

Virological and Epidemiological Studies on Encephalitis
in Chiang Mai Area, Thailand in the Year of 1982
V. Seroepidemiological survey on humans

Toshihiko FUKUNAGA

*Department of Preventive Medicine, Research Institute for Microbial
Diseases, Osaka University, Osaka, Japan*

Akira IGARASHI

*Department of Virology, Institute for Tropical Medicine,
Nagasaki University, Nagasaki, Japan*

Takayuki OGATA

*Department of Virology and Rickettsiology, National Institute of
Health of Japan, Tokyo, Japan*

Nobuya FUJITA

*Department of Microbiology, Kobe University School of Medicine
Kobe, Japan*

Ongart CHAROENSOOK, Sujarti JATANASEN

Division of Epidemiology, Ministry of Public Health, Bangkok, Thailand

Charnchudhit CHANYASANHA, Kanai CHATTYANONDA

*Virus Research Institute, Department of Medical Sciences,
Ministry of Public Health, Bangkok, Thailand*

Supatra PEERAKOME, Jiraporn SUPAWADEE, and Kampol PANASAMPOL

*Department of Microbiology, Faculty of Medicine, Chiang Mai University,
Chiang Mai, Thailand*

Abstract: Prevalence of antibody titers were measured by the hemagglutination-inhibition test (HI) against Japanese encephalitis (JE) and dengue antigens, and also by the enzyme-linked immunosorbent assay (ELISA) against JE antigen, for healthy inhabitants at 5 locations in Chiang Mai Area, Thailand. Fang, which belonged to Mae Kong Valley, showed lower antibody prevalence than other 4 places in Chiang Mai Valley. Prevalence of IgM-ELISA antibodies against JE was different among sampling places, indicating inhomogeneity of JE virus circulation in the study area.

Key words: Seroepidemiology, Chiang Mai, Thailand, Encephalitis

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INTRODUCTION

Infection by JE virus has been a great public health problem in Thailand since its outbreak into epidemics in 1969 (Yamada *et al.*, 1971; Grossman *et al.*, 1973a). Seroepidemiological survey on antibodies among healthy humans was conducted as an important part of our virological and epidemiological studies on encephalitis in Chiang Mai Area, in order to know the prevalence of JE and other related flaviviruses (Igarashi *et al.*, 1983). Since ELISA has been introduced as a new serological method in various infectious diseases (Sever and Madden, 1977), we applied it together with classical HI test for the antibody assay, and the results are reported in this paper.

MATERIALS AND METHODS

Serum specimens: Specimens were collected at 5 locations as shown in Fig. 1; Fang, Mae Taeng, Doi Saket, and Sarapee in Chiang Mai Province, and Pasang in Lamphoon Province. These places were rural areas and surrounded by rice fields in Chiang Mai Valley except Fang, which belonged to Mae Kong Valley. Human subjects were divided into 9 age groups and approximately 20 individuals from each group were bled totalling 985 specimens (Table 1). Procedures of bleeding is essentially the same as reported before (Fukunaga *et al.*, 1974). Fingertips of these individuals were cleaned by 70% alcohol and blood was taken by stainless steel disposable lancets (Feather Co. Gifu, Japan) and absorbed into bleeding filter paper No. 1 (Toyo Roshi Co. Tokyo, Japan). Three to four filter papers were used for a single individual in order to obtain enough amount of blood for further studies by the neutralization tests. The volume of the blood in the absorbing area of the filter paper is approximately 0.1 ml, corresponding to 0.04 ml of the serum. After drying, the filter papers were

