15 minutes, store at 4 °C.

Preparation of 2 % MEM pH 7.1-7.4

1. Plain MEM solution	500	ml
2. 7.5 % $NaHCO_3$	10 - 12	ml
3. 3 % L-glutamine	5	ml
4. FCS (Fetal calf serum)	10	ml

Mix together, store at 4 °C

Preparation of 10 % MEM pH 7.1 - 7.4

1. Plain MEM	500	ml
2. 7.5 % $NaHCO_3$	10 - 12	ml
3. 3 % L-glutamine	5	ml
4. FCS	50	ml

Mix together, store at 4 °C

Preparation of Synder I diluent

1. Sucrose	75	gm
2. Kh_2PO_4	0.52	gm
3. Na_2HPO_4	1.22	gm
4. L-glutamic acid	0.72	gm

Method for preparation of Synder I diluent

1. Dissolve sucrose in 750 ml of distilled water.
2. Add buffer salts in order, then glutamic acid.
3. Add distilled water to 1,000 ml.
4. Adjust pH to 7.4 with 10M NaOH.
5. Dispense in 50 ml quantities and autoclave for 20 min at 10 lb pressure.
6. Store at 4°C.

Preparation of PBS for cell culture pH 7.3 - 7.65

1. PBS powder	9.6	gm
2. Distilled water	1000	ml

Mix together, autoclave at 121 °C for 15 minutes and store at 4 °C

Preparation of 7.5 % $NaHCO_3$

1. $NaHCO_3$	7.5	gm
2. Distilled water	100	ml

Mix together and filter with Millipore 0.45 µm, store at –10 or -20 °C.

Preparation of 3% L-glutamine

1. L-glutamine	3	gm
2. Distilled water	100	ml

Mix together and filter with Millipore 0.2 µm, store at –10 or -20 °C.

Preparation of 1X PBS working solution, pH 7.4

1. NaCl	8.00	gm
2. KCl	0.20	gm
3. $NaHPO_4$	1.15	gm
4. K_2HPO_4	0.20	gm
5. Distilled water	1000	ml

Store at room temperature.

Recovering L-929

1. Remove L-929 cells from -80 °C freezer and thaw in a water bath at 37 °C. Transfer the cells to a 15 ml centrifuge tube.
2. Centrifuge at 1000 rpm for 10 minutes at room temperature.
3. Discard the supernatant and add 5 ml of 10% MEM, resuspended and then transfer to first tissue culture bottle (25 cm^2).
4. Incubate at 37 °C in CO_2 incubator for 24 hours.
5. Change media by pouring off the first media and adding 5 ml of 10% MEM (1^{st}media change).
6. Change 2^{nd} media when complete 48 hour.
7. Incubate at 37 °C in CO_2.

8. Change 3rd media when complete 72 hour.
9. Incubate at 37 °C in CO_2 (observe cell growth until a monolayer has been formed, then change media to 2% MEM).
10. After changing media with 2% MEM for 3 -4 days we can pass cell in tissue culture bottle (75 cm^2).

Passage of L-929 cells

1. Place tissue culture bottle has L-929, and pour off the first media.
2. Wash bottle with 10 ml of PBS and mix gently, pour off PBS.
3. Add 0.5-1 ml of 0.25% trypsin and place it at room temperature for≤ 5 minutes.
4. Tap the tissue culture bottle to make the cells peel from the surface of the bottle.
5. Add 5 ml of 10% MEM and strongly mix (to separate clumping cells into single cells).
6. Add 25-30 ml of 10% MEM in new tissue culture bottle by media cover the surface.
7. Add 1ml of cell (No.5) into the tissue culture bottle (No.6) (ratio 1:5).
   Mix with pipette and incubate at 37 °C for 7 days.

Inoculation of seed into L-929

1. Remove seed antigen from -80 °C freezer and thaw in a water bath 37 °C.
2. Add 5 ml of PBS to the seed antigen and grind with grinder 30 times.
3. Prepare L-929 by discard the media and wash with 10-15 ml of PBS and pour off again.
4. Transfer seed antigen (No.2) to adsorb into L-929 tissue culture bottle (No.3).

5. Incubate at 35 °C for 1 - 2 hour, gently titling every 30 minutes when complete time pour off first media.
6. Add 30 ml of 2% MEM and incubate at 35 °C for 7 - 10 days.
7. Change media every 3 days.

