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STUDIES ON AN EPIDEMIC OF JAPANESE ENCEPHALITIS IN THE NORTHERN REGION OF THAILAND IN 1969 AND 1970[1]

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SUMMARY In 1969 and 1970, a big epidemic of Japanese Encephalitis (JE) occurred in the northern region of Thailand, especially in the Chiang Mai province. Virologically and ecologically studies were performed on the epidemic. The epidemics began in May, reached their peak in July and then gradually declined in August. This is the rainy season in Thailand. In 1969, there was an epidemic of Thai Hemorrhagic Fever (THF) at the same time in the Chiang Mai province. The cases of JE were under twenty years old and 50% of young healthy people of this age have no antibody for JE. The incidence rate in 1969 due to JE per 100,000 head of population was 20.3, which had never seen previously in Thailand. Two strains of virus were isolated from the brains of fatal cases in these years. The causal agent was confirmed to be Japanese Encephalitis virus and the strain had very similar character-

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istics to the JaGAr #01 strain, which is a reference strain of JE. The scale of the epidemic had some correlation with the density of livestock and the scale of rice fields. Pigs, cattle and horses are considered as the amplifier of JE virus. The weather conditions may also have influenced the scale of the epidemic. HI tests on sera of patients showed some cross reactions with Dengue viruses. This makes some problems for diagnosis of JE in south-east Asia.

INTRODUCTION

In 1969, an epidemic of encephalitis became a matter of grave concern in the Chiang Mai Province in Thailand, the northern region of Thailand. The scale of the epidemic was very large and have never experienced in Thailand. A similar epidemic occurred the following year, 1970, in the same place and at the same time.

This paper reports results of virological and ecological studies on these epidemics. Some of the problems and factors involved are discussed. This is the first big epidemic of Japanese Encephalitis to be confirmed virologically in Thailand.

MATERIALS AND METHODS

1. *Neutralization test (NT)*

The 50% plaque reduction method for measuring the serum NT titer was performed essentially by the method of Hashimoto and Prince (1963). Primary cultures of chick embryo cells were prepared by the method of Porterfield (1960).

2. *Hemagglutination inhibition test (HI) and complement fixation test (CF)*

These tests were done by the methods of Hammon (1964). Sera for the HI test were treated with acetone, and sera for the CF test were inactivated at 56C for 30 minutes. Antigens were extracted from the bcains of infected suckling mice with acetone-ether or sucrose-acetone method.

3. *Virus*

Reference strains of virus were supplied from NIH of Japan and propagated in suckling mice brains. For virus isolation, a 20% homogenate of fresh brain from a fatal case of JE was inoculated into the 1–3 day old suckling mice.

4. *Antisera*

Hyperimmune rabbit sera of reference arbo-virus were used for sreum tests. Rabbits were immunized by intravenous injection of 5 ml of the supernatant of 20% homogenate of infected mouse brain. Four injections were given at one week intervals. One week after the last injection, the serum was separated.

5. *β-Mercapto-ethanol treatment*

This was carried out essentially by the method of Ishida (Experimental Code for Microbiology and Serology 1966). The incubation time was one hour at 37C.

6. *Serum specimens*

1) Sera of patients

Most sera were collected from Chiang Mai University Hospital and McCormic Hospital in Chiang Mai City. Some specimens were collected from other hospitals. Paired sera were exanined.

2) Sera of healthy subjects

Sera of healthy subjects in Chiang Mai City were collected in Chiang Mai University Hospital in March, 1970. Sera of healthy subjects in Bangkok and Thonburi city were collected in Siriraj Hospital of Mahidol University from December 1968 to June 1969.

3) Sera of livestock

Pig sera were obtained monthly from a slaughter house of Chiang Mai City. Twenty samples of sera were collected each time. Sera of breeding pigs were collected from the jugular vein. Horse and cow sera were obtained monthly from the same group of animals on Thai Army Farm at Mae-Sa in the suburbs of Chiang Mai City.

7. *Fluorescent antibody technique (FAT)*

Specimens from the brains of fatal cases were taken as soon as possible after death. The specimens in 1969 were taken at autopsy and those in 1970 were taken by brain puncture (Kimoto et al., 1968). The direct FAT technique was done by

the method of Aoyama (1967). Anti-JaGAr #01 rabbit serum was used for FITC conjugation.

8. *Electron microscopy*

Virus preparations were examined by negative staining method using phosphotungstate (Brenner and Horne, 1959) in a Hitachi electron microscope, model HU-11d.

9. *Collection of mosquitoes*

Mosquitoes were collected at TA-WANG-TAN Village in Saraphi county in Chiang Mai. A light trap was placed under a house near a pig-sty. Sometimes mosquitoes were also caught in a hand net supplementary.

10. *Statistical data*

Statistical data were obtained from the National Statistical Office and the Meteorological Department of the Government of Thailand.

RESULTS

1. *General observations on the epidemic*

1) Geographical and seasonal occurrence

According to data from Chiang Mai University Hospital, the epidemic of JE in 1969 in Chiang Mai began in May, reached a peak in July and gradually declined in August. An epidemic of THF also occurred at the same time (Fig. 1).

As shown in Fig. 2 and 3, Thailand is usually divided into four regions (quoted from the data of the Statistical Office of Thailand) i.e. the northern, central, north-eastern and southern regions. The epidemics of JE in 1969 and 1970 occurred mainly in the northern region. However, the epidemic of THF occurred in the central and north-eastern regions in both

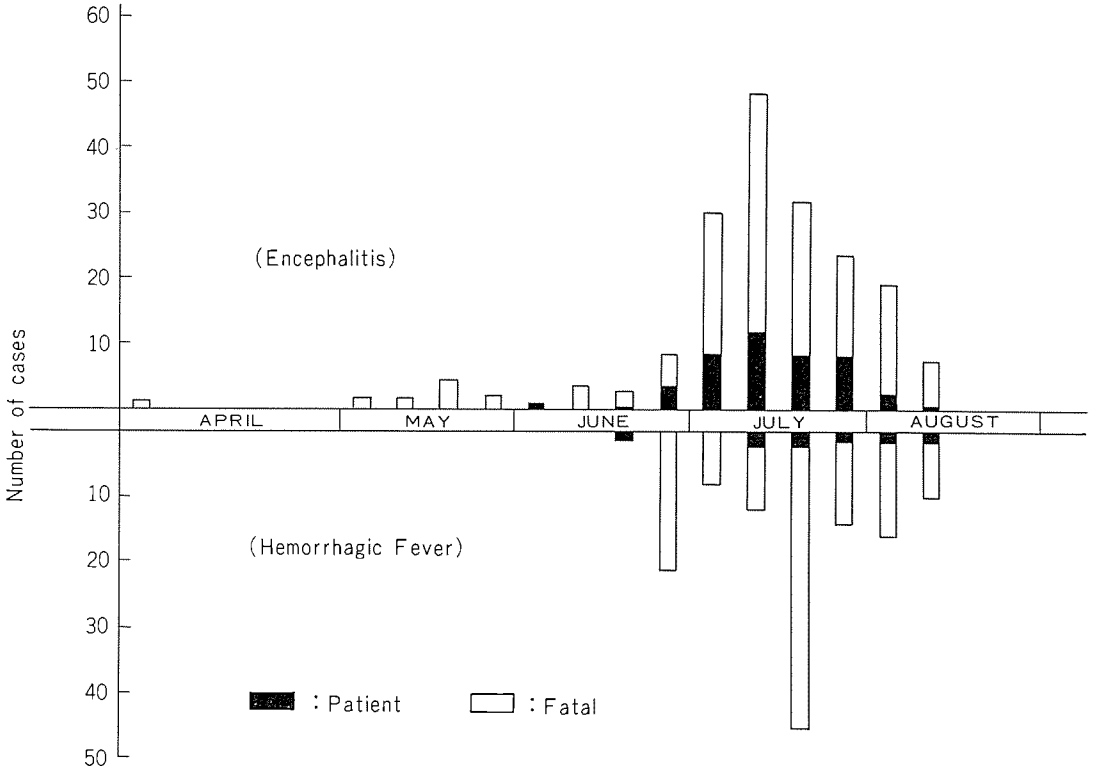

FIGURE 1. *Weekly distribution of cases of Encephalitis & Hemorrhagic Fever in Chiang Mai (1969).*

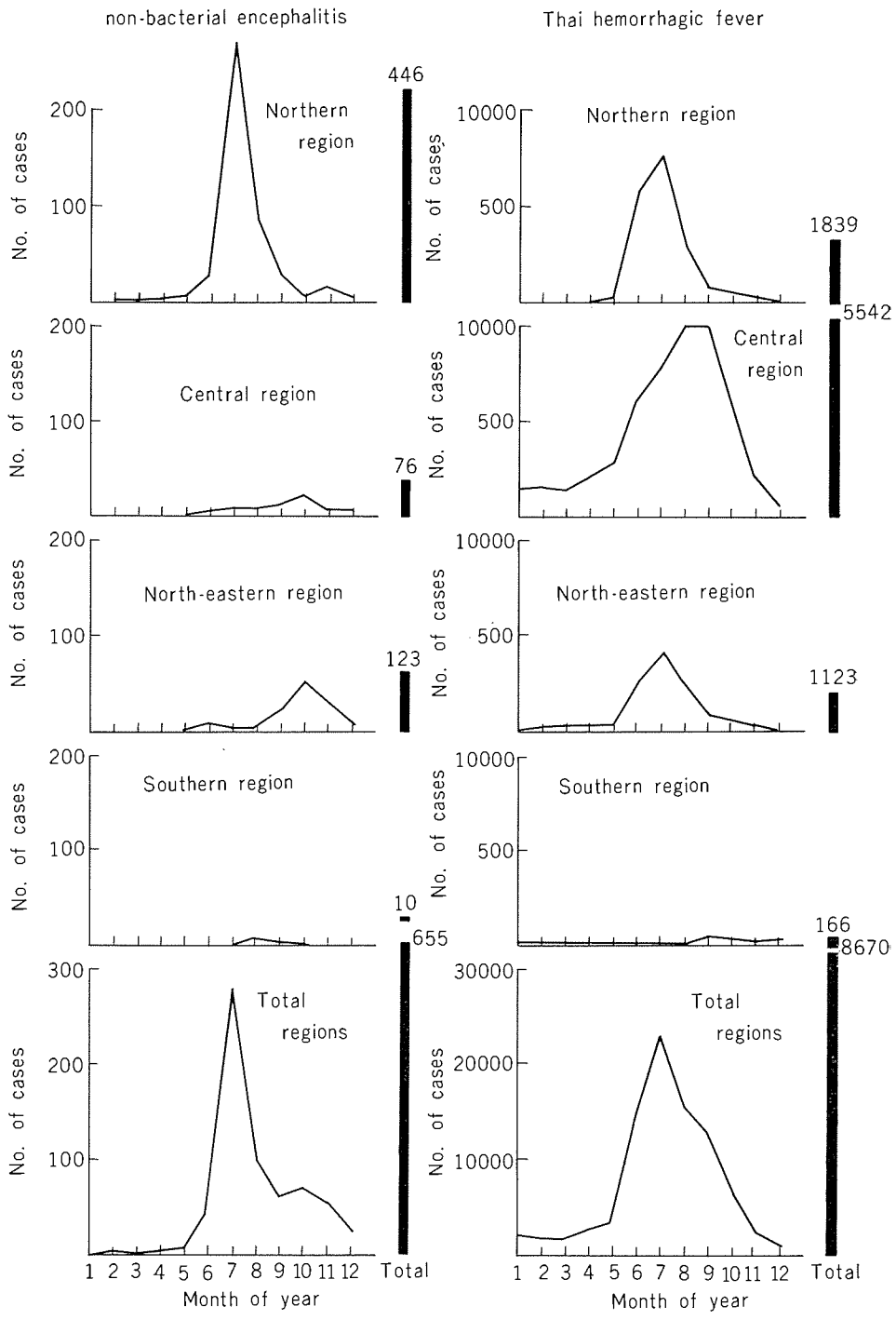
non-bacterial encephalitis
Thai hemorrhagic fever
No. of cases
Northern region
Central region
North-eastern region
Southern region
Total regions
446
76
123
10
655
1839
5542
1123
166
8670
Total
Month of year
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 2.

