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– Content –

A Study of Scrub Typhus in Dengue Fever Suspects in Chiayi County

Abstract

Scrub typhus (tsutsugamushi disease) is one of the reportable diseases in Taiwan. It is an acute febrile eruptive disease caused by the infection of Rickettsia. In recent years, most cases of scrub typhus have been reported primarily from Penghu, Hualien and Taitung counties, only a few cases from other counties. However, in September 1991, positive cases of scrub typhus were identified from specimens of suspected dengue fever patients in Chiayi County. Serum specimens of all suspected dengue fever patients in Chiayi County between January 1991 and June 1992 were, therefore, tested for scrub typhus antibody. Of the 15 cases, seven were identified positive, giving a positive rate of 47%. These seven positives all live in the mountainous townships, giving a positive rate of 77% among cases living in the mountainous townships districts. Of the 15 patients, only one was dengue fever positive case. There was no duplication of diseases among the seven scrub typhus cases so identified.

The study suggests that some scrub typhus patients in places other than penghu, Hualien and Taitung counties have not been noted, and intends to alert physicians and the public the possibility of tsutsugamushi infection in order to have adequate prevention.

Key words: Scrub typhus, tsutsugamushi disease
Rickettsia tsutsugamushi
Indirect immunofluorescence assay, IFA

1. Introduction

Scrub typhus (tsutsugamushi disease) is an acute febrile infection caused by the bites of larval mites infected with *Rickettsia tsutsugamushi*. The disease occurs more frequently in eastern and southeastern Asia[1,2]. Though it was made one of the reportable diseases in Taiwan in 1955, for lack of information, only 71 cases were reported between

1955 and 1984, and no case was reported in 1960, 1965-181, 1983 and 1984[3]. Information became more available since 1985 when the former Taiwan Provincial Institute of Infectious Diseases took the responsibility of surveillance and testings for scrub typhus, and also conducted surveys and training. More cases were then reported. Since 1990, the National Quarantine Service has been responsible for the surveillance, and the National Institute of Preventive Medicine for the confirmation testings. In 1990 and 1991, more cases were reported from Penghu, Hualien and Taitung, and only a few cases from other countries and cities.

In 1990, one scrub typhus case was reported from Minhsiung township of Chiayi County. The case, however, was identified negative after laboratory testings. In September and October of 1991, two suspected dengue fever patients in Chiayi County, after laboratory testings for scrub typhus antibody, were found to be scrub typhus and not dengue fever patients. Serum specimens of suspected dengue fever patients living in Chiayi County between January 1991 and June 1992 were thus tested with indirect immunofluorescence assay (IFA) for scrub typhus antibody to study the prevalence of this disease in Chiayi County for the future planning of pretive measures.

2. Materials and Methods

(1) Serum specimens

Serum specimens collected by hospitals and clinics between January 1991 and July 1992 of suspected dengue fever patients living in Chiayi County were taken 50 $\mu\ell$ each and diluted with phosphate buffer saline (PBS containing 0.4% bovine serum albumin and 0.05% sodium azide) to 5 times and placed in refrigerator under $-20°C$.

(2) Preparation of antigen glass slides

Antigens used are Karp, Gillium and Kato strains of *Rickettsia tsutsugamushi*. These strains came from the Okinawa University of Japan. They are multiplied in L929 cell strain and then placed under $-70°C$ for storage. When use, the three strains are dripped separately in the same well of the fluorescence slide, dried under room temperature, fixed for 10 minutes with $-20°C$ acetone, dried under room temperature and then placed in refrigerator under $-20°C$ for use. The glass slides should not be kept for more than two months.