   (complete 3 - 4 days make 1st IFA and change media by pour off first media and add new 2 % MEM 30 ml, incubate again)

Starting infected cell.

1. Take off infected cell from the surface by cell scraper (batortechnik, Germany).
2. Mixed gently and then transfer to 30 ml centrifuge tube.
3. Centrifuge at 8,000 rpm for 30 minutes.
4. Pour off supernatant.
5. Add 1 ml of Synder I, mixed gently and transfer to a cryogenic tube and stored at -80°C.

Storing L-929 infected cell

1. Place L-929 and pour off first the media.
2. Wash with PBS 10 ml and pour off.
3. Add trypsin 0.5-1 ml/75 cm^2 to trypsinize cells, keep at room temperature for $\leq$ 5 minutes.
4. Tap the bottle and remove cells from the bottle (check by microscope).
5. Add 5 ml of 10% MEM, then mix to separate clumping cells into single cells.
6. Transfer solution (No.5) to a 15 ml centrifuge tube and wash tube (No.5) again with 5 ml of 10% MEM and transfer solution to a centrifuge tube.

7. Centrifuge at 1000 rpm for 10 minutes at room temperature.
8. Pour off supernatant and add 10% DMSO (0.5-1 ml). Mix with pipette.
9. Transfer to a cryogenic tube and store at -80 °C.

For preparation of *Rickettsia typhi* antigen, the method was the same as above except that cells were infected with the seed of *Rickettsia typhi* (strain Wilmington).

APPENDIX C

Table 35 Number of patients distribution in IgM antibodies titers to each strain of scrub typhus infection in acute sera classified by age group (x = reciprocal titers = 50).

Age group (years)	No. of test	Gilliam									Karp									Kato								
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
0-9	48	43	-	-	1	1	-	-	2	1	43	2	-	-	-	1	-	1	1	44	-	-	1	-	1	-	1	1
10-19	126	109	6	1	2	2	4	-	1	1	110	4	-	5	3	1	2	-	1	110	4	3	2	3	2	1	1	-
20-29	82	65	7	3	4	-	1	-	1	1	73	3	2	2	-	-	1	1	-	72	4	1	2	-	1	-	2	-
30-39	71	58	1	4	4	1	3	-	-	-	63	1	2	3	-	1	1	-	-	65	3	-	1	-	-	2	-	-
40-49	31	21	4	1	3	-	1	1	-	-	26	2	-	2	-	1	-	-	-	25	3	1	1	-	1	-	-	-
50-59	18	15	2	-	-	-	-	1	-	-	16	1	-	-	-	-	-	1	-	17	-	-	-	-	-	-	1	-
≥60	17	15	1	-	1	-	-	-	-	-	16	1	-	-	-	-	-	-	-	16	1	-	-	-	-	-	-	-
Total (cases)	393	326	21	9	15	4	9	2	4	3	347	14	4	12	3	4	4	3	2	349	15	5	7	3	5	3	5	1

Table 36 Number of patients distribution in IgM antibodies titers to each strain of scrub typhus infection in convalescence sera classified by age group (x = reciprocal titers = 50).

Age group (years)	No. of test	Gilliam									Karp									Kato								
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
0-9	21	19	-	-	-	2	-	-	-	-	19	-	-	-	2	-	-	-	-	19	-	-	-	2	-	-	-	-
10-19	67	62	1	1	2	-	-	-	1	-	61	3	1	-	1	-	1	-	-	63	1	1	1	-	1	-	-	-
20-29	45	33	5	1	3	-	1	2	-	-	38	2	-	2	1	-	2	-	-	37	2	-	3	-	1	2	-	-
30-39	39	27	5	3	3	-	1	-	-	-	27	8	2	1	-	1	-	-	-	31	3	3	1	-	-	1	-	-
40-49	13	10	2	-	1	-	-	-	-	-	12	1	-	-	-	-	-	-	-	12	1	-	-	-	-	-	-	-
50-59	8	6	-	-	1	-	-	1	-	-	6	-	-	1	-	1	-	-	-	6	-	-	1	-	1	-	-	-
≥60	6	6	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-
Total (cases)	199	163	13	5	10	2	2	3	1	0	169	14	3	4	4	2	3	0	0	174	7	4	6	2	3	3	-	-

Table 37 Number of patients distribution in IgG antibodies titers to each strain of scrub typhus infection in acute sera classified by age group (x = reciprocal titers = 50).

Age group (years)	No. of test	Gilliam									Karp									Kato								
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
0-9	48	40	1	-	1	6	-	-	-	-	39	3	2	-	1	3	-	-	-	40	3	1	1	2	1	-	-	-
10-19	126	100	2	1	8	6	4	5	-	-	100	1	2	4	5	8	4	2	-	102	2	3	7	4	4	4	-	-
20-29	82	52	6	2	4	8	8	1	-	1	54	3	2	5	7	3	4	3	1	55	7	2	4	6	4	3	-	1
30-39	71	44	3	4	6	5	6	3	-	-	45	3	3	3	3	5	8	1	-	44	6	2	6	3	5	4	1	-
40-49	31	16	2	-	2	4	3	3	-	1	17	3	-	3	1	4	2	-	1	17	4	-	3	3	3	-	-	1
50-59	18	10	1	1	1	1	3	-	-	-	9	2	1	-	1	3	1	-	1	10	1	1	2	2	1	-	-	1
≥60	17	10	-	1	2	1	2	1	-	-	11	-	1	-	3	1	1	-	-	10	1	1	2	1	1	1	-	-
Total (cases)	393	272	15	9	24	31	26	13	-	3	275	15	11	15	21	27	20	6	3	278	24	10	25	21	19	12	1	3

Table 38 Number of patients distribution in IgG antibodies titers to each strain of scrub typhus infection in convalescence sera classified by age group (x = reciprocal titers = 50).

Age group (years)	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
0-9	21	18	-	-	-	2	-	-	1	-	18	-	-	-	2	1	-	-	-	18	-	-	-	2	-	1	-	-
10-19	67	58	2	1	1	4	-	1	-	-	58	1	-	4	1	3	-	-	-	59	2	1	4	1	-	-	-	-
20-29	45	25	2	3	4	3	3	3	-	2	27	2	1	2	3	3	4	1	2	27	1	2	3	3	5	2	-	2
30-39	39	17	2	1	6	4	4	3	2	-	18	3	1	2	1	8	5	-	1	17	3	1	3	8	6	-	1	-
40-49	13	4	-	-	1	2	3	3	-	-	4	1	-	1	1	3	3	-	-	4	1	-	2	2	4	-	-	-
50-59	8	3	1	-	1	1	-	-	2	-	3		1	-	1	1	-	2	-	3	-	1	1	1	-	-	2	-
≥60	6	2	-	-	-	1	2	-	1	-	1	-	1	-	-	2	2	-	-	1	-	1	-	2	1	1	-	-
Total (cases)	199	127	7	5	13	17	12	10	6	2	129	7	4	9	9	21	14	3	3	129	7	6	13	19	16	4	3	2

Table 39 Number of patients distribution in IgM antibodies titers to each strain of scrub typhus infection in acute sera classified by sex (x = reciprocal titers = 50).

Sex	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Male	203	169	8	7	8	2	4	1	3	1	179	8	2	7	1	2	1	2	1	180	9	1	5	1	4	-	2	1
Female	190	157	13	2	7	2	5	1	1	2	168	6	2	5	2	2	3	1	1	169	6	4	2	2	1	3	3	-
Total (cases)	393	326	21	9	15	4	9	2	4	3	347	14	4	12	3	4	4	3	2	349	15	5	7	3	5	3	5	1

Table 40 Number of patients distribution in IgM antibodies titers to each strain of scrub typhus infection in convalescence sera classified by sex (x = reciprocal titers = 50).

Sex	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Male	101	77	8	3	9	2	-	1	1	-	84	6	2	4	3	-	2	-	-	85	3	3	6	2	1	1	-	-
Female	98	86	5	2	1	-	2	2	-	-	85	8	1	-	1	2	1	-	-	89	4	1	-	-	2	2	-	-
Total (cases)	199	163	13	5	10	2	2	3	1	-	169	14	3	4	4	2	3	-	-	174	7	4	6	2	3	3	-	-

Table 41 Number of patients distribution in IgG antibodies titers to each strain of scrub typhus infection in acute sera classified by sex (x =reciprocal titers =50).

Sex	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Male	203	135	10	2	11	23	17	4	-	1	133	10	8	6	12	21	9	3	1	137	17	6	10	14	13	4	1	1
Female	190	137	5	7	13	8	9	9	-	2	142	5	3	9	9	6	11	3	2	141	7	4	15	7	6	8	-	2
Total (cases)	393	272	15	9	24	31	26	13	-	3	275	15	11	15	21	27	20	6	3	278	24	10	25	21	19	12	1	3

Table 42 Number of patients distribution in IgG antibodies titers to each strain of scrub typhus infection in convalescence sera classified by sex (x = reciprocal titers = 50).

Sex	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Male	101	58	3	3	7	12	8	7	2	1	57	3	4	3	7	15	10	-	2	57	3	5	7	14	10	3	1	1
Female	98	69	4	2	6	5	4	3	4	1	72	4	-	6	2	6	4	3	1	72	4	1	6	5	6	1	2	1
Total (cases)	199	127	7	5	13	17	12	10	6	2	129	7	4	9	9	21	14	3	3	129	7	6	13	19	16	4	3	2

Table 43 Number of patients distribution in IgM antibodies titers to each strain of scrub typhus infection in acute sera, classified by occupation (x = reciprocal titers = 50).

Occu-pation	No. of test	Gilliam									Karp									Kato								
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Agri-culture	219	170	14	8	12	2	8	2	1	2	188	8	4	8	3	2	3	2	1	189	11	4	4	2	3	2	4	-
Look for anything from the forest	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Employee	45	39	4	1	1	-	-	-	-	-	42	2	-	1	-	-	-	-	-	42	2	1	-	-	-	-	-	-
Business	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Student	109	98	3	-	1	2	1	-	3	1	98	4	-	2	-	2	1	1	1	99	2	-	2	1	2	1	1	1
Do not work	18	17	-	-	1	-	-	-	-	-	17	-	-	1	-	-	-	-	-	17	-	-	1	-	-	-	-	-
Total (cases)	393	325	21	9	15	4	9	2	4	3	347	14	4	12	3	4	4	3	2	349	15	5	7	3	5	3	5	1

Table 44 Number of patients distribution in IgM antibodies titers to each strain of scrub typhus infection in convalescence sera classified by occupation (x = reciprocal titers = 50).

Occu-pation	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Agri-culture	116	93	9	3	9	-	2	3	-	-	93	12	2	4	1	2	2	-	-	98	5	3	5	-	2	3	-	-
Look for anything from the forest	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Employee	25	22	2	1	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-	25	-	-	-	-	-	-	-	-
Business	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Student	51	46	1	1	-	2	-	-	1	-	45	2	1	-	2	-	1	-	-	46	1	1	-	2	1	-	-	-
Do not work	7	5	1	-	1	-	-	-	-	-	6	-	-	-	1	-	-	-	-	5	1	-	1	-	-	-	-	-
Total (cases)	199	163	13	5	10	2	2	3	1	-	169	14	3	4	4	2	3	-	-	174	7	4	6	2	3	3	-	-

Table 45 Number of patients distribution in IgG antibodies titers to each strain of scrub typhus infection in acute sera classified by occupation (x = reciprocal titers = 50).

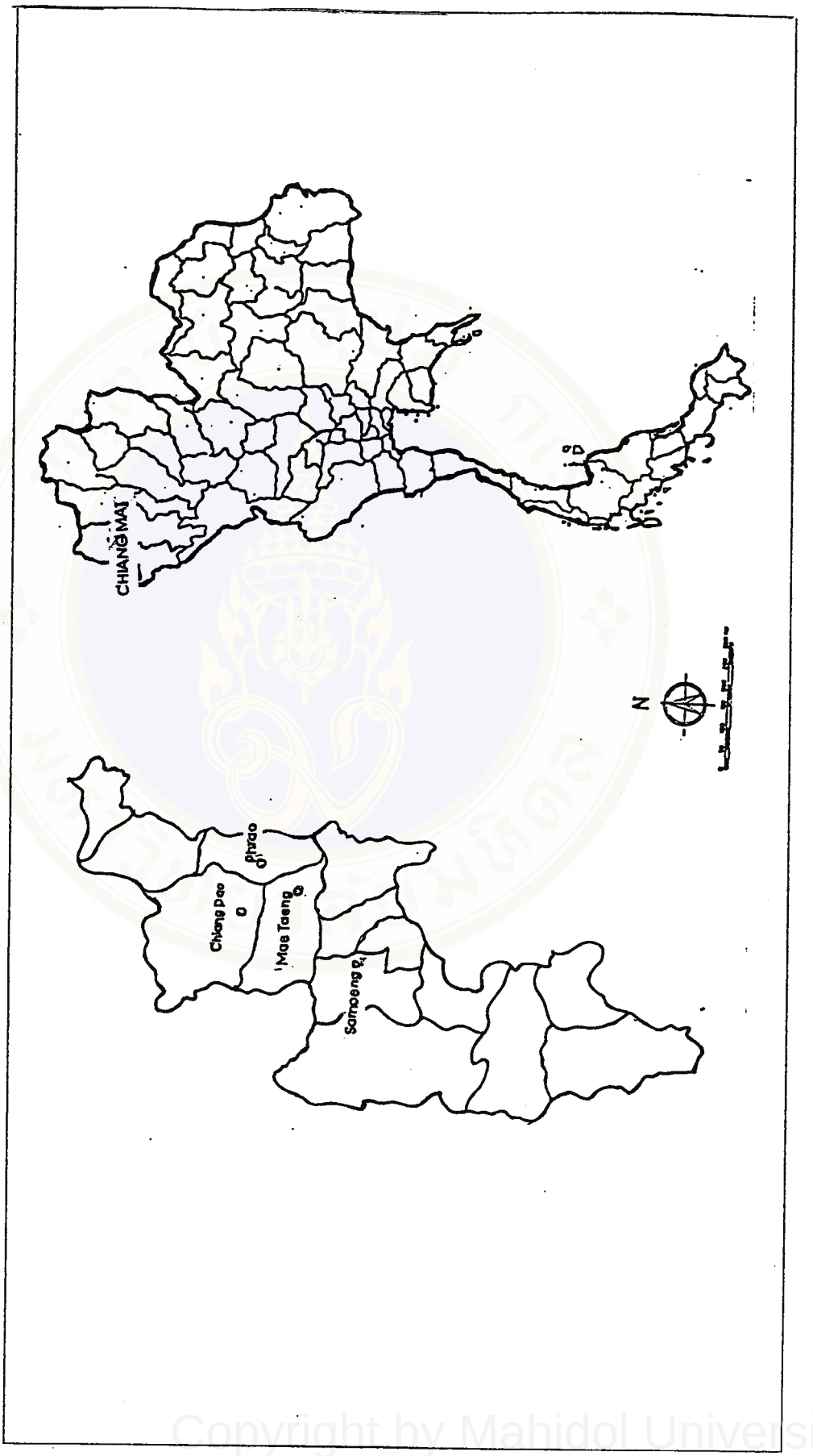
Occu-pation	No. of test	Gilliam									Karp									Kato							
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x
Agri-culture	219	131	10	7	16	21	20	11	-	3	134	11	6	11	12	19	17	6	3	137	16	4	17	16	14	11	1	3
Look for anything from the forest	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Employee	45	36	1	1	3	-	4	-	-	-	37	1	-	3	2	-	2	-	-	37	2	1	2	1	2	-	-	-
Business	1	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Student	109	90	3	1	4	9	-	2	-	-	89	3	4	1	5	6	1	-	-	90	5	4	4	4	1	1	-	-
Do not work	18	13	1	-	1	1	2	-	-	-	13	-	1	-	2	2	-	-	-	12	1	1	2	-	2	-	-	-
Total (cases)	393	272	15	9	24	31	26	13	-	3	275	15	11	15	21	27	20	6	3	278	24	10	25	21	19	12	1	3

Table 46 Number of patients distribution in IgG antibodies titers to each strain of scrub typhus infection in convalescence sera classified by occupation (x = reciprocal titers = 50).

Occupation	No. of test	Gilliam										Karp										Kato									
		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x		<x	x	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x		<x	X	2x	2^2x	2^3x	2^4x	2^5x	2^6x	2^7x	
Agriculture	116	60	5	3	10	13	9	9	5	2		62	6	3	5	7	14	13	3	3		61	5	4	9	15	14	3	3	2	
Look for anything from the forest	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	
Employee	25	20	-	2	1	1	1	-	-	-		20	-	1	1	1	1	1	-	-		20	-	2	1	2	-	-	-	-	
Business	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	
Student	51	43	2	-	1	3	-	1	1	-		44	1	-	1	1	4	-	-	-		44	2	-	2	2	-	1	-	-	
Do not Work	7	4	-	-	1	0	2	-	-	-		3	-	-	2	-	2	-	-	-		4	-	-	1	-	2	-	-	-	
Total (cases)	199	127	7	5	13	17	12	10	6	2		129	8	4	9	9	21	14	3	3		129	7	6	13	19	16	4	3	2	

APPENDIX D

Figure 7 The map of Chiang Mai Province.



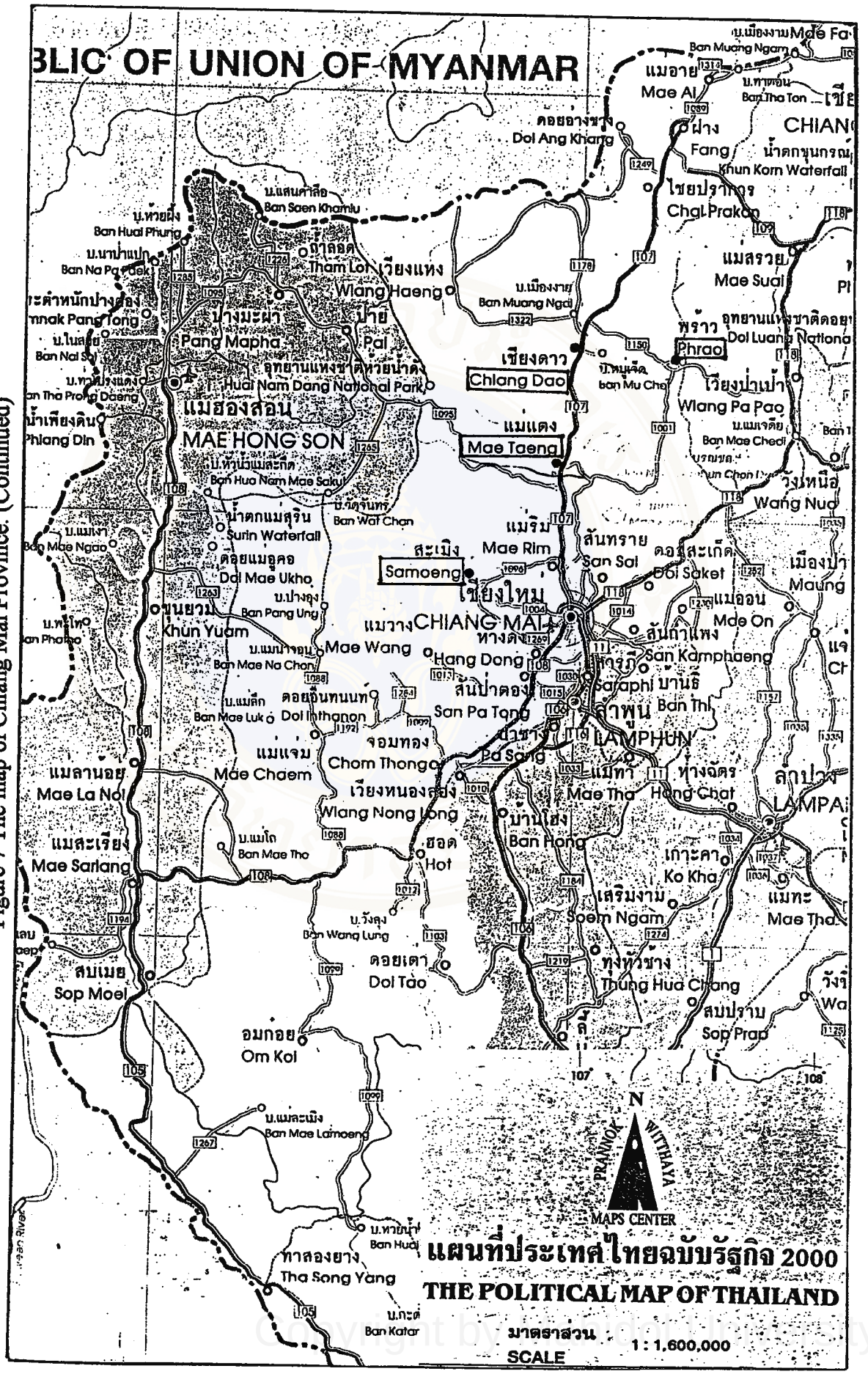


Figure 7 The map of Chiang Mai Province. (Continued)

BIBIOGRAPHY

NAME	Miss. Uraiwon Muktaree
DATE OF BIRTH	16 January 1973
PLACE OF BIRTH	Ayutthaya Province, Thailand
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