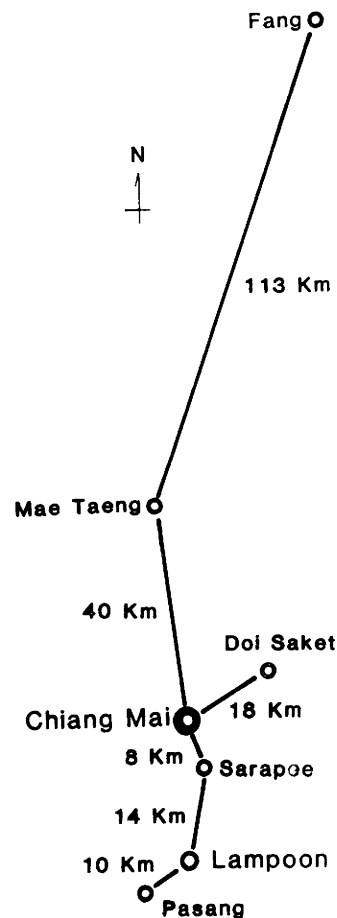


Fig. 1. Sampling places of healthy human specimens

Table 1. Number of blood specimens collected from healthy people

Age group (years)	Collection site					Total
	Chiang Mai				Lampoon	
	Fang	Mae Taeng	Doi Saket	Sarapee	Pasang	
0- 3	23	23	21	21	22	110
4- 6	23	23	23	19	21	108
7- 9	22	21	17	18	23	101
10-14	26	20	22	20	21	106
15-19	21	23	24	20	22	114
20-29	25	19	21	23	21	109
30-39	21	25	24	22	21	113
40-49	22	22	21	24	21	110
50-	22	22	26	21	20	111
Total	205	198	199	192	191	985

brought back to Chiang Mai University, and one of the filter papers from each individual was processed for the HI and ELISA. One-fourth of the absorbing area (corresponding to 0.01 ml of the serum) was cut out and put into 1 ml of PBS-T (phosphate-buffered saline, pH 7.2, containing 0.05% Tween 20 and 0.01% NaN_3) to make 1:100 dilution of the serum for use in the ELISA. The remaining three-fourth was cut into small pieces of 2-3 mm and the serum was extracted with 0.3 ml of BS (0.05 M borate buffer, 0.15 M NaCl, pH 9.0) for overnight at 4°C. The extract was mixed with 0.3 ml of 25% kaolin (Fischer, Acid-washed) in BS and then centrifuged. The supernatant was absorbed with one drop of 50% goose red blood cells in BS and centrifuged. The supernatant was used for the HI as 1:20 diluted serum.

ELISA procedures: Indirect micromethod of Voller et al. (1976) was followed with slight modifications (Igarashi *et al.*, 1981), using formalin-inactivated purified JE vaccine concentrate (Takaku *et al.*, 1968) as assay antigen as described in the accompanying paper (Fujita *et al.*, 1983). ELISA titer of test specimen was estimated by comparing the color density developed by the specimens with those by serial dilution of standard positive serum (Igarashi *et al.*, 1981).

HI: The method of Clarke and Casals (1958) was followed with modification to microtiter system (Sever, 1962). Sucrose-acetone extracted antigen of JE virus, JaGAr-01 strain, was kindly supplied by Chemoserotherapeutic Institute, Kumamoto, Japan. The Mochizuki strain of dengue virus type 1 (D1), and TR1751 strain of dengue virus type 2 (D2) were used to prepare sucrose-acetone extracted antigens from infected suckling mouse brains.

RESULTS

Table 2 shows the age distribution of HI titers among healthy inhabitants at 5

Table 2. Age distribution of HI titers among healthy people against JE, Dengue 1, and Dengue 2 antigens