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FIGURE 2. *Incidence of cases of Encephalitis and Thai Hemorrhagic Fever in four regions of Thailand in* 1969
(based on data from the Ministry of Pub. Health, Thailand).

years. Attention should be paid that cases of THF were also seen in the northern region in 1969 at the same time as the epidemic of JE. The geographical distributions of JE in the two years are shown in Fig. 4. More than 30 of the cases were mainly occurred in the northern region. But in the north-eastern region, more than 30 cases were seen in Buri Ram Province only. (Cases of Equine Encephalitis occurred in the Kon Kaen Province which is next to the Buri Ram Province, and the disease was confirmed virologically as JE).

2) Yearly incidence of JE

As shown in Fig. 5, before 1969 the incidence rate per 100,000 head of population was about 2–3. But in 1969, it increased to 20.3 and in 1970 it was 14.4. This incidence rate is very high compare with that in Japan (Miura, 1967).

3) Age and sex distribution

The data on the epidemic in 1969 given in Fig. 6, show that the cases of JE were under twenty years old. The peak in the age-incidence distribution was from 10 to 14 years old. More cases were men than women, but fatality was similar in the two sexes (Hiraishi, 1967; Kono, 1969). The peak in the age distribution of cases of THF was from 5 to 9 years old, which is a little younger than that of JE.

4) Distribution of JE in the different counties (Amphoe) in Chiang Mai

The distribution of cases of JE in the various counties in Chiang Mai is shown in Fig. 7. The incidence rate in the central part is clearly higher than the other parts.

5) The environment of epidemic area in Chiang Mai

The styles of the farm-houses in Chiang Mai area are one-storied with high floors and every house has a pig-sty. There are little ponds and rice fields round the houses where mosquitoes such as Culex tritaeniorhynchus can readily breed.

2. *Clinical manifestations*

Most of the patients showed disturbance of conciousness, difficulty in swallowing and convulsions. Convalescent patients suffered from malnutrition and the deformation of the extremities. Their mental faculties were reduced, especially the ability to calculate. The clinical findings, symptoms and signs on these cases are summarized in Fig. 8 and Tables 1 and 2. These data agree very well with those on JE.

3. *Serological examination*

The results of serological examinations of patients are given in Table 3. (6 cases at the beginning of the epidemic in 1969). Every case had high antibodies against JE in each test. But sometimes, cross antibody against Dengue group antigens was recognized. (e.g. Sample ES-6 and ES-7). So, to confirm that the epidemic was due to JE exactly, the virus isolation was done.

4. *Virological examination*

As shown in Table 4 and 5, six cases were examined, 3 cases in 1969 and 3 in 1970. The samples in 1969 were obtained at autopsy, and those in 1970 by using special brain puncture (Kimoto, 1968).

1) Fluorescent antibody test (FAT)

The direct method of FAT was used. Specific fluorescence were recognized in 5 of 6 cases.

2) Virus isolation

One-three day old suckling mice were used for virus isolation. A 20% homoginate of human brain was inoculated into the brains of the mice. Virus was isolated from 2 of the 6 specimens and these were temporarily named Chiang Mai X-1 and X-4 (Tables 4 and 5). As the representative strain, Chiang Mai X-1 was used for further examination. Spherical

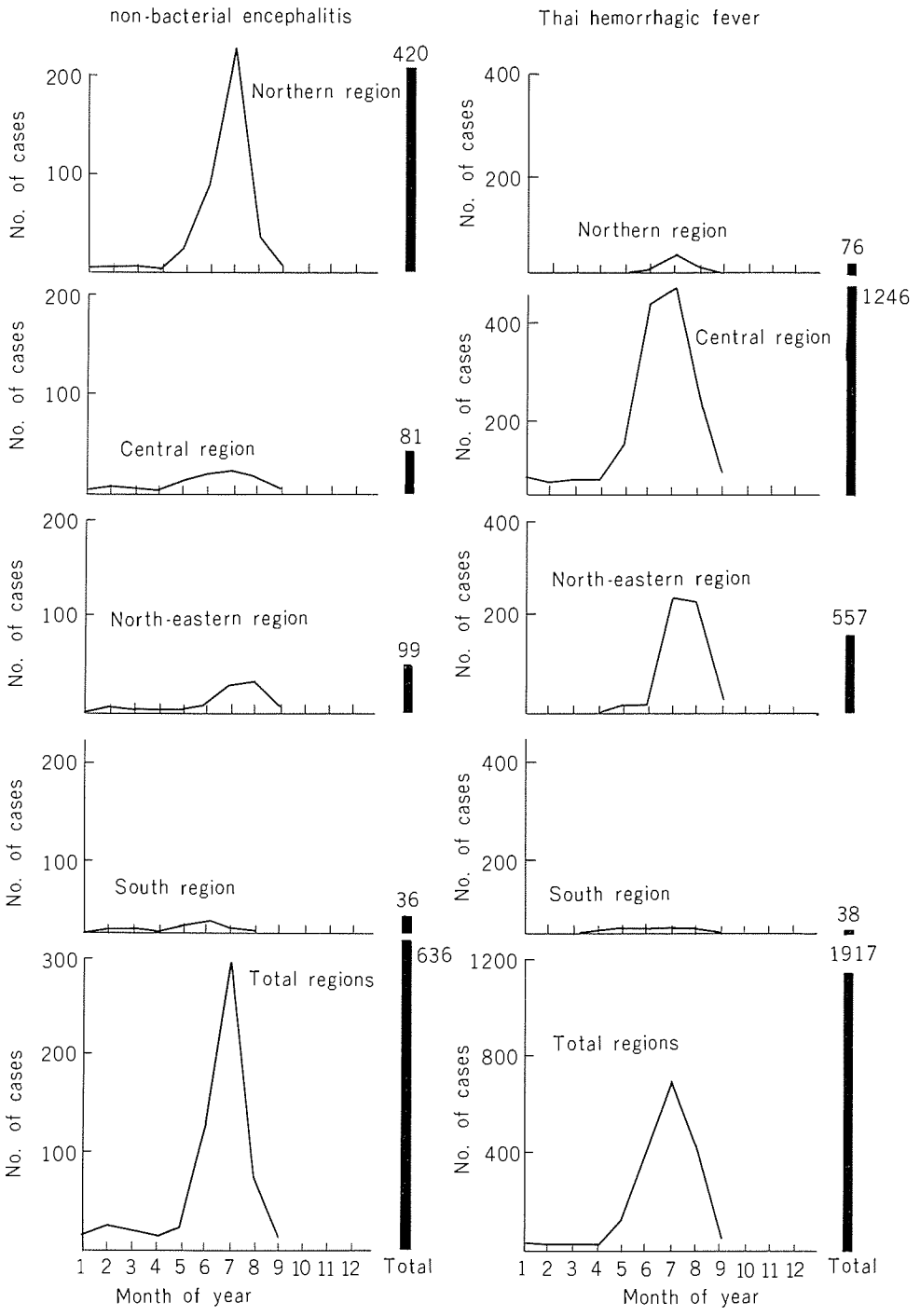
non-bacterial encephalitis
Thai hemorrhagic fever
No. of cases
Northern region
Central region
North-eastern region
South region
Total regions
420
81
99
36
636
76
1246
557
38
1917
Total
Month of year
1 2 3 4 5 6 7 8 9 10 11 12

FIGURE 3.

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FIGURE 3. *Incidence of cases of Encephalitis and Thai Hemorrhagic Fever in four regions of Thailand in 1970* based on data from the Min. of Pub. Health, Thailand).

particles of about 35 mμ are recognized in an electronmicrograph of the isolated virus.

3) Identification of virus

Chiang Mai X-1 was identified as JE virus (Table 6 and Fig. 9) as follows; namely, the results of the HI test indicates that Chiang Mai X-1 is a strain of group B arbo-virus. The CF test shows that it is JE virus, because the box-patterns of JE (JaGAr #01) and Chiang Mai X-1 antigens are very similar comparing with those of other viruses. Chiang Mai X-1 was compared with reference strains of JE, the Nakayama and JaGAr #01 strains. The optimum pH ranges of JaGAr #01, Chiang Mai X-1 and Chiang Mai Ogata (isolated by Dr. Ogata. T. in Chieng Mai in 1964 from a sporadic case.) are similar (pH 6.6 to 6.8) but that of Nakayama is pH 6.2 (Table 7). The HI patterns of these strains were also examined, as shown in Table 8, JaGAr #01, Chiang Mai X-1 and Chiang Mai Ogata had the same patterns, but the patterns of Nakayama was different. Therefore, serologically Chiang Mai X-1 seems to be the JaGar #01 type virus. (Aizawa, 1968)

5. *Ecological study*

1) Antibody level of JE in healthy people

To know the background of the JE epidemic in Chiang Mai, the HI antibody levels against JE in healthy people were examined. As shown in Fig. 10, 50% of the children of 0-10 years old examined had no antibody against JE (<10 HI units). But in young adults of 11 to 20 years old 93% had JE antibody. These data are consistent with the observation that JE only occurred in young people. For comparison with data on Chiang Mai, the antibody levels in Bangkok and Thonburi cities were examined. As shown in Fig. 11, almost subjects in all age groups had antibody against JE.

2) Antibody level of JE in livestock

With regard to factors causing a JE epidemic, a relation between people, pigs (or livestock) and mosquitoes has been confirmed (Konno, 1965; Nakamura, 1966). Pigs seem to be amplifiers of JE virus. As mentioned above, there were many pigs in the Chiang Mai area, so the antibody level against JE in Pigs was examined. As shown in Table 9, all serum specimens collected in July, 1969 at a slaughter house of Chiang Mai showed a very high antibody titer against JE. Specimens from 2 of 5 breeding pigs, showed JE antibody. The antibodies against each Dengue virus type were examined simultaneously, but the value were much less than that against JE. The seasonal change in the antibody level in pigs was also examined (from August, 1969 to September, 1970). As shown in Fig. 12, from June to December, the antibody level remained at a high plateau and this period was during and just after the time of the epidemic. From January the level began to decrease gradually and started to increase again from April. In May, β-mercapto-ethanol sensitive antibody was detected. This sensitive antibody suggests the occurrence of fresh infection of pigs with JE virus. The same phenomenon was seen in sera of horses and cows though less clearly.

3) Mosquitoes examination

Culex tritaeniorhynchus is considered to be the most important species of mosquito as the carrier of JE (Scherer, 1959). The species of mosquitoes in Chiang Mai were examined. Culex tritaeniorhynchus was found in the Chiang Mai area (Table 10).