(3) Staining the fluorescent antibody

Serum specimens are consecutively diluted to two times beginning from 1:20 with 0.01 M PBS at pH 7.4. The antigen glass slide is taken out and dried. In each well of it, some diluted serum is added. The slide is then placed in a incubator for reaction at 37°C for 30 minutes. It is then removed and washed with PBS and immersed in PBS. This washed process is repeated 5 minutes later. The slide is removed, washed with PBS and distilled water, and dried under room temperature. Then, some fluorescein conjugated goat antihuman antiserum (anti-IgG bought from Jackson of Philadelphia,

USA or anti-IgM bought from Zymed of California, USA) is added to each well. The slide is placed in a 37°C incubator for 30 minutes, washed with PBS and distilled water, dried, and added some 0.01M PBS containing glycerol (1:1) at pH 8.0. The slide is placed under fluorescent microscope at 400 times for studying the florescent reaction.

(4) Reading the findings

(a) A case is identified positive if the IgG antibody value for his serum B (in the convalescent stage) is 8 times higher than serum A (in the acute stage) or that of the second serum specimen is 8 times higher than the first serum specimen.

(b) Even if the IgG antibody value of serum B is not 8 times higher than serum A, the finding is considered positive when one of the testings shows IgM antibody value $\geq$ 1:40.

(c) When there is only one serum, and its IgM antibody value is $\geq$ 1:40, the finding is also considered positive.

3. Results

(1) The specimens

Between January 1991 and July 1992, 15 serum specimens of suspected dengue fever patients in Chiayi County have been collected. Except No. 006, two serum specimens of seven days and longer intervals are available for testings (see Table 1).

Table 1. Specimens Collected from Chiayi County (January 1991 — July 1992)

No	Sex	Age	Locality	Date of onset	No. of blood specimens collected	Days of blood collected
001	M	35	Minhsiung	Jan 1991	2	5, 21
002	M	28	Talin	July 1991	2	8, 16
003	M	13	Liuchiao	July 1991	2	6, 17
004	F	39	Chuchi	July 1991	2	10, 24
005	M	24	Lichiao	Aug 1991	2	7, 17
006	F	62	Tapu	Sep 1991	1	14
007	F	56	Hsinkang	Sep 1991	2	5, 16
008	M	51	Meishan	Sep. 1991	2	4, 13
009	F	39	Fanlu	Sep 1991	2	12, 20
010	F	48	Chungpu	Oct 1991	2	7, 20
101	M	14	Chungpu	Jan. 1992	2	3, 19
102	M	52	Liuchiao	Feb 1992	2	7, 28
103	F	45	Tapu	Apr 1992	2	7, 16
104	F	32	Chuchi	Apr 1992	2	5, 16
105	M	32	Chungpu	June 1992	2	10, 24

(2) Antibody value of scrub typhus positive cases

Of the 15 specimens tested with IFA for scrub typhus antibody, the IgG and IgM antibody values of eight specimens were found to be less than 1:40 and were identified negative. The rest seven specimens showed high antibody values and were considered scrub typhus cases (see Table 2), giving a positive rate of 47%. The antibody values of the seven positive cases can be grouped into three for discussion:

Table 2. Antibody Value of Scrub Typhus Positive Cases (January 1991 — July 1992)

No.	Frequency blood collected	Days of blood collected	IFA antibody value						Reading
			Karp		Gillium		Kato		
			IgG	IgM	IgG	IgM	IgG	IgM	
006	A	14	≥640	(≥640)	≥640	(≥640)	≥640	(≥640)	Positive
009	A	12	1280	(640)	≥2560	(≥2560)	640	(≥640)	Positive
	B	20	1280	(640)	≥2560	(≥2560)	1280	(≥2560)	
010	A	7	2560	(1280)	5120	(2560)	5120	(1280)	Positive
	B	20	1280	(1280)	≥20480	(≥2560)	5120	(≥2560)	
105	A	10	160	(320)	640	(640)	160	(160)	Positive
	B	24	160	(320)	1280	(1280)	160	(160)	
004	A	10	<40	(<40)	<40	(<40)	<40	(<40)	Positive
	B	24	640	(320)	≥2560	(≥2560)	640	(320)	
008	A	4	<40	(<40)	40	(80)	40	(<40)	Positive
	B	13	80	(≥2560)	2560	(≥2560)	320	(160)	
103	A	7	80	(<40)	160	(<40)	80	(<40)	Positive
	B	16	1280	(640)	≥2560	(1280)	≥2560	(320)	

(a) Case No. 006 refused to be taken blood, only one specimen was available. However, blood taken on the 14th day of onset in the convalescent stage already showed an IgM antibody value of 1:640, so this was identified positive.