Age group		Fang						Mae Taeng						Dol Saket						Sarapee						Pasang									
		<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320
JE	0- 3	20	1						19	1	2					19							19						18	1	1				
	4- 6	19	1	1	1	1			14	5				1	1	19	2	2					13	4					17	1					
	7- 9	17	4	1					11	5	3	2				15	1						18						13	6	2	1			
	10-14	16	6	3	1				5	5	6	1	2			12	4	4	1	1			15	4	1				4	7	2	7			1
	15-19	11	3	1	5	1			4	1	6	5	5	1		12	5	4	1	1	1		6	5	7	6			2	3	7	4	3	1	2
	20-29	12	3	7	1				3	1	5	7	2	1		7	4	8	1	1			4	5	13	1			5	8	6	1	1		
	30-39	8	2	7		1		1	4	3	4	7	5	1	1	5	7	6	1	2			4	8	7	3			2	8	6	4			1
	40-49	6	8	5	2	1			1	3	3	8	3	3		7	4	6	4				1	5	6	8	4		1	11	3	4			
	50-	3	9	4	4	1	1		2	1	8	8	3	1		2	8	11	5				3	6	9	2			5	7	5	2			
	Total	112	36	36	14	5	1	1	63	20	42	38	20	8	2	98	35	41	16	6	1		80	34	40	27	6		55	29	47	32	14	2	4
Dengue 1	0- 3	21	1	1					11	4	5	2				15	1	2	1			11	2	2	3	1		15	2	2				1	
	4- 6	13	1	5	1		1		7	3	4	5		2		8	5	10				11			4		1	17	1						
	7- 9	15	5	2					4	6	7	4				7	4	3	2		1		15	2		1	1		15	5	2				
	10-14	15	4	3	2	1			2	2	10	3	1	1		5	3	5	2	6	1		4	3	3	3	5	1	7	4	6	2	1	1	
	15-19	12	3	1	4	1			4		2	5	7	4		5	3	5	6	3	2		2	5	8	6	3	1	6	6	4	2	3		
	20-29	17	1	5					1	1	7	5	3	1	1	2	2	5	3	9			1	2	1	10	7	2	2	2	3	11	3		
	30-39	13	1	1	1		2	2	2		7	4	4	5	2	2	2	8	7	4	1		2	2	4	8	4	1		1	3	7	8	1	1
	40-49	7	7	5	1		1		1	3	6	7	3		1	3	1	3	6	8			2		4	10	7	1	1	8	6	2			2
	50-	7	10	1	3		1		2	2	7	7	2	3		2	1	8	12	3			5	5	5	5	1	5	1	11	2				
	Total	120	32	24	14	2	4	3	34	21	55	42	20	14	6	49	22	49	38	34	2	3	46	13	24	48	40	12	3	63	5	33	49	21	5
Dengue 2	0- 3	5	5	8	2	1																9	2	5	2	1									
	4- 6	4	8	5	4	1																4	6	3	2	1	1								
	7- 9	6		11	4	1																4	2	8	4										
	10-14	3	1	12	6	3																2	4	9	2	1	1								
	15-19	2	1	8	7	1	3															1		4	11	5	3								
	20-29	10	1	5	6	1																		7	14	2									
	30-39	7	4	3	2	2																2	1		6	7	5								
	40-49	2	1	5	5	5		4														1		2	5	11	4	1							
	50-	3	3	5	5	3	1	2															1	5	8	5	2								
	Total	42	24	62	41	18	4	6															21	13	27	51	49	21	4						