DISCUSSION

The JE epidemic that exploded in Chiang Mai in 1969 was the biggest epidemic there has ever been in Thailand. The incidence rate was 20.3 which was higher than it has ever experienced in Japan (Miura, 1967). Many facts were found about the epidemic. The epidemic occurred in young people (Fig. 6). This tendency has also been noted in the data of Korea

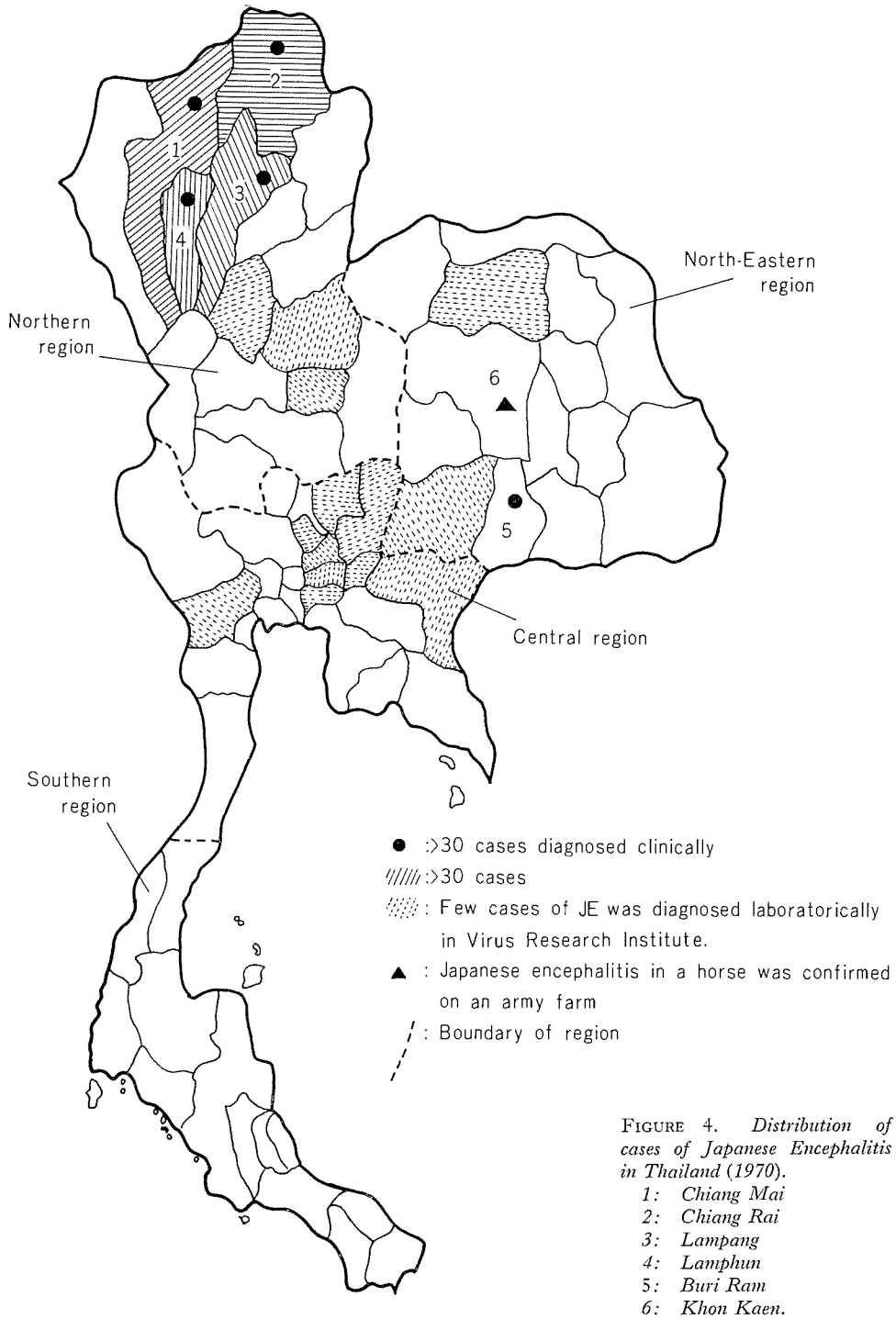


FIGURE 4. *Distribution of cases of Japanese Encephalitis in Thailand (1970).*
1: Chiang Mai
2: Chiang Rai
3: Lampang
4: Lamphun
5: Buri Ram
6: Khon Kaen.

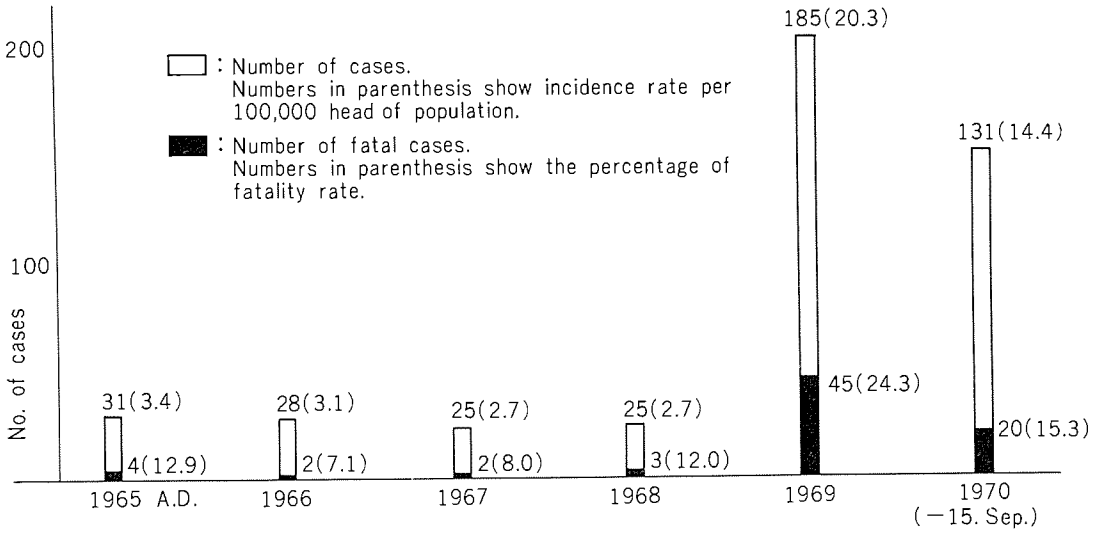


FIGURE 5. *Cases of Japanese Encephalitis diagnosed clinically in Chiang Mai University Hospital.*

and Taiwan (Kono, 1969). The age distributions of cases in these areas are compared in Fig. 13. In Thailand this tendency was explained by the fact that the antibody level against JE is low in young people (Fig. 10). As shown in Fig. 2 and 3, the epidemic of JE was almost entirely limited to the northern region of Thailand. On the other hand, epidemics of THF occurred in the central and north-eastern regions, but in 1969, cases were also seen in the northern region. No cases of either disease were seen in the southern region. Why did the epidemics have these different distributions? Many factors may be considered as causes. As shown in Table 11, there are not so detectable differences or rules in the regions. For example, the densities of population are not so different. However, in the central and southern regions, the incidence rate is not high, although the densities of pigs in these regions are high. The influence of the climate type may also be considered. According to data from the Meteorological Department of Thailand, the southern region has a tropical monsoon, or tropical rainforest climate. On the other hand, other regions have a Savanna climate. It is curious that in the southern region, there are no cases of JE or THF and this requires a future study including the vector distribution. It is said that epidemics of similar arboviruses do not occur simultaneously, because of their interfere reaction. For example, where there is an epidemic of Dengue, there will not be an epidemic of JE (Miura, 1967). Certainly, we did not find a big JE epidemic in a region where there was an epidemic of THF, except in the northern region in 1969 (Fig. 2, 3 and Table 12). And the important causal agent of THF is reported as Dengue virus by Halstead (1963, 1966). But its effect must be affected by an interference reaction between JE and Dengue. Usually a high and broad antibody response against group B arboviruses is seen after THF infection. This phenomenon has been called a "secondary type" of antibody reaction to group B arbovirus antigens (Igarashi, 1969; Halstead, 1966). As shown in Fig. 11, the antibody level of JE in Bangkok is very high. This may depend on the effect of the existence of THF (Table 12).

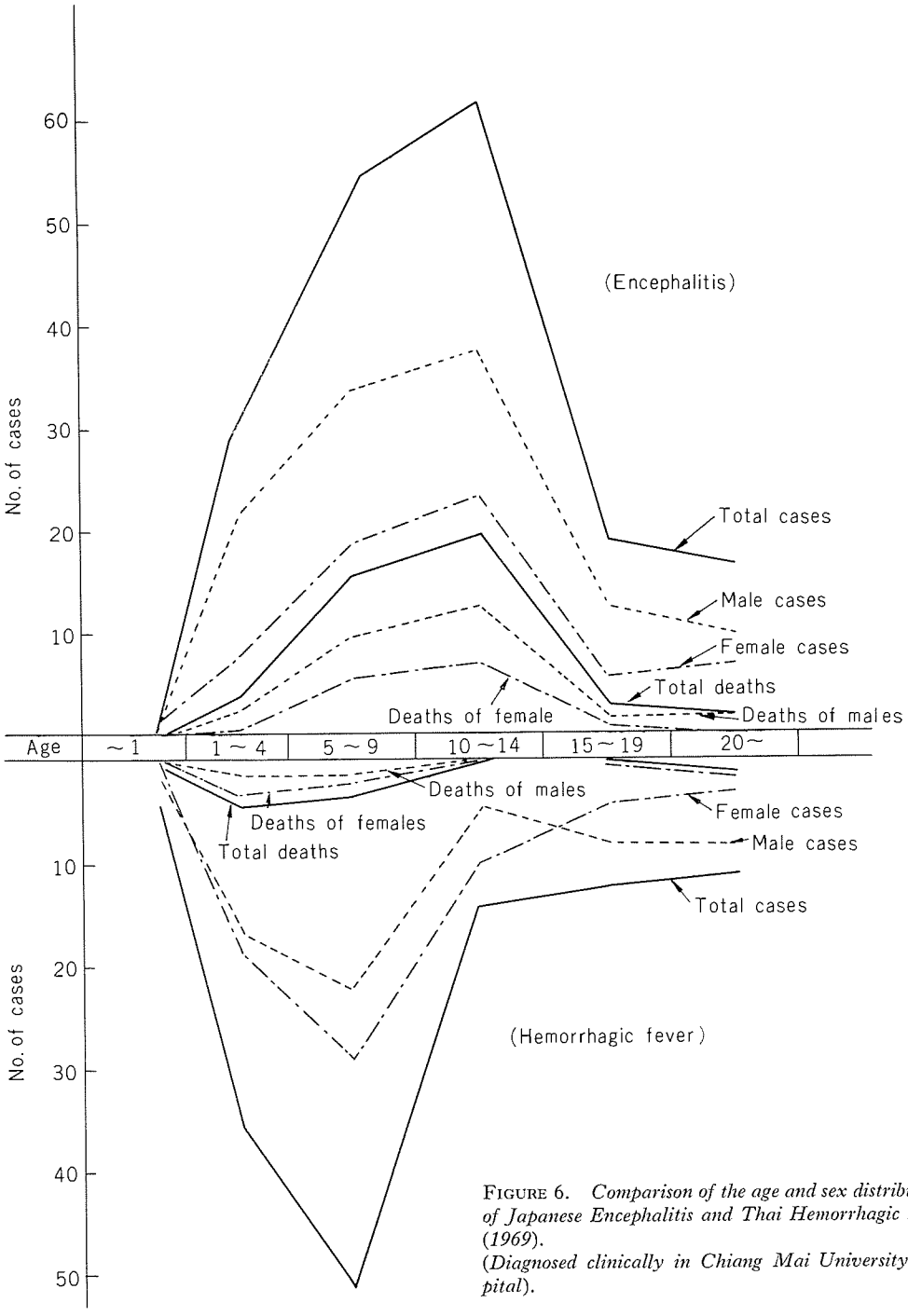


FIGURE 6. *Comparison of the age and sex distributions of Japanese Encephalitis and Thai Hemorrhagic Fever (1969).*
(Diagnosed clinically in Chiang Mai University Hospital).

