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Author(s)	Fukunaga, Toshihiko; Igarashi, Akira; Okuno, Yoshinobu et al.
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A SEROEPIDEMIOLOGICAL STUDY OF JAPANESE ENCEPHALITIS AND DENGUE VIRUS INFECTIONS IN THE CHIANG MAI AREA, THAILAND

TOSHIHIKO FUKUNAGA^{1,4}, AKIRA IGARASHI², YOSHINOBU OKUNO¹, TSUYOSHI ISHIMINE¹, MASAYUKI TADANO¹, YUTAKA OKAMOTO¹ and KONOSUKE FUKAI³

¹ Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, 3-1, Yamadaoka, Suita, Osaka 565, Japan

² Department of Virology, Institute for Tropical Medicine, Nagasaki University, Sakamotocho, Nagasaki 852, Japan

³ Research Foundation for Microbial Diseases of Osaka University, 3-1, Yamadaoka, Suita, Osaka 565, Japan

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SUMMARY As part of a virological and epidemiological survey of encephalitis in the Chiang Mai area, the neutralizing (N) antibody levels of healthy persons to Japanese encephalitis (JE) and dengue (DEN) type 1-4 viruses were examined. A total of 985 blood samples was collected by the filter paper method from subjects of nine age groups in five districts, four (Pasang, Sarapee, Doi Saket and Mae Taeng) in the Chiang Mai Valley and one (Fang) in another valley separated by several ranges of mountains from the Chiang Mai Valley. From analyses of the results of N tests on the specimens, the following conclusions were drawn about the prevalences of JE and DEN viruses in the Chiang Mai area: (1) In the Chiang Mai Valley, the percentage incidences of N antibodies to JE and DEN viruses increased with age and by the age of 15, two thirds or more of the residents had been infected with JE and all DEN viruses except DEN type 2 virus, which showed the lowest prevalence. (2) In the Fang district, the percentage incidence of N antibody to JE virus increased with age, but those to DEN viruses did not, indicating much lower prevalences in the past of all four serotypes of DEN viruses in this district than in the Chiang Mai Valley. (3) At present, most infants in the Chiang Mai area, including the Fang district, seem to be exposed to DEN viruses first and later to JE virus.

INTRODUCTION

Since a large outbreaks of Japanese encephalitis (JE) throughout northern Thailand was first recognized in 1969 (Yamada et al., 1971; Grossman et al., 1973), the Chiang Mai Valley has been known to be an area where JE and dengue (DEN) viruses co-exist and repeated

⁴ Address reprint requests to: Dr. Toshihiko Fukunaga, Department of Virology, Faculty of Medicine, University of the Ryukyus, 207, Ueharamiyazato, Nishihara, Okinawa 903-01, Japan

outbreaks of JE and dengue hemorrhagic fever (DHF) occur simultaneously during the rainy season. For establishing methods to prevent these outbreaks, such as by vaccination, it is essential to clarify the seroepidemiological status of the people associated with virus transmission in the area. However, JE and DEN viruses are very closely related and cross-react serologically and thus they cannot be differentiated by the most commonly used hemagglutination inhibition (HI) test. Moreover, there are four serotypes of DEN viruses and these cannot be distinguished by HI tests. The most specific serological test available is the N test, but this test is much more complicated and time-consuming than the HI test, and so it is difficult to perform seroepidemiological studies by the N test in an area where the JE and DEN viruses co-exist. Therefore, we developed a rapid micro-method based on the N test (Okuno et al., 1978), and used this method in the present work, which forms part of a virological and epidemiological study on encephalitis in the Chiang Mai area, Thailand (Igarashi et al., 1983).

The purpose of the study was to determine the extents of past and present exposures of humans to JE and DEN viruses and to obtain information on the seroepidemiological relations between JE and DEN virus transmissions in the Chiang Mai area.

This paper reports the seroepidemiological data obtained by N tests on JE and DEN viruses in specimens from healthy persons in the Chiang Mai area and some conclusions on transmissions of the viruses deduced from the data.