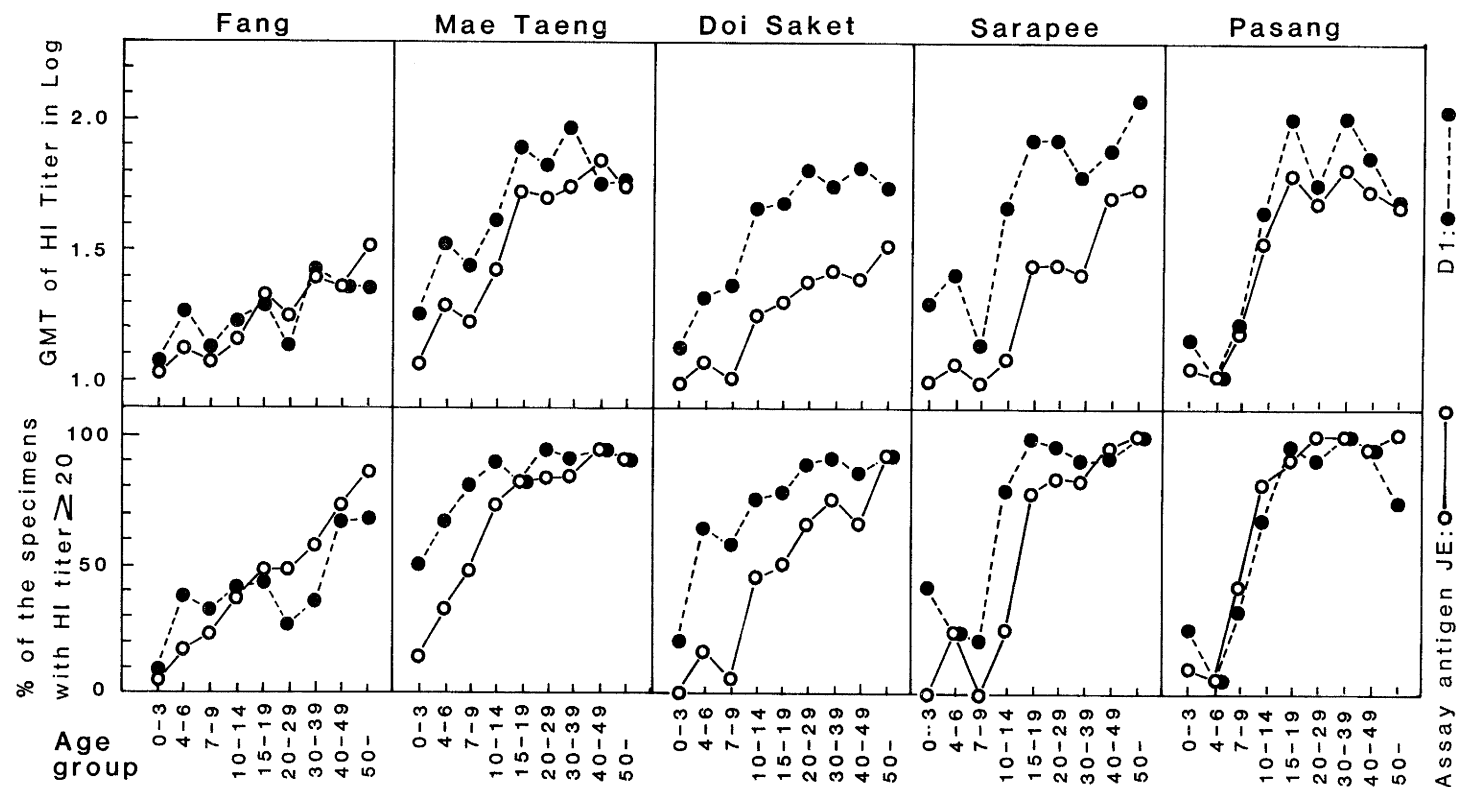


Fig. 2. Age distribution of HI antibodies as measured by JE and D1 antigens in 5 sampling places. Geometrical mean titer (GMT) in the upper panels and antibody positive rates in lower panels were shown as measured by the JE(○—○) and D1(●- - ●) antigens, respectively.

sampling places according to the antigens used in the tests. Because of the limited period of stay in Chiang Mai, the titer against D2 antigen was assayed only for the specimens from Fang and Sarapee. The data in Table 2 were summarized into percentage positives and geometrical mean titer (GMT) in each age group at 5 sampling places (Fig. 2). The result shows that both the antibody positive rate and GMT were lowest in Fang. These 2 parameters as measured by JE antigen were lower than the corresponding values measured by D1 antigen, in the case of Doi Saket, Sarapee, and less markedly in Mae Taeng. In contrast, specimens from Fang and Pasang showed that these values were similar whether assayed by JE or D1 antigen. The results appear to indicate that dengue virus circulation is more prevalent than JE virus in Doi Saket, Sarapee, and Mae Taeng. This reasoning is supported by the result in Table 3, which shows correlation between the HI titers assayed by JE antigen and that by D1 antigen for 5 sampling places, and also the correlation between the HI titer assayed by D1 and that by D2 antigen for Fang and Sarapee. Although many specimens from Doi Saket, Sarapee and Mae Taeng showed higher titers by D1 than by JE antigen, the tendency was less marked for Fang and Pasang. The titers assayed by D2 antigen appear to be higher than the titers assayed by D1 in Fang and Sarapee.

Fig. 3 shows the percentages of the specimens in each age group in 5 sampling places according to their presence of HI antibodies assayed by JE and D1 antigens. The result also shows that in Fang percentage of the people without JE and D1-HI antibodies was significantly higher, and that with both JE and D1-HI antibodies was lower, than those in other 4 sampling places. It can also be observed that in Mae Taeng, Doi Saket, and Sarapee, significant portion of the people possessed D1-HI antibodies without showing positive HI antibodies against JE, especially in younger people. The result of Fig. 3 was summarized into Table 3, which shows that percentage of the specimens showing monospecific HI antibodies to JE was high in Fang and Pasang, on the other hand, the value was low in other 3 sampling places, where, percentages of people showing D1 antibodies without JE-HI antibody titers were higher.