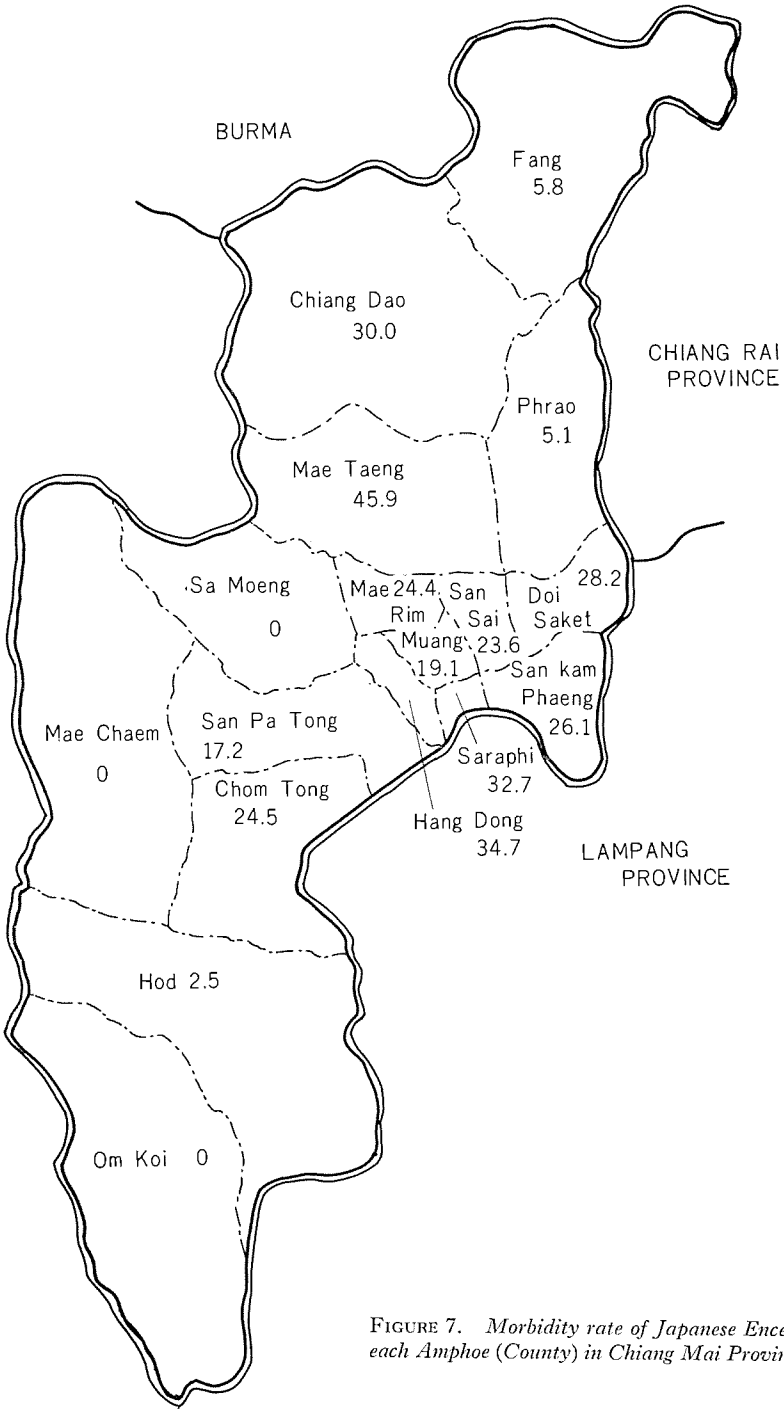


FIGURE 7. *Morbidity rate of Japanese Encephalitis in each Amphoe (County) in Chiang Mai Province (1969).*

TABLE 1. *Findings on cerebro-spinal fluid of cases of Japanese encephalitis in 1969 (Chiang Mai University Hospital)*

Cerebro-spinal Fluid	Total cases		Fatal cases	
	No. of Cases	%	No. of Cases	%
Cell No. 10	11	9.0	5	10.7
11–100	40	32.5	13	28.3
101–500	64	52.0	26	56.5
501–1000	6	5.0	1	2.2
1001–1500	2	1.5	1	2.2
Poly 50%	28	25.0	13	31.7
Lymph 50%	83	75.0	28	68.3
Protein normal	62	50.0	20	45.5
slightly up	62	50.0	24	54.5
Sugar normal	95	88.5	28	90.3
slightly down	12	11.5	3	9.7

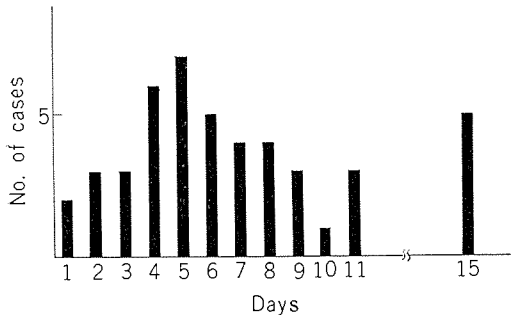


FIGURE 8. *Days from onset to death of cases of Japanese Encephalitis.*
(Chiang Mai University Hospital).

TABLE 2. *The symptoms and signs in cases of Japanese encephalitis in 1969 (Chiang Mai University Hospital)*

Symptoms and sign		%	
Conciousness impaired		100	
Fever		97.7	
Convulsions and / or spasticity		68.8	
Meningeal signs	+	44.1	63.8
	±	19.7	

This is consistent with the fact that no epidemic of JE was seen in Bangkok, although JE virus was found in the region (Gould, 1966). On the other hand, in the northern region, there has not been a big epidemic of THF (Table 12). The reason for this cannot be discussed since few data are available. And the antibody level of JE in Chiang Mai is not so high as that in Bangkok. This indicates that THF may have less influence on the antibody level of JE in the Chiang Mai area.

What is the etiology of THF? A second infection with a different type Dengue is considered as the cause of THF (Russell, 1969; Halstead, 1966; Okuno, 1966). Then is it impossible to make the assumption that THF may also be caused by other B group arboviruses including JE? THF is probably not caused by repeated infections with JE, because in Japan, where usually only JE epidemics occur, there have been no cases of THF. Then is it possible that THF might occur on infection with JE after previous infection with Dengue? If this is so, then the epidemics of JE and THF in 1969 might both be caused by JE virus. That is to say, a subject who had previously been infected with Dengue might manifest the symptoms of THF while a subject who had no antibody of any kind against B group arboviruses might manifest the symptoms of JE. This is only a speculation at present and requires further study.

The next problem is why the incidence rate of JE in different countries in Chiang Mai Province differed. As mentioned above, most cases were in the central part of Chiang Mai Province (Fig. 7). It has been reported that the scale of JE epidemics is influenced by the density of pigs in the region, and pigs are considered as amplifiers of JE. A clear correlation of direct proportion was found between the incidence rate of cases of JE and density of pigs in Osaka (Report of an investigation on forcasting epidemics of Japanese Encephalitis in Osaka III 1968, in Japanese). To see what animals are important as amplifiers of JE in Chiang Mai, we studied the number of live-

TABLE 3. *Serological examination of patients' sera (13 samples from the beginning of the epidemic in 1969)*

Patient	Sex	Age	No. of sample	Days from onset	Neutralization		Hemagglutination inhibition test						CF-Test
					JE Nakayama	JE JaGAr #01	JE Nakayama	JE JaGAr #01	Dengue −1	Dengue −2	Dengue −3	Dengue −4	JE Nakayama
M. M.	F	12	ES-1	3	240	380	40	80	<20	40	40	<20	<4
			ES-2	4	190	460	40	160	<20	20	40	<20	<4
			ES-3	12	700	1400	160	2560≤	<20	80	80	<20	16
N. V.	M	9	ES-4	3	200	<100	40	40	<20	<20	<20	<20	<4
			ES-5	15	490	360	320	640	<20	20	40	<20	<4
N. K.	M	5	ES-6	4	<100	130	80	160	<20	40	80	—	<4
			ES-7	18	4500	4500	1280	2560≤	80	320	320	80	32
P. C.	F	10	ES-8	5	970	230	160	80	<20	20	40	<20	<4
			ES-9	16	520	230	1280	1280	<20	40	—	<20	16
P. N.	M	4	ES-10	3	270	170	80	80	<20	<20	40	<20	<4
			ES-11	11	3600	480	1280	2560≤	<20	40	40	20	8
P. J.	F	3	ES-12	7	860	460	320	640	<20	20	20	<20	8
			ES-13	14	1600	150	320	1280	<20	40	40	<20	8
					105[a] PFU	93[a] PFU							

a Challenge dosis per 0.2 ml. *Abbreviations: JE=Japanese Encephalitis; PFU=Plaque forming units*

stock there (Table 13). From the reports of Ueba (1968), Committee on investigation for forcasting epidemics of Japanese Encephalitis in Osaka (1968), Halstead (1965), Toshikawa (1960) and Toshioka (1960), it seems that in Japan, the most important mosquito for the vector of JE is Culex tritaeniorhynchus (Culex tritaen.). And in Thailand, Culex. tritaen. and Culex gelidus are found. As their habit, they like to bite pigs or other livestock such as cows and horses, but don't attack chickens much. On the other hand, Aedes aegypti, the vector of Dengue does not attack big livestock. This is also suspected from the data in Table 9, showing that the HI titers against Dengue in pigs are very low compared with those against JE. And this mean the vector of Dengue does not attack pigs so much. Thus, big livestock seem to be the amplifiers of JE in Thailand and of these, pigs are probably the most important because of long duration of their viremia (Ueba, 1968). To see if this was the case in Chiang Mai, Chiang Mai Province was divided in to three regions (Fig. 14) and culculated each value (Fig. 15, Table 13 and 14). The incidence rate of cases of JE was directly proportional to the density of pigs or livestock. (Fig. 15). The area of rice fields may also be correlated with the incidence rate of JE, because we can commonly guess that in areas where there are many farmhouses, there are many rice fields and livestock. Rice fields are suitable places for Culex tritaen. to breed. Same correlation was seen between the area of rice fields and the incidence of JE in Chiang Mai. (Fig. 14 and Table 14). Thus in Chiang Mai, there is probably a cycle of the