MATERIALS AND METHODS

1. *Districts where the blood samples were collected*

In the Chiang Mai Valley, four districts (Pasang, Sarapee, Doi Saket and Mae Taeng) were selected for collection of samples from healthy persons. The Valley is approximately 1500 km² in area and is 300–350 meters above sea level. It is crescent-shaped, extending in a north-south direction along the Ping

River which joins the Chao Phya (Mae Nam) River. In the villages, rice cultivation is ubiquitous with paddy fields, and various fruit and vegetables are grown, and there are many pigs and other domestic animals. The Fang district is located in another valley about 160 km north of Chiang Mai City. The Fang Valley is separated by several mountain ranges from the Chiang Mai Valley and rivers from the surrounding mountains run into the Mekong River. On the northern border of this district is the Shan State, Burma. There are no marked differences in the modes of life or environmental conditions of people in the Fang district and Chiang Mai Valley. The Fang district and the Sarapee, Doi Saket and Mae Taeng districts are in the Chiang Mai Province, while the Pasang district is in the Lamphoon Province. The locations of these five districts are shown in Fig. 1.

2. *Blood samples*

In all, 985 blood samples were collected from healthy persons in July and August, 1982. The subjects were divided into 9 age groups, and samples from about 20 individuals of each age group in each district were obtained (Table 1). For obtaining a sample, the finger was cleaned and disinfected with 70% ethyl alcohol and then pricked with a stainless steel disposable lancet (Feather Co., Gifu, Japan). The blood obtained by pricking was fully absorbed into bleeding filter paper No. 1 (Toyo Roshi, Tokyo, Japan). The filter paper strips were not exposed to sunlight, but air-dried in the shade, stored at –20 C and brought to Japan on dry-ice.

The specimens on filter paper were cut into 8–10 pieces with scissors at room temperature. The pieces were soaked in 1.2 ml of Eagle's minimum essential medium (MEM) supplemented with 5-fold concentrated streptomycin, penicillin and fungison in a tube to give 1:30 dilution of serum. The tube was kept at 4 C overnight to allow extraction of the antibody components into the medium. Then the filter paper was removed by centrifugation at 3,000 rpm for 20 min and the supernatant was heat-inactivated at 56 C for 30 min before neutralization tests.

3. *Neutralization (N) tests*

The neutralizing antibody titers of the serum specimens were measured against JE and DEN viruses by the focus reduction method applying the peroxidase-anti-peroxidase (PAP) staining tech-