Table 4 shows age distribution of ELISA titers against JE antigen among healthy individuals at 5 sampling places. The data were summarized into Fig. 4 in order to show percentage positives of IgG- and IgM-ELISA as well as GMT of IgG-ELISA. Here, we arbitrarily chose the positive limit of IgG-ELISA as 1000 and that of IgM-ELISA as 100, according to the previous experience in Japan (Bundo et al., 1981; 1982). Both the percentage positives and GMT showed age-dependent increase, however, the values observed for Fang was lower than those in other 4 sampling places, supporting the findings by the HI. More than 80-90% of individuals over 15 years in Mae Taeng, Doi Saket, Sarapee, and Pasang possessed anti-JE IgG-ELISA antibody titers over 1000, whereas, in Fang the value was reached at the age of 40 years old. The distribution of IgM-ELISA was quite different from those of IgG-ELISA. Antibody positive rate of IgM-

Table 2. Correlation between the HI titer against JE and Dengue antigens

JE		Fang							Mae Taeng							Doi Saket							Sarapee							Pasang						
D1		<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640
	<20	94	17	8	2				29	2	1	1				44	2	1	1				40	1	2					50	6	4	2	1	1	
	20		1	10	3				15	2	5	1				19	2	1				12	2	1						1	3					
	40			7	6	5	2	1	13	14	20	8	2			23	16	10	1				8	8	7	1				3	10	19	1			
	80				7	3	4	2	2		2	1	13	17	7		7	9	16	5	2		11	9	17	11			1	9	20	19				
	160					1	8	5			2	1	3	7	3	3	1	4	6	12	10	2		11	6	10	9	4			1	3	8	9		
	320						1	1			2		1	4	6	1					1			1	2	3	5	1				1	2	2		
	640							2					1	1	3	1				2	1			1		1	1					2	1	4		
	Total	110	36	31	12	5		3	63	20	43	39	19	7	2	97	35	40	17	7	1		83	29	40	27	6			55	29	47	32	14	2	4

D2	D1	<20 20 40 80 160 320 640							<20 20 40 80 160 320 640							<20 20 40 80 160 320 640							<20 20 40 80 160 320 640											
		<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160	320	640	<20	20	40	80	160
	<20	39	1	1	1																	17	1	1	1	1								
	20		20	2		2																	11	1	2									
	40			41	2	7	7	1															12	3	6	3	2							
	80				13	5	9	7	10														6	4	13	18	12							
	160					5	1	3	1	1													2	1	3	24	17	1	1					
	320						1	1			3														1	2	8	6	2					
	640							2	2	1																	4	1						