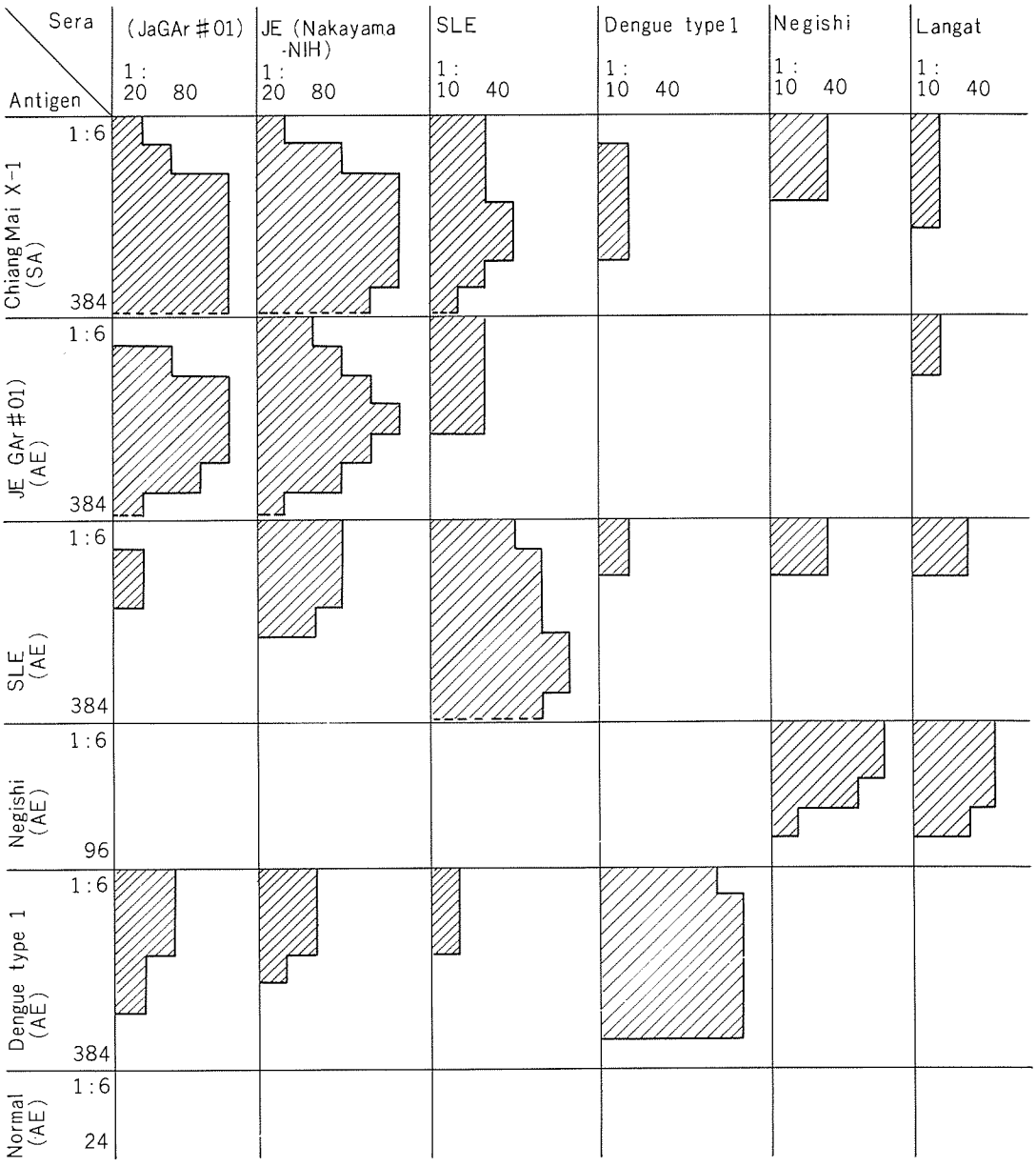


FIGURE 9. *Results of CF test for identification of Chiang Mai X-1 strain.*
The dotted line indicates that the antigen titer is equal or more than given.
(SA): Sucrose-acetone antigen (AE): Acetone-ether antigen.
SLE: st. Louis encephalitis.

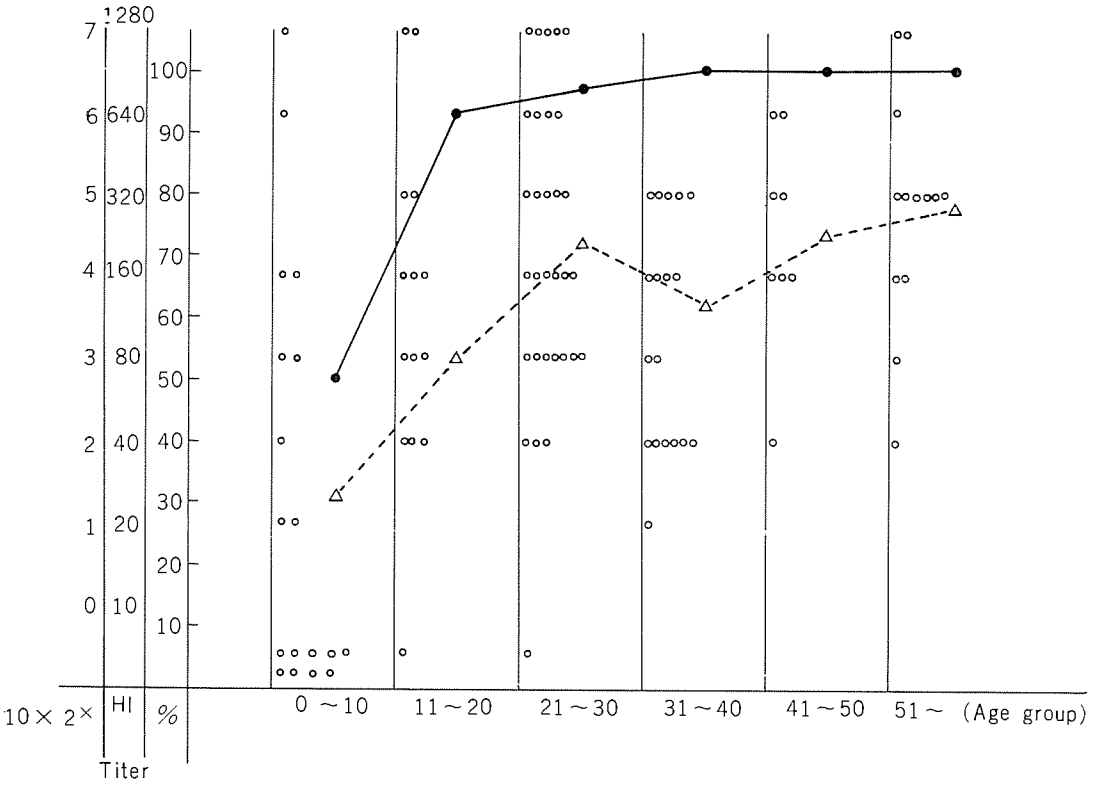


FIGURE 10. *Distribution of HI antibody against Japanese Encephalitis in healthy people (Chiang Mai City, March, 1970).*

Titers were estimated with JaGAr Ol antigen.

●——●: *Percentage of cases with HI titers of more than 10.*

△------△: *Geometrical mean titer.* ○: *Antibody titer.*

virus between mosquito, pig and man. The above data are on the epidemic in Chiang Mai area. With regard to the whole of Thailand, the data in Table 11 show that there is no clear relation between these factors and the incidence of JE in the four regions. The question of what factors control epidemics of JE and THF in the four big regions of Thailand requires further study. Konno et al., (1965), proposed a model for the mechanism of epidemics in Japan drawing attention to the fact that the rainy season (the so called " Tsu-yu " in Japan) provided suitable places for mosquitoes to breed. In Chiang Mai, where the big epidemic occurred, a similar situation seems to have occurred judging the facts describe above. (Fig. 16). That is; by the information of Ministry of Public Health of Thailand (Tuchinda, 1970), SEATO Laboratory of Bangkok confirmed that the number of Culex tritaen. increases from April and decreases from October, April to October being the rainy season in Thailand. SEATO succeeded to isolate JE virus from Culex tritaen. in April. One month later in May when the first case of JE occurred, JE virus was isolated from a fatal case at the Virus Research Institute. Since the epidemic of JE in Chiang Mai started in

TABLE 4. *Examination of brain specimens (1969)*

Case	Sex	Age	Hospital & No.	Laboratory No.	Onset	Death	Autopsy	Materials collected from	FA	Virus isolation: 1st Inoc. SM	Virus isolation: 2nd Passage SM	Virus isolation: 2nd Passage AM
T. D.	♂	6½	McCormic Hospital #44352	Chiang Mai X-1	13 July	19 July	after 2 hours	Cerebellum	−	pooled	+	+
								Morter cord	−			
								Frontal cortex	−			
								Parietal cortex	+	+		
								Basal ganglia	+			
								Brain stem	−			
P. A.	♂	4	Chiang Mai Hospital #335310	Chiang Mai X-2	10 Aug.	17 Aug.		Cerebellum	−	pooled	n.t.	n.t.
								Brain stem & cord	−			
								White matter	−	−		
								Hippocampus	−			
								Sensory cortex	−			
								Substantia nigra	−			
T. J.	♂	12	Chiang Mai Hospital #333228	Chiang Mai X-3	24 July	4 Aug.	after 36 hours	Cerebellum	−	pooled	n.t.	n.t.
								Brain stem	−			
								Cord	−	−		
								Hippocampus	−			
								Frontal cortex	+			
								Basal ganglia	+			

Abbreviations and symbols: SM=1-3 day old suckling mice; AM=adult mice; +=virus isolation or fluorescent antibody test positive; n.t.=not tested; Inoc.=inoculation; −=virus isolation or fluorescent antibody test negative

May, the epidemic circulation between pigs, mosquitoes and humans was probably as follows; In April, when the rainy season begin, the mosquitoes increase in number and gradually JE virus spread among them. (The problems of where the JE virus came from and what the reservoir of JE virus is are still unsolved.) Then, pigs or other livestock are infected with JE virus by mosquito. The virus multiply in pigs (viremia of pigs lasts for about 5 days, Ueba et al., 1968). By repeating this cycle, large numbers of mosquitoes are infected with JE virus. Then human cases of JE occurs. On infection of pigs with JE virus, the JE antibody begins to increase in pigs. When all the pigs have high JE antibody, they can no longer harbor the virus, so the mosquitoes cannot get any virus from pigs. Therefore, the humber of cases JE gradually decreases. It seems that there is not much difference in mechanisms of epidemics in Japan and Thailand. However, in Japan there is a winter season when there are very few mosquitoes. Any JE virus can not be isolated. While, in Thailand there is not such the cold season. Never the less, the epidemic occurred from June to August. The similar phenomenon is seen in Taiwan and Korea (Kono, 1969; Hsu, 1969). The reason for this is also an interesting problem.

It is possible to guess mechanism of the epi-

TABLE 5. *Examination of brain spcimens (1970)*

Case	Sex	Age	Hospital	Laboratory No.	Onset	Death	Autopsy	Collected by	FA	Virus isolation		
										1 st Inoc.	2nd Passage	
										SM	SM	AM
B. S.	♀	8	Chiang Mai Hospital	Chiang Mai X-4	12 May 1970	21 May 1970	not done	special biopsy needle 1 hr after death	+	+	+	+
D. S.	♀	13	Chiang Mai Hospital	Chiang Mai X-5	28 June 1970	6 July 1970	not done	special biopsy needle immediately after death	+	−	n.t.	n.t.
S. C.	♂	12	Chiang Mai Hospital (Chiang Rai)	Chiang Mai X-6	17 July 1970	31 July 1970	not done	special biopsy needle 6 hr after death	+	−	n.t.	n.t.

Abbreviations and symbols: SM=1-3 day old suckling mice; AM=adult mice; n.t.=not tested; Inoc=inoculation; +=positive; −=negative

demic but it is uncertain why a big epidemic suddenly occurred in 1969. Many factors may have been contributory causes. One of these is the annual change in the antibody level. Committee on investigation for forecasting epidemic diseases in Osaka (1968) reported that, " when the temperature in February is higher than normal for the month, a big epidemic may occur ", (A longer period of observation may be necessary to confirm this). Therefore, the climatic conditions in Chiang Mai were studied. In Fig. 17, the temperatures of monthly normal are shown as a continuous straight line and the difference in the actual average temperatures for each month from the

TABLE 6. *HI test for identification of Chiang Mai X-1 strain among the arbovirus group*

Group	Antibody	Antigen	
		JE JaGAr # 01[a]	Chiang Mai X-1[b]
A	Chikungunya (BaH 306)	<20[c]	<20
	Sindbis	<20	<20
B	SLE	1280	1280
	Dengue-1	640	1280
	Langat	2560	2560
	WN (Eg. 101)	2560	2560
	JE (JaGAr #01	1280	1280
	„ (Mizushima)	640	1280
	„ (JaTH)	1280	1280
C	Oriboca	<20	<20
Simbu	Akabane	<20	<20
Bunyamwera	Batai	<20	<20

▶
a Acetone-ether antigen.
b Sucrose-acetone antigen.
c Antibody titer when 4 units of HAnin are used.