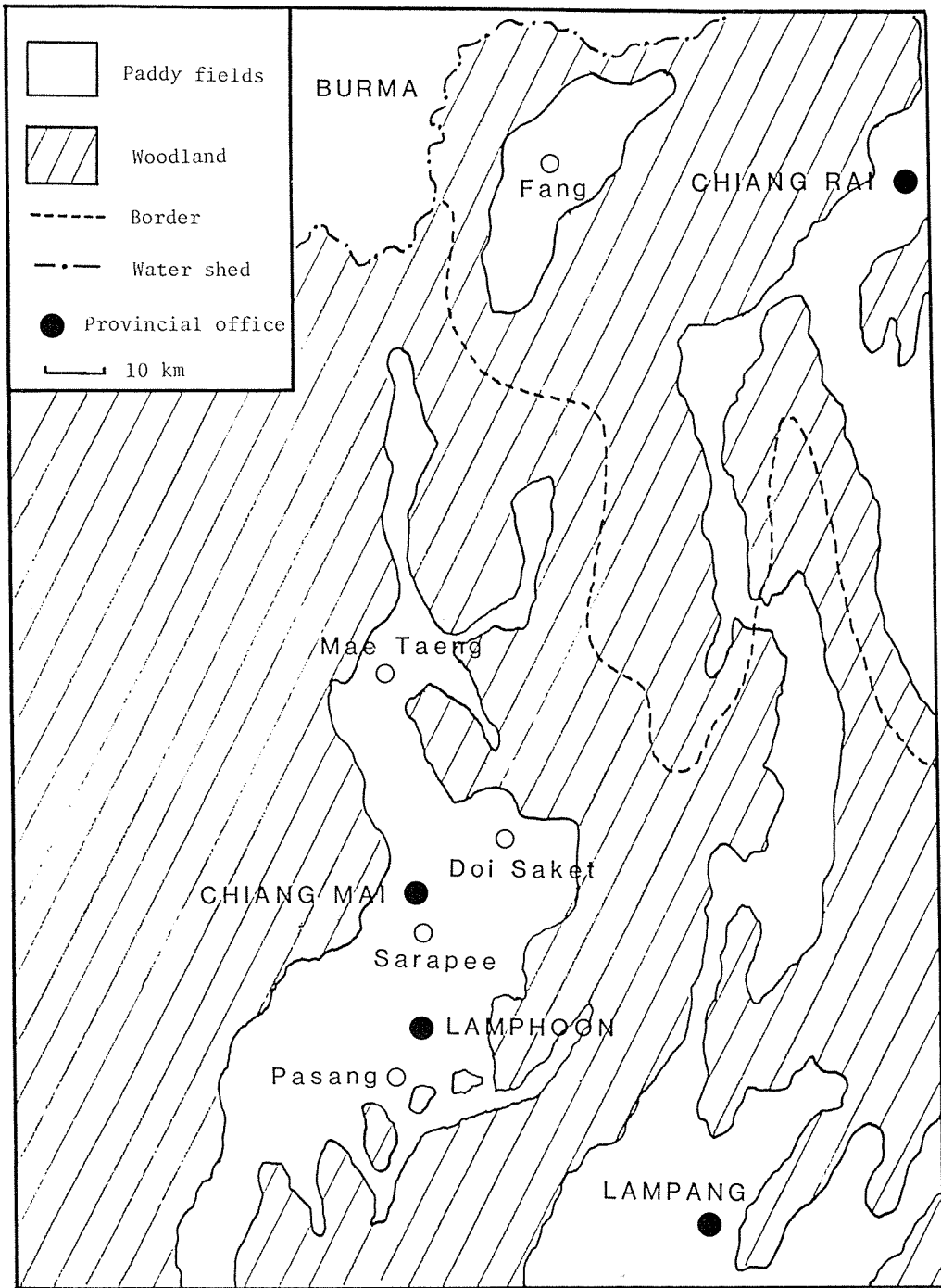


FIGURE 1. Locations of districts where blood samples were collected.

TABLE 1. *Number of blood specimens collected*

Age group (year)	SR	DS	PS	MT	FN	Total
1-3	21	21	22	23	23	110
4-6	19	23	20	23	23	108
7-9	18	17	23	21	22	101
10-14	20	22	21	20	26	109
15-19	24	24	22	23	21	114
20-29	23	21	21	19	25	109
30-39	22	24	21	25	21	113
40-49	24	21	21	22	22	110
50-	21	26	20	22	22	111
Total	192	199	191	198	205	985

SR, Sarapee; PS, Pasang; DS, Doi Saket; MT, Mae Taeng; FN, Fang.

nique (Okuno et al., 1978). The viruses used were the JaGAR#01 (JE), Hawaiian (DEN type 1), New Guinea B (DEN type 2), H-87 (DEN type 3) and

H-241 (DEN type 4) strains. For the tests, BHK-21 cells cultured on Lab-Tek 8 chamber slides (Miles, Ill., U.S.A.) were used. Titers were expressed as 50% focus reduction rates (FR₅₀).

RESULTS

1. *Incidences of neutralizing (N) antibodies to JE and DEN type 1-4 viruses*

The percentage incidences of N antibodies to JE and DEN type 1-4 viruses are shown in Fig. 2. In the Chiang Mai Valley (Pasang, Sarapee, Doi Saket and Mae Taeng), the percentage incidence of N antibody to JE virus rose with age to 70-86% at 10-14 years old. In the Fang district, the incidence also increased with age, but more slowly than in the Chiang Mai Valley. These results indicated that JE virus transmission has been occurring for a considerable time in the entire Chiang