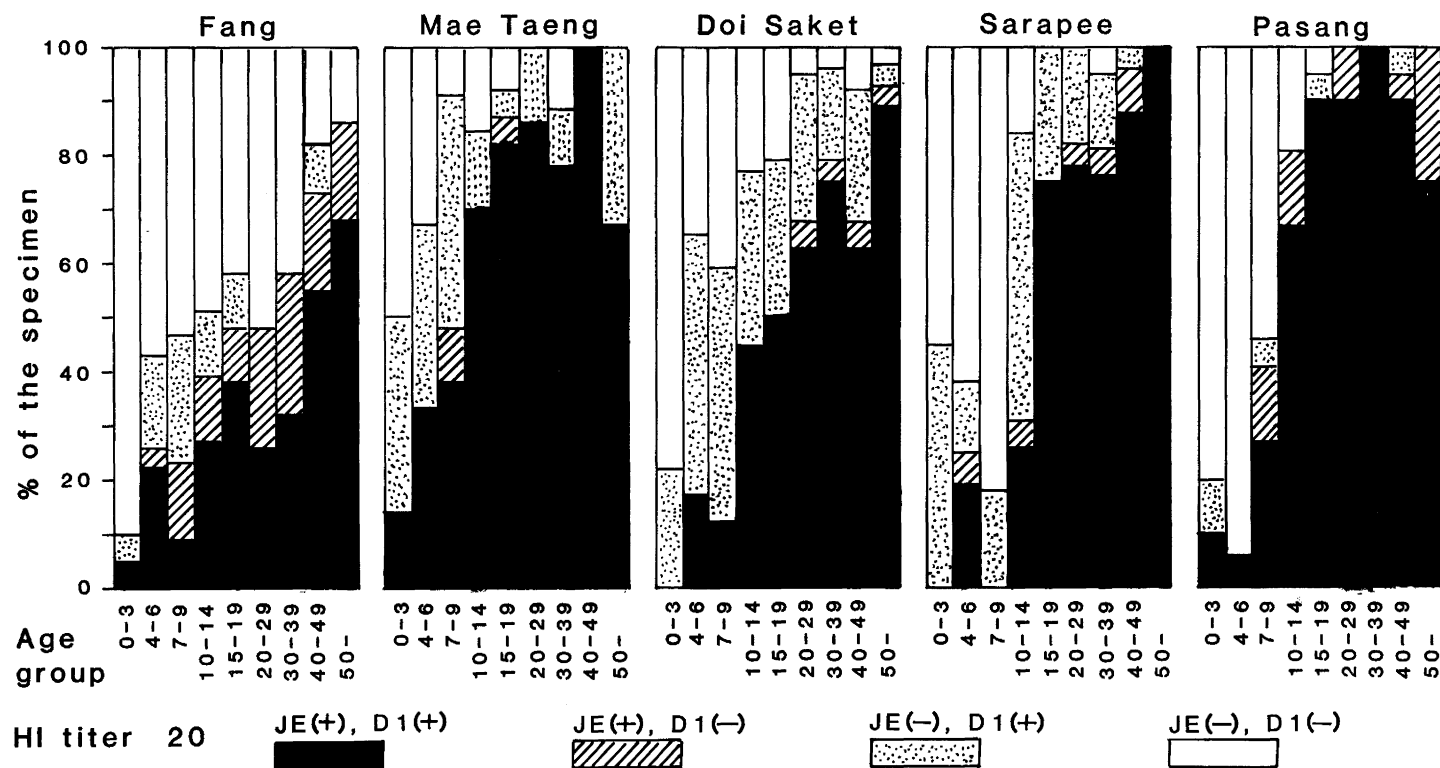


Fig. 3. Percentage of the specimens possessing HI antibodies in each age group in 5 sampling places measured by JE and D1 antigens.