TABLE 7. *Comparison of pH range of strains of Japanese encephalitis virus*

	PH \ HA	800	1600	3200	6400	12800	25600	51200	102400	C
JaGAr #01[a]	6.0	+	+	+	±	−	−	−	−	−
	6.2	+	+	+	±	−	−	−	−	−
	6.4	+	+	+	+	−	−	−	−	−
	6.6	+	+	+	+	±	−	−	−	−
	6.8	+	+	+	+	±	−	−	−	−
	7.0	+	+	+	±	−	−	−	−	−
	PH \ HA	80	160	320	640	1280	2560	5120	10240	C
Chiang Mai X-1[c]	6.0	+	+	+	±	−	−	−	−	−
	6.2	+	+	+	±	−	−	−	−	−
	6.4	+	+	+	+	−	−	−	−	−
	6.6	+	+	+	+	+	−	−	−	−
	6.8	+	+	+	+	+	±	−	−	−
	7.0	+	+	+	+	+	±	−	−	−
	PH \ HA	80	160	320	640	1280	2560	5120	10240	C
Chieng Mai Ogata[d]	6.0	+	+	+	+	+	+	⊕	−	−
	6.2	+	+	+	+	+	+	⊕	−	−
	6.4	+	+	+	+	+	+	+	±	−
	6.6	+	+	+	+	+	+	+	+	−
	6.8	+	+	+	+	+	+	+	+	−
	7.0	+	+	+	+	+	+	+	+	−
	PH \ HA	800	1600	3200	6400	12800	25600	51200	102400	C
Nakayama[b]	6.0	+	+	+	+	−	−	−	−	−
	6.2	+	+	+	+	−	−	−	−	−
	6.4	+	+	+	+	−	−	−	−	−
	6.6	+	+	⊕	−	−	−	−	−	−
	6.8	+	±	−	−	−	−	−	−	−
	7.0	−	−	−	−	−	−	−	−	−

a Reference strain of Japanese Encephalitis (acetone-ether).
b Reference strain of Japanese Encephalitis (acetone-ether).
c Current strain isolated in Chiang Mai, 1969 (acetone-ether).
d Strain isolated in Chiang Mai by Dr. T. Ogata, 1965 (sucrose-acetone).

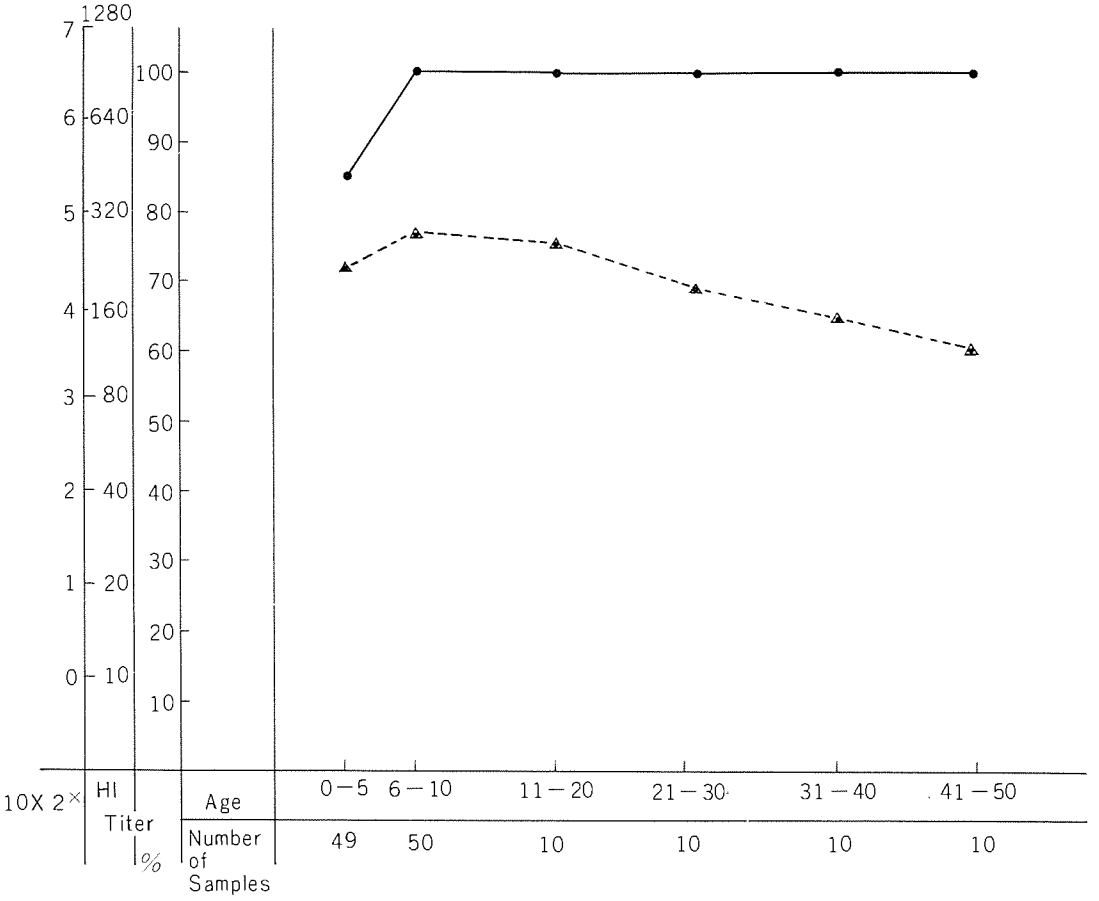


FIGURE 11. *Distribution of HI antibody against Japanese Encephalitis in healthy people (Bangkok & Thonburi Cities, Dec. '68–Jun. '69).*
Challenge Virus: HI antigen of JaGAr #01.
●——●: *Percentage of subjects with an HI titer of more than 10 units (Reciprocal of dilution).*
△------△: *Geometrical mean titer.*

TABLE 8. *Comparison of HI paterns of strains of Japanese encephalitis virus*

Serum \ Dilution	NAKAYAMA 80						20480			JaGAr #01 40							10240	
Anti-Nakayama-R	−	−	−	−	−	−	⊕	+	+	−	−	−	−	−	−	−	+	+
Anti-JaGAr #01-R	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+
Anti-Chiang Mai-R-1	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	+	+
Anti-Chiang Mai-R-2	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+
Patients' Sera ES-3[b]	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+
Patients' Sera ES-13	−	−	−	−	+	+	+	+	+	−	−	−	−	−	+	+	+	+
Patients' Sera ES-42	−	+	+	+	+	+	+	+	+	−	−	±	+	+	+	+	+	+
Anti-Dengue-1-R	±	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+
Anti-Dengue-2-R	−	−	⊕	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+
Anti-Dengue-3-R	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+
Anti-Dengue-4-R	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+

a Reciprocal of dilution
b Convalescent serum of patient
Abbreviation and symbols: R=rabbit; ES=Sample of Japanese Encephalitis; +=HA positive;

TABLE 9. *Antibody distribution in pig sera in Chiang Mai (July, 1969)*

HI Titer \ No. of sample	Pig-1	Pig-2	Pig-3	Pig-4	Pig-5	Pig-6	Pig-7	Pig-8	Pig-9	Pig-10	Pig-11	Pig-12
JE JaGAr #01	640	640	1280	1280	5120	1280	2560	640	1280	1280	5230≤	5120≤
JE Nakayama	320	320	640	640	1280	640	1280	320	640	640	1280	1280
Dengue-1	≤40	≤40	40	≤40	80	40	≤40	≤40	1280	≤40	40	≤40
Dengue-2	≤40	40	80	40	160	80	84	≤40	160	80	160	80
Dengue-3	≤40	40	80	40	160	80	80	40	160	40	160	80
Dengue-4	≤40	40	80	40	80	80	80	≤40	160	40	160	10240

Sera of pigs 1 to 20 were from a slaughter house in Chiang Mai (collected 29, July 1969).
Sera of pigs 21 to 25 were collected from breeding pigs in Ta-Wang-Tan Village in Saraphi County in

Chiang Mai X-1									Chieng Mai Ogata									
40								10240	40								10240	C
−	−	−	−	−	−	±	+	+	−	−	−	−	−	−	±	+	+	−
−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+	−
−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	⊕	+	+	−
−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	+	+	−
−	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−
−	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−
−	−	+	+	+	+	+	+	+	−	−	⊕	+	+	+	+	+	+	−
−	−	−	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	−
−	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−
−	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−
−	−	−	−	+	+	+	+	+	−	−	−	−	+	+	+	+	+	−

− = HA negative

Pig-13	Pig-14	Pig-15	Pig-16	Pig-17	Pig-18	Pig-19	Pig-20	Pig-21	Pig-22	Pig-23	Pig-24	Pig-25
1280	2560	1280	2560	1280	320	2560	1280	≤40	≤40	320	40	≤40
640	1280	640	1280	320	160	1280	640	≤40	≤40	160	40	≤40
≤40	40	≤40	40	≤40	≤40	40	40	≤40	≤40	≤40	≤40	≤40
160	160	80	160	≤40	≤40	80	80	≤40	≤40	≤40	≤40	≤40
80	160	80	80	40	≤40	160	80	≤40	≤40	≤40	≤40	≤40
80	80	80	80	40	≤40	320	80	≤40	≤40	≤40	≤40	≤40

Chiang Mai. The pigs were less than 6 months old.

TABLE 10. *Species of mosquitoes collected in Ta-Wang-Tan Village in Chiang Mai Province (Aug. 1969)*

Anopheles philippinensis	◀
Aedes Sp. (unidentified)	*(Identified by Dr. S. Ito, Osaka Prefectural Institute of Public Health, Osaka, Japan)*
Culex tritaeniorhynchus	
Culex pseudovishnui	
Culex whitmorei	
Culex sinensis	
Culex pipiens fatigans	
Culex annulus	
Culex fuscocephala	
Culex bitaeniorhynchus	

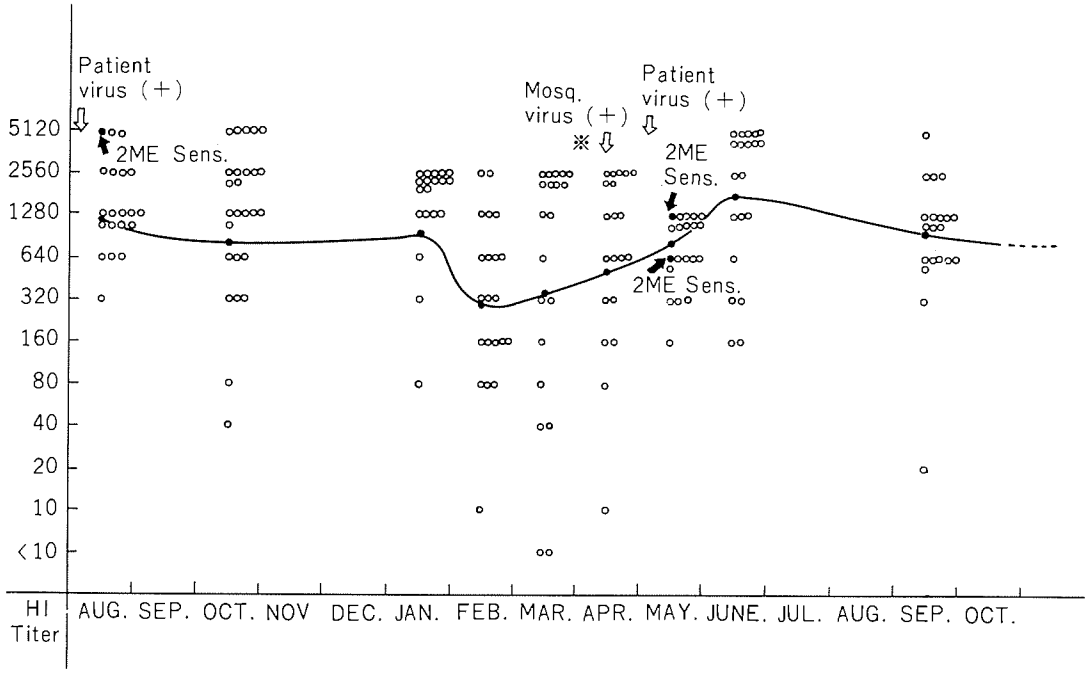


FIGURE 12. *Annual variation in antibody of pigs from a slaughter house in Chiang Mai City (from Aug. 1969 to Sep. 1970).*
●——●: *Geometrical mean titer.* ○: *Antibody titer.* ※: *SEATO's data.*
2 ME sens: Antibody sensitive to 2(β)-Mercapto-ethanol.