(b) Although the IgG antibody values of cases Nos. 009, 010 and 105 did not show an 8-time increase, their first blood specimens were collected on the 12th, 7th and 10th days of onset close to the convalescent stage, their IgG antibodies had already appeared and would not increase significantly any more, and their IgM antibody values were higher than 1:640, so they were identified positive.

(c) The IgG antibody values of the second specimens of cases Nos. 004, 008 and 103 were 8-times higher than those of the first specimens. The first specimens were

collected on the 10th, 4th and 7th days of onset, and antibody values were low. The second specimens were collected on the 24th, 13th and 16th days of onset. They showed a significant increase in antibody between the acute and the recovery periods. The IgM antibody values also increased significantly. These cases were, therefore, identified positive.

(3) Geographical distribution

Mt. Ali and other high mountains are in chiayi County (Figure 1). The 15 specimens collected were grouped into plain and mountain townships. The IgG and IgM antibody values of the six cases in the plain townships, Minhsiung, Talin, Liuchiao and Hsinkang, were lower than 1:40 of the negative reaction. The positive cases were all in the mountain townships: two each in Chungpu and Tapu, one each in Meishan, Fanlu and Chuchi (see Table 3). Of the nine cases in the mountain townships, seven were identified positive, giving a positive rate of 77%.

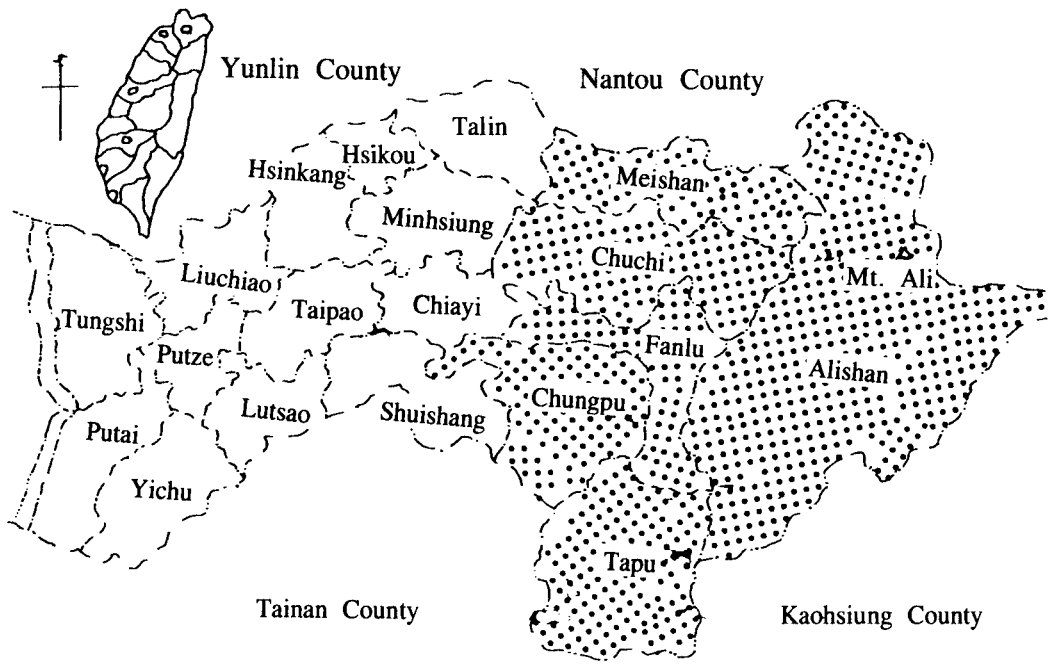

Figure 1. Map of Chiayi County

Table 3. Findings of Laboratory Testings (January 1991 — July 1992)