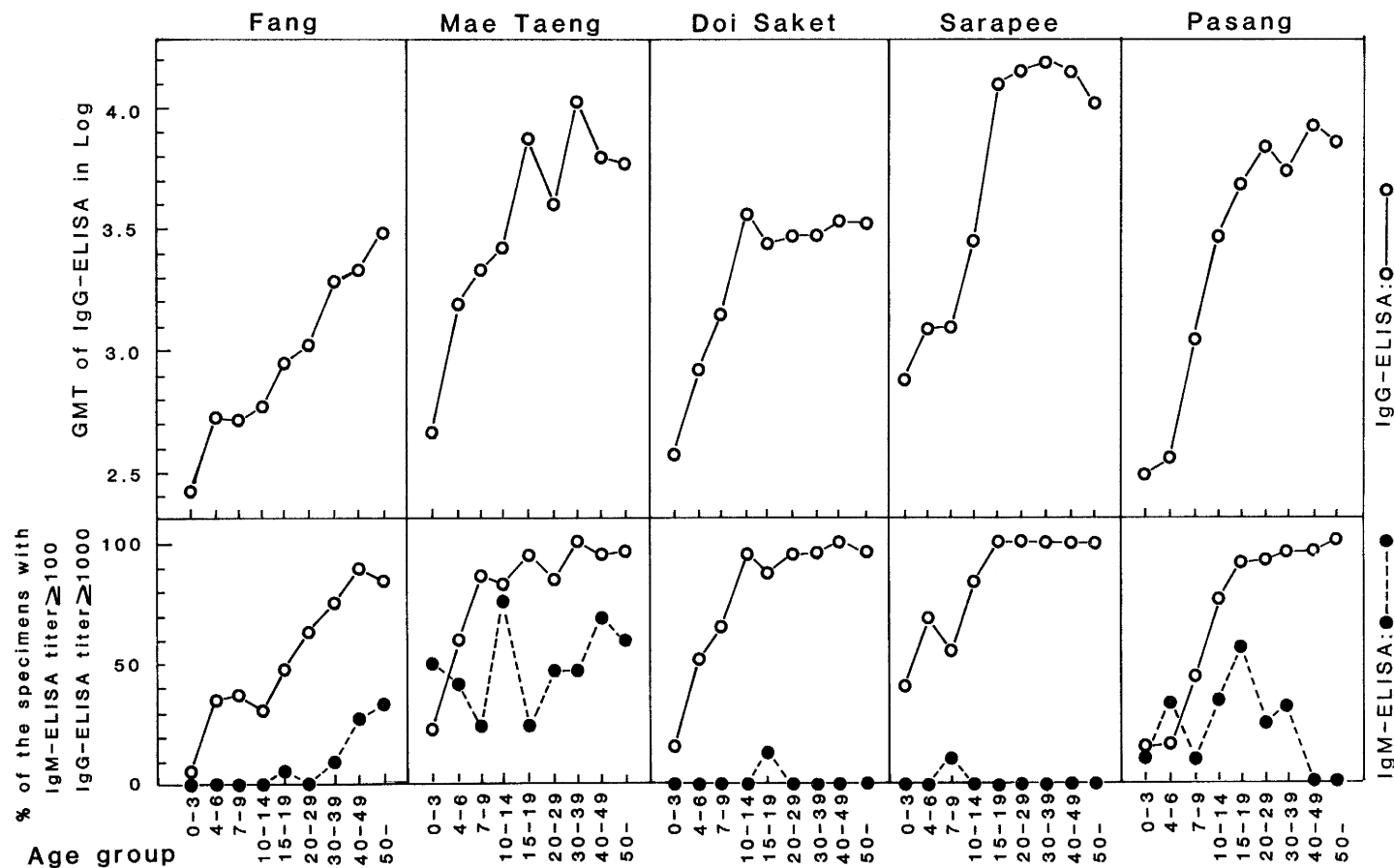


Fig. 4. Age distribution of ELISA antibodies against JE antigens in 5 sampling places. Geometrical mean titer (GMT) of IgG-ELISA in the upper panels and antibody positive rates of IgG-ELISA (\circ — \circ), and IgM-ELISA antibodies (\bullet \bullet) in the lower panels were shown.

Table 4. Antibody positive rates among healthy inhabitants as measured by JE and D1 antigens in the HI tests

Sampling place	Fang	Mae Taeng	Doi Saket	Sarapee	Pasang
Total number	196	193	197	185	183
Positive %					
JE & D1	52.6	85.0	77.7	78.4	72.7
JE only	13.8	2.1	2.0	1.6	7.7
D1 only	8.2	17.6	26.9	23.2	2.7

ELISA was highest in Mae Taeng, followed by Pasang, then Fang. In contrast, only a few individuals possessed significant levels of IgM-ELISA titer against JE virus in Doi Saket and Sarapee. The results indicate that the recent infection by JE virus was more frequent in Mae Taeng, followed by Pasang, and in Fang, at the time of our sampling period.

DISCUSSION

The age distribution of HI antibody positive rate (Fig. 2) as measured by JE antigen was lower than the value measured by D1 antigen in Mae Taeng, Doi Saket, and Sarapee. The result is opposite to the data reported by Grossman *et al.* (1973b). They showed that the antibody positive rate by JE was higher in 3 out of 4 villages studied, especially in 2 villages, while another village showed similar antibody positive rate both by JE and dengue antigen, like Pasang and Fang in our study. Thus, dengue virus appeared to have circulated more frequently after 1970 than before. High antibody positive rate and high titered HI antibodies against dengue antigen, especially D2 in Sarapee, could be explained by dengue epidemic in 1977 in this area (Statistics of the Ministry of Public Health, Thailand). The antibody positive rate in Sarapee was similar to the values reported by Yamada *et al.* (1971) for Chiang Mai, or for Bangkok Metropolitan Area in 1962 by Halstead *et al.* (1969a). Still the value was lower than those observed in Khon Kaen, Northeastern Thailand, by Fukunaga *et al.* (1974). Halstead *et al.* (1969b) showed that frequency of dengue antibodies by the HI test was lowest (36%) in Chiang Mai among 14 sampling places in Thailand, in 1962 and 1964. Lower antibody positive rate in Fang may reflect some ecological characteristics of this area, which belongs to Mae Kong Valley, while 4 other sampling places belong to Chiang Mai Valley. Also it is possible that flavivirus infections did not invade so much across the watteridge into Mae Kong Valley. Interesting is the finding that IgM-ELISA positive rate was different among 5 sampling places, even among 4 places in Chiang Mai Valley. The data