TABLE 11. *Natural features of each region in Thailand*

Region	Number of cases (JE)[a]	No. of deaths[a]	Fatality rate[d]	Population[b]	Incidence rate[e]
Central R.	76	16	21.0	8,271,302	0.9
North-East	123	47	38.2	8,991,543	1.4
North	466	86	18.5	5,723,106	8.1
South	10	3	30.0	3,271,965	0.3
Total	675	152	22.5	26,257,916	2.6

Region	Area of Region[f]	Density of Population[g]	Number of pigs[c]	Density of pigs[g]	Area of[e] rice fields[f]	% of areas rice fields
Central R.	103,579	79.5	1,406,461	13.6	19,363	18.7
North-East	170,226	52.9	895,109	5.3	30,562	18.0
North	170,006	33.7	891,893	5.2	15,688	9.2
South	70,189	46.7	949,605	13.5	5,280	7.5
Total	514,000	51.1	4,143,068	8.1	70,893	13.8

a Data in 1969
b Data in 1960
c Data in 1968
d Numbers are percentages
e Per 100,000 head of population
f In square Kilo-meters
g Per areas

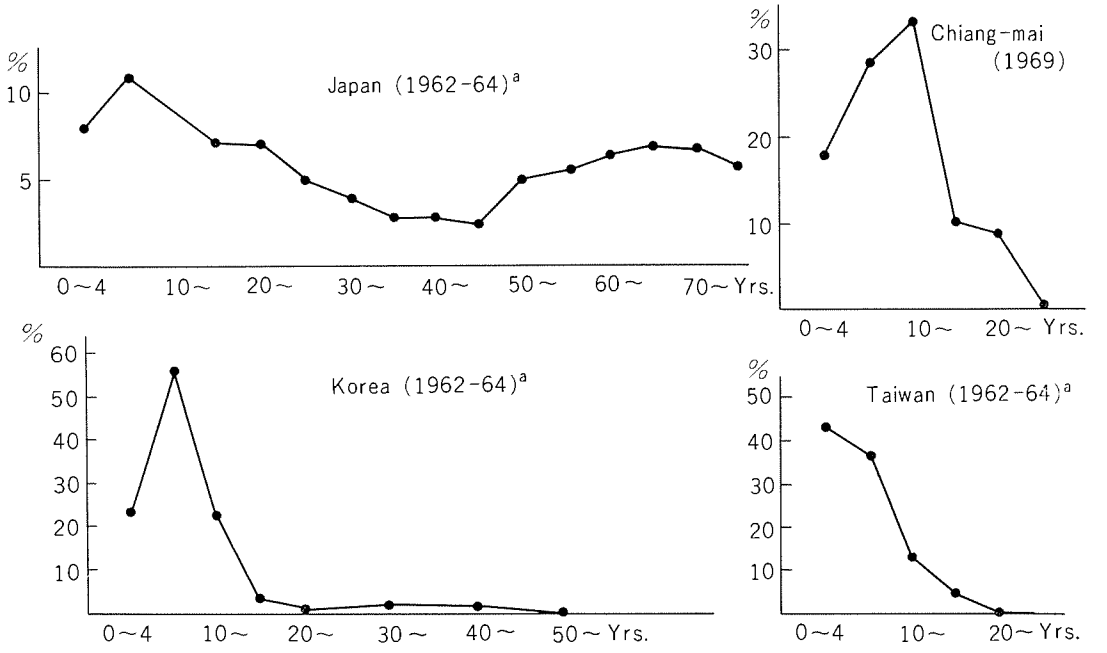
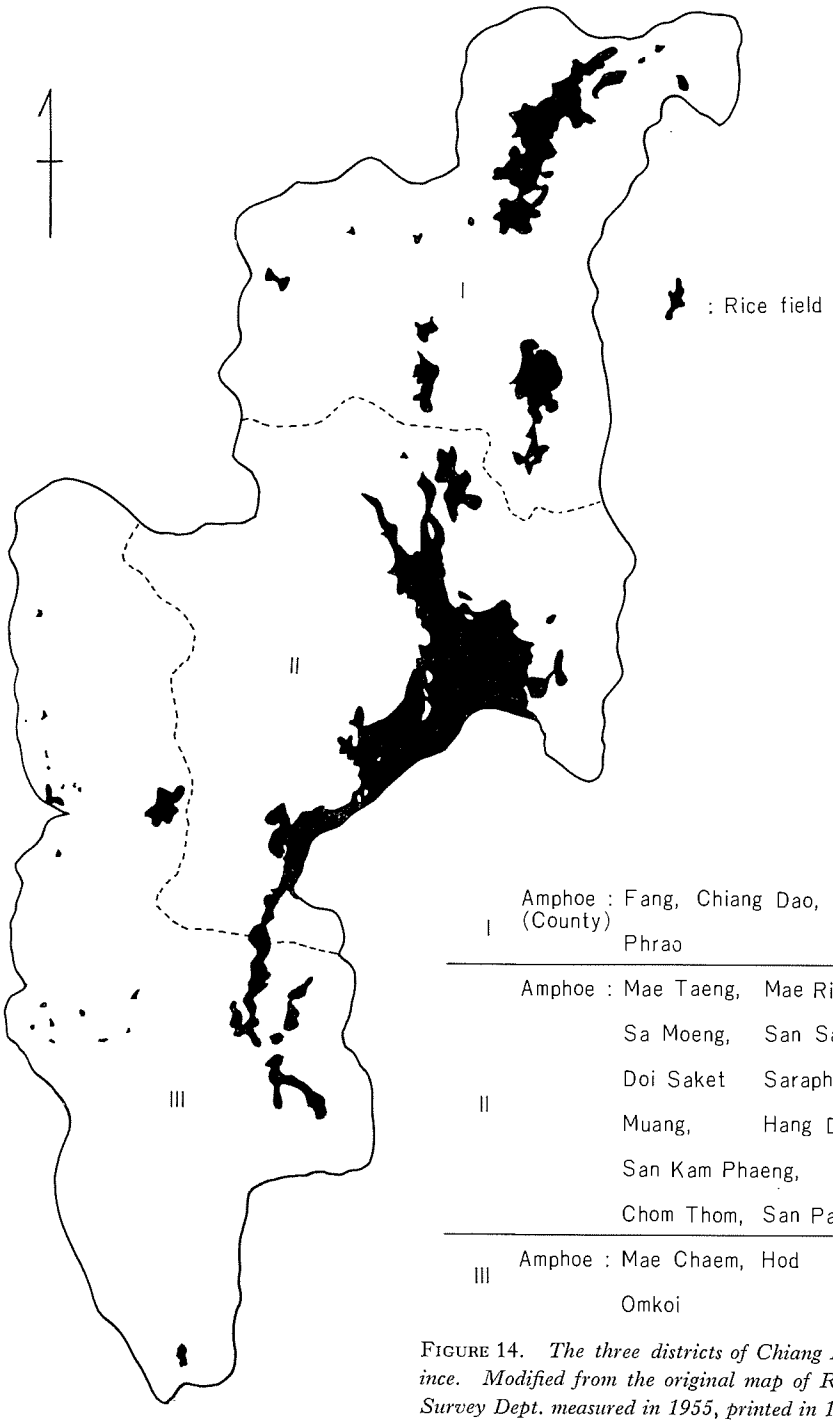


FIGURE 13. *Age distribution among JE patients in four Asian Countries.*
a: from WHO Bulletin (Kono et al.).



I	Amphoe (County) :	Fang, Chiang Dao, Phrao
II	Amphoe :	Mae Taeng, Mae Rim Sa Moeng, San Sai Doi Saket Saraphi Muang, Hang Dong San Kam Phaeng, Chom Thom, San PaTong
III	Amphoe :	Mae Chaem, Hod Omkoi

FIGURE 14. *The three districts of Chiang Mai Province. Modified from the original map of Royal Thai Survey Dept. measured in 1955, printed in 1967.*

TABLE 12. *Yearly incidence of Thai hemorrhagic fever and Japanese encephalitis in Bangkok and Chiang Mai (by clinical diagnosis)*

Thai hemorrhagic fever

	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970
Bangkok	481	4,185	1,657	5,403	1,994	3,046	834	1,107	1,298	158
Chiang-Mai	—	—	—	69	7	11	—	1	83	2

Japanese encephalitis

	1965	1966	1967	1968	1969	1970
Bangkok	—	—	—	—	9	18
Chiang-Mai	31	28	25	25	235	131

— not reported

From Statistics of the Dept. of Health, Thailand.

TABLE 13. *Number of Livestock in Chieng Mai*

	District / Total	I	II	III
Pigs (1963)	125,921	25,030	81,791	19,100
Cattle (1965)	173,759	38,910	108,688	26,161
Buffaloes (1965)	188,803	60,628	96,067	32,108
Horses (1965)	7,411	1,488	5,082	841
Ducks (1963)	120,133	19,168	100,088	877
Chickens (1963)	1,421,814	272,526	968,406	180,882

TABLE 14. *Relationship between the incidence of JE and some environmental factors*

District (Chiang Mai Pro.)	No. of cases 1969	Population 1969	Incidence cases per 100,000 people	No. of pigs 1963	Area of district A (km^2)	Density of pigs per (km^3)	Area of ricefield B (km^2)	% $\frac{B}{A}\times 100$
I	18	163,963	11.0	25,030	6,503	3.8	227.5	3.4
II	166	730,412	22.7	81,791	10,596	7.7	689.1	6.3
III	1	91,565	1.1	19,100	9,079	2.1	51.2	0.6

(*Chiang Mai Province, Thailand*)

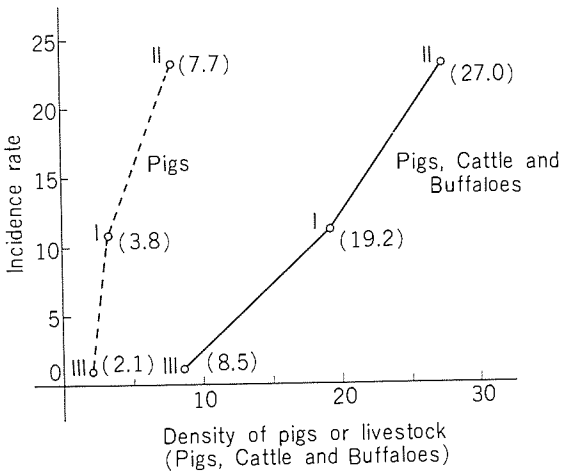


FIGURE 15. *Relationship between the incidence rate of cases of JE and the density of pigs or livestock (Chiang Mai Province).*

FIGURE 17. *Annual weather in Chiang Mai City (Meteorological Dept. of Thailand).*

------ : *Rain-fall.* ⊙ : *Incidence rate per 100,000 people.*

——— : *Difference in average temperature for the month from the normal monthly average.*