Group	No.	Sex	Age	Locality	Date of onset	Reading
Plain	001	M	35	Minhsiung	Jan 1991	*
Township	002	M	28	Talin	July 1991	
	003	M	13	Lichiao	July 1991	
	005	M	24	Liuchiao	Aug 1991	
	102	M	52	Lichiao	Feb 1992	
	007	F	56	Hsinkang	Sep 1991	
Mountain	105	M	32	Chungpu	June 1992	Scrub typhus
Township	010	F	48	Chungpu	Oct 1991	Scrub typhus
	103	F	45	Tapu	April 1992	Scrub typhus
	006	F	62	Tapu	Sep 1991	Scrub typhus
	008	M	51	Meishan	Sep 1991	Scrub typhus
	009	F	39	Fanlu	Sep 1991	Scrub typhus
	004	F	39	Chychi	July 1991	Scrub typhus
	104	F	32	Chuchi	April 1992	*
	101	M	14	Chungpu	Jan 1992	

* Confirmed by the National Institute of Preventive Medicine as dengue fever patient.

(4) Distribution by month

The seven cases occurred between April and October, with more cases in September.

(5) Distribution by sex

Of the seven cases, five were female and two male.

(6) Distribution by age

The ages of the seven cases ranged from 32 to 62 years.

(7) Traveling

With the exception of case No. 009 who had been to Tungkang of Pingtung County and Taipei City ten days prior to the onset, the rest (six cases) had never been to anywhere within two weeks before onset. They could be considered indigenously infected.

(8) Distribution by fiscal year

Of the 10 suspected dengue fever patients between January and December 1991, five were confirmed scrub typhus patients, giving a positive rate of 50%. Of the five patients in January through June 1992, two were confirmed scrub typhus patients, giving a positive rate of 40%.

4. Discussion

The major symptoms of scrub typhus disease are: (1) during the incubation period of 9-12 days, a "punched out" skin ulcer (eschar) will appear at the site of the bite; (2) local lymphadenopathy; (3) high fever (more than 39°C) for as long as 14 days; (4) headache, profuse sweating and conjunctival injection; and (5) a dull red maculo-papular eruption appears 4-5 days after onset on the trunk extends to the extremities and disappears in a few days[1,2]. Though scrub typhus is easily identifiable by the specific eschar, the eschar may be unnoticed if it appears at a private spot or on female patient, and only 50-80%[4,5] of patients will develop eschar. Physicians in diagnosis should carefully ask the patients about their symptoms, resident and occupation for confirmation.

Clinical diagnosis for scrub typhus includes Weil-Felix reaction, IFA, isolation of rickttsia, complement-fixation reaction, toxicity neutralization, and polymerase chain reaction. Of them, the Weil-Felix reaction is the easiest, and can be used to detect antibody 7-14 days after onset, and to detect at the same time various Rickettsial diseases, such as scrub typhus, typhus fever, spotted fever and Q-fever[6]. It does not require special facilities, and, therefore, can be performed by many hospitals and clinics. It, however, can detect only 50% of scrub typhus, and also shows non-specific reactions[2,5]. Other laboratory methods should also be employed for confirmation. We have compared both the Weil-Felix and the IFA methods and found that the sensitivity of the former is only 48% of the latter[7]. IFA is currently used by the National Institute of Preventive Medicine. As this method is sensitive, cost-effective and convenient, and can also detect IgM for earlier confirmation, it is used widely[8]. However, the method requires the preparation of antigens and some sophisticated facilities such as the fluorescent microscope, it may not be practicable for some hospitals and clinics. To improve the identification rate of scrub typhus for early prevention and treatment, physicians are requested to, after testings by the Weil-Felix method, send specimens to the Institute for further confirmation[9].