Table 5. Age distribution of anti-JE ELISA titers among healthy people (Titer $\times 10^{-2}$)

	Age group	Fang							Mae Taeng							Doi Saket							Sarapee							Pasang						
		2	5	10	20	40	80	160	2	5	10	20	40	80	160	2	5	10	20	40	80	160	2	5	10	20	40	80	160	2	5	10	20	40	80	160
Ig	0- 3	19	2				1	11	6	2	1	1	1		10	5	2		1			3	9	4	2		2		18		1		1	1		
	4- 6	13	2	3	1	2	2	4	5	2	2	2	7		7	4	4	4	2	2		4	3	2	3	3	3		12	4	2		1			
	7- 9	10	4	4	3		1	3		4	5	2	7		2	4	2	3	5		1		8	2	4	2	2		5	8	3		1	4	2	
	10-14	11	7	2	1	4	1	3	1	1	4	3	9			1	3	3	6	8	1	1	2	3	1	5	6	1	3	2	1	1	4	8	2	
	15-19	7	4	2	2	3	3		1			7	4	9		3	5	3	6	6	1				2	1	21		2		4	3	7	4		
	20-29	5	4	6	3	4	2		3	3		3	6	4		1	3	2	11	3					1	1	21		2	1	1	4	1	12		
	30-39	1	4	5	1	3	6			1	1		10	14		1	2	6	12	3						1	21		1		4	4	5	5		
	40-49	2	2	3	2	4	4	1		1	2	1	1	11	5			1	6	11	3					4	20		1	2	1	1	1	14		
	50-		4	3	2	7	5	3		1	3	1	2	7	8		1		6	17	2					1	6	14		2	4		3	11		
	Total	68	33	28	15	27	25	4	21	18	18	15	21	62	40	19	20	22	33	71	27	3	8	22	11	12	13	25	98	38	20	12	15	19	30	50
IgM		1	2	4				1	2	4					1	2	4				1	2	4					1	2	4						
	0- 3							11																					2							
	4- 6							9																					6							
	7- 9							4	1													1		1					2							
	10-14							16																					7							
	15-19	1						4	1						2	1													11							
	20-29							9																					5							
	30-39	2						12																					6							
	40-49	5						12	2																											
	50-	8						13																												
Total	16							90	4					2	1						1		1					39								

seem to indicate that JE virus circulation or recent infection rate in the inhabitants was not uniform throughout the Chiang Mai Valley. On the other hand, IgG-ELISA against JE antigen probably reflects antibodies against flaviviruses, including JE and dengue as shown by laboratory studies (Roehrig, 1982). More precise studies on the prevalence of type-specific antibodies to JE and dengue viruses should be studied by the neutralization tests.

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1982年タイ国チェンマイ地区における脳炎のウイルス学的疫学的調査 V. 健康人の血清疫学調査

福永利彦 (大阪大学微生物病研究所防疫学部門)

五十嵐 章 (長崎大学熱帯医学研究所ウイルス学部門)

緒方隆幸 (国立予防衛生研究所ウイルスリクエッチャ部)

藤田宣哉 (神戸大学医学部微生物学教室)

Ongart CHAROENSOOK, Sujarti JATANASEN (タイ国公衆衛生省疫学部)

Charnchudhit CHANYASANHA, Kanai CHATYANONDA (タイ国公衆衛生省医科学局ウイルス研究所)

Supatra PEERAKOME, Jiraporn SUPAWADEE, Kampol PANASAMPOL (タイ国チェンマイ大学医学部微生物学教室)

タイ国チェンマイ地区の5地点において健康人住民の抗体価調査を日本脳炎 (JE) とデングウイルス抗原に対する血球凝集抑制反応および、JE に対する免疫酵素測定法 (ELISA) により実施した。メコン河流域に属する Fang はチェンマイ渓谷にある他の4地点に比べ抗体価陽性率および平均抗体価共に低い結果が得られた。JE に対する IgM-ELISA 陽性率は採血地点により異なり、調査地域において JE ウイルスの散布が均一でない事が示唆された。

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