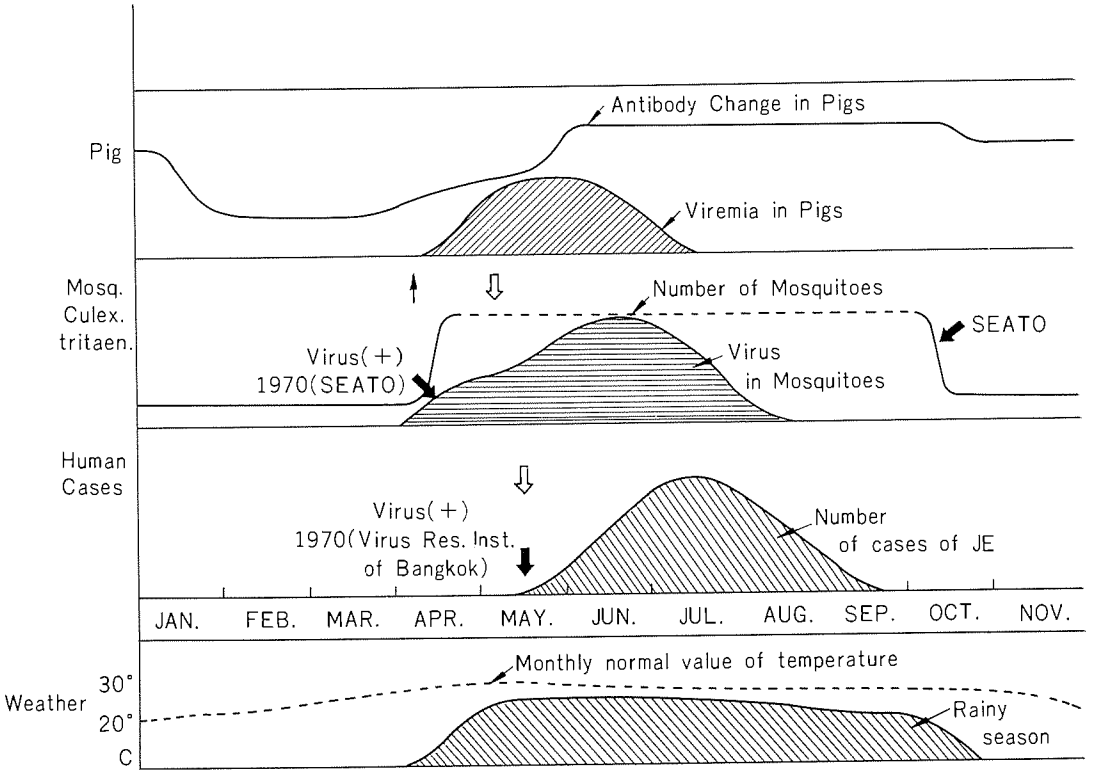
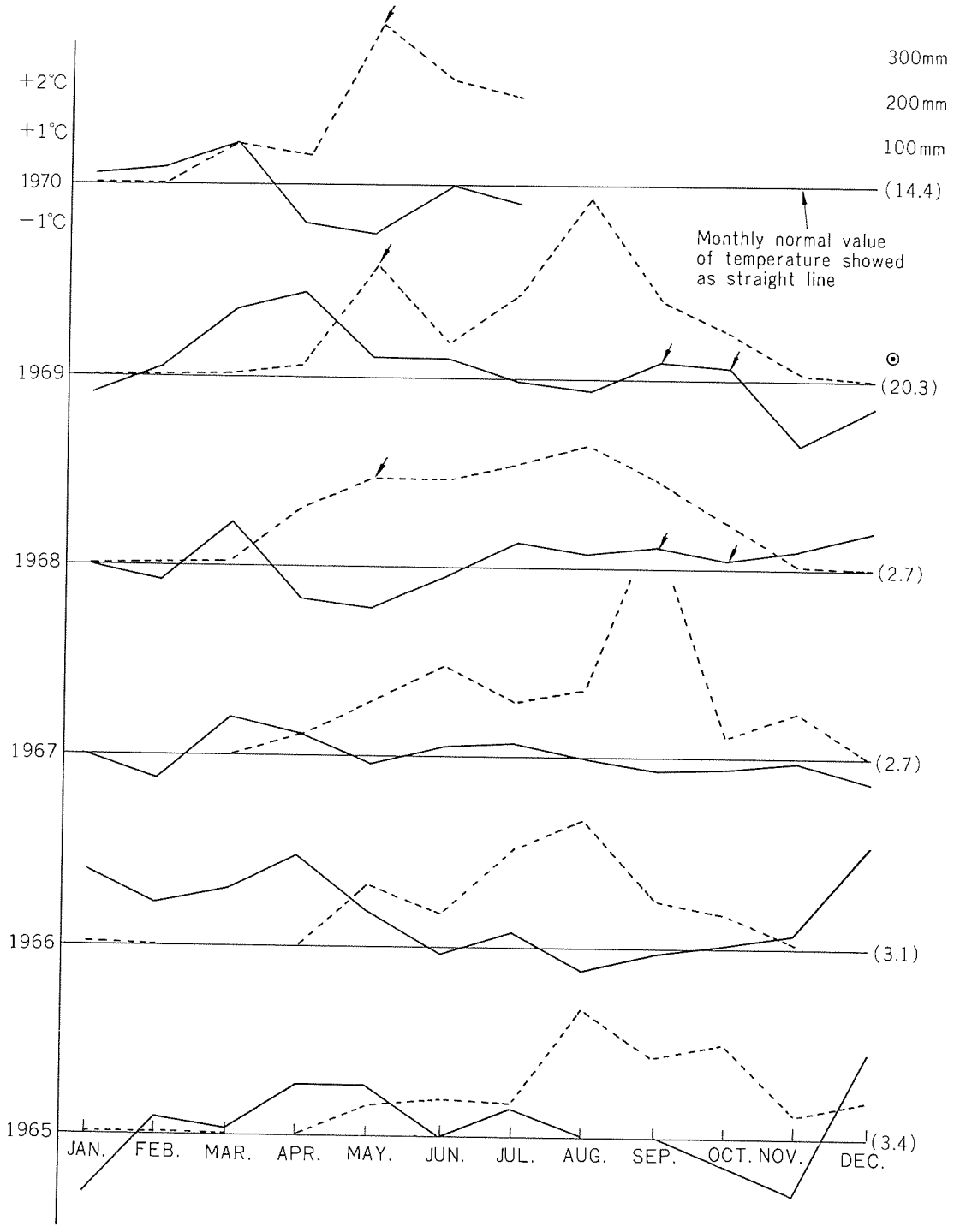


FIGURE 16. *Presumed mechanism of JE epidemic in Chiang Mai.*


+2℃
+1℃
1970
−1℃
300mm
200mm
100mm
(14.4)
Monthly normal value
of temperature showed
as straight line
1969
(20.3)
1968
(2.7)
1967
(2.7)
1966
(3.1)
1965
(3.4)
JAN.
FEB.
MAR.
APR.
MAY.
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.

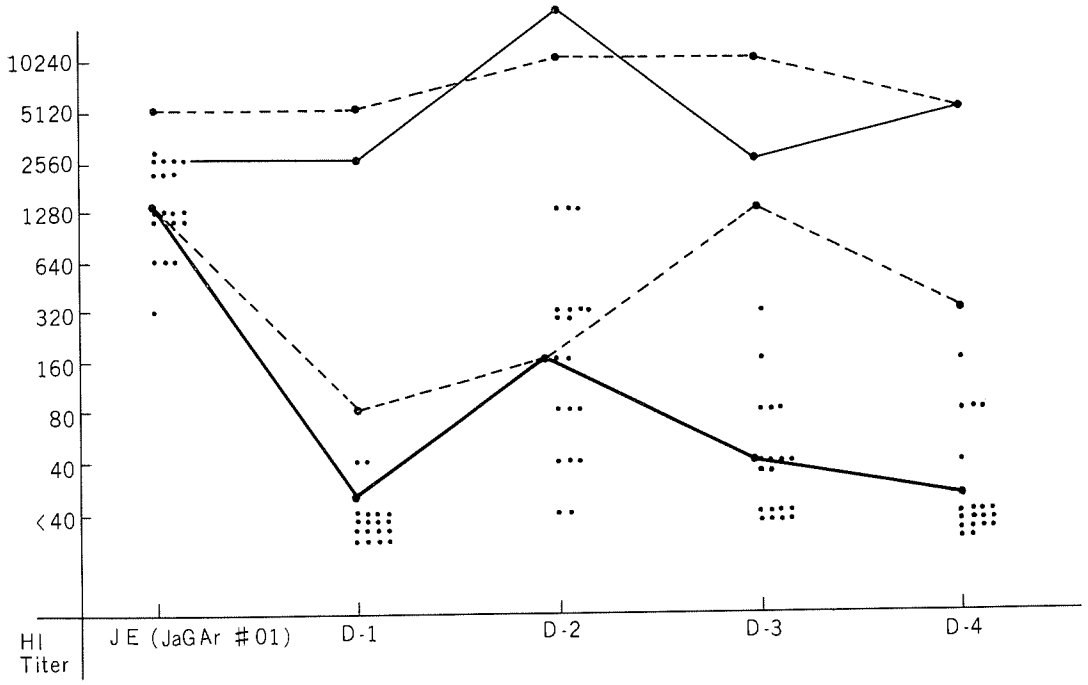


FIGURE 18. *Cross reaction of JE and Dengue antigen with JE and THF patient's sera.*
●——●: *Geometrical mean titer of JE cases.*
●——●: *Special case of JE.* ●------●: *Titer of THF cases.*

temperatures of monthly normal is plotted as a continuous bent line. The monthly total of rain-fall is plotted as a dotted line. It can be seen that the monthly rain-falls in May in 1968, 1969, and 1970 were very great. The average temperatures in September and October in 1968 and 1969 were higher than normal through these 2 months. These facts may be related to the scale of the epidemic since high rain-fall provides good conditions for mosquitoes to breed. Moreover, high temperatures in September and October facilitate survival of mosquitoes until the next year. Thus these doubled conditions favor spread of the virus. Further prolonged observations on these factors are required.

JE is usually diagnosed by the HI test in Japan. But in south-east Asia, mosquito borne hemorrhgic fever is prevalent (Halstead, 1966), and as reported by Igarashi (1968), Halstead (1966), Okuno (1966) and Hammon (1964), after THF infection, patients have very high antibody with broad specificity against group B arboviruses. This makes serological diagnosis of JE difficult in south-east Asia (Ishii, 1967). As shown in Table 15, cases of type I are easy to diagnose but those types II, III, and IV are sometimes very difficulty. The HI titers of cases of JE are plotted in Fig. 18 and the behavior of the sera can be recognized. Cross reaction with Dengue 2 is dominant. Further studies are required on this problem. However, from careful clinical examination combined with exact serum analyses, the diagnosis of JE in this area should not be impossible.

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We express our gratitute to Dr. Yoshiomi Okuno (Director, The Research Institute for Microbial

TABLE 15. *Results of serological examination of sera of cases (July 1969 Chiang Mai)*

C.D.	Type	Age	Sex	Days from onset	HI Test				
					JaGAr #01	D-1	D-2	D-3	D-4
Japanese Encephalitis	I	9	♂	3	40	<20	20	20	<20
				15	640	<20	<20	<20	<20
		4	♂	3	80	<20	<20	40	<20
				11	2560≤	<20	40	40	20
		9	♂	5	80	<40	<40	<40	<40
				17	640	<40	<40	<40	<40
	II	6	♀	3	160	<40	160	<40	<40
				9	1280	<40	320	<40	<40
		12	♂	5	160	<40	160	<40	<40
				10	1280	<40	1280	<40	<40
		3	♂	6	20	<40	<40	<40	<40
				13	1280	<40	80	<40	<40
	III	10	♀	6	640	<40	160	<40	<40
				15	1280	<40	320	80	80
		6	♂	3	160	<40	40	40	<40
				16	2560≤	<40	320	80	80
	IV	20	♀	5	2560≤	320	10240	1280	640
				13	2560≤	2560≤	20480≤	560	5120
Thai hem. fever	V	11	♂	4	640	<40	40	40	40
				13	5120≤	5120≤	10240	10240	5120
		1	♂	8	320	80	160	1280	160
				16	1280	80	160	1280	320

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