The first suspected scrub typhus case of Taiwan was reported in 1908 in Hualien[10]. Thereafter, more suspected cases were reported in Chiayi, Kaohsiung and Hualien[11,12,13]. The first official report, however, was made by J. Hatori in 1915[14]. During the Japanese occupatioon, many surveys on scrub typhus had been made by Japanese scholars. Many cases had been reported in practically every county and city with more cases in Hualien, Kaohsiung and Penghu counties[15,16,17]. After the end of the Second World War, NAMRU-2 (US Naval Medical Research Unit No. 2) and the ROC military had conducted jointly many surveys of scrub typhus in Penghu[18,19,20,21].

Other researchers also focused their studies on Penghu, the Orchid Island and Hualien[22,23,24]. Data from these areas, therefore, are more available, and more cases have been reported from these areas. These studies also confirmed an epidemic in 1962 in Penghu[25] and another one in 1970 in Fengping Township of Hualien County[24]. Data concerning the prevalence of this disease in other parts of Taiwan, however, are not available.

The first two suspected scrub typhus patients in Chiayi area occurred in 1911[11]. The first confirmed case was reported in 1918[26,27]. In 1914[28] and 1921[29], two cases and one case were reported respectively. The first death case was reported in 1930[30]. Most cases reported during this period either lived along the Mt. Ali railway, worked in Mt. Ali or for Mt. Ali railway, or had been to Mt. Ali. They all seemed to be related to Mt. Ali. In 1944, a Japanese physician reported that in addition to the area along the Mt. Ali railway and Chungpu Township at the foot of Mt. Ali, cases had also been detected in the coastal areas and plain townships such as Chiayi City, Talin, Hsinkang and Tungshi townships[31]. No further surveys, however, have been conducted thereafter, so information concerning the distribution of scrub typhus in Chiayi County is not available.

Testings for scrub typhus antibody were conducted among military draftees in 1984[32]. Draftees from Chiayi County had a low positive rate of 5.0%, lower among the 21 cities and counties of the Province. Of the nine positive cases, seven were from the mountain townships of Alishan, Meishan, Chungpu and Tapu, only two from Lutsao and Yichu townships. In December 1990, the Institute received a report from a hospital in the northern part of Taiwan of a 49-year female patient living in Alishan Township of Chiayi County suffering from fever, chill land eruption. The physician administered Weil-Felix test on the serum of the convalescent stage to find Proteus OXK at 1:160. The patient reported that many in her village had the same symptoms of fever and eruption. Since the second serum in the convalescent stage tested with Weil-Felix method did not show a significant increase, serum specimen was not sent to the Institute for confirmation. The present study did not detect any cases in townships along Mt. Ali and in the coastal townships, so the findings can not be compared with information obtained previously. However, most positive cases detected in the present study come from the mountain townships, it can be said with certainty that scrub typhus occurs in Chiayi County and primarily in the mountain areas of the County. Physicians in the County should, therefore, be more alert to this fact. Physicians should, in the future, collect either serum or blood specimen of suspected cases and send it to the Institute for confirmation. Health workers at the local level should also promote health education to encourage people for blood collection for early detection of the disease.

The seven positive cases are all above the age of 32 years and five of them being female. Dose this fact mean that infection rate is related to the work environment? Has the current living condition changed the distribution of mites? What is the distribution of scrub typhus in other neighboring cities and counties? These questions will have to be answered by further studies. The distributions of both scrub typhus and mites in the Province as a whole, and the difference in their distribution between mountain and plain areas should also be further studied. It is also hoped that the findings of the present

study would call more attention of clinicians to the Rickettsial diseases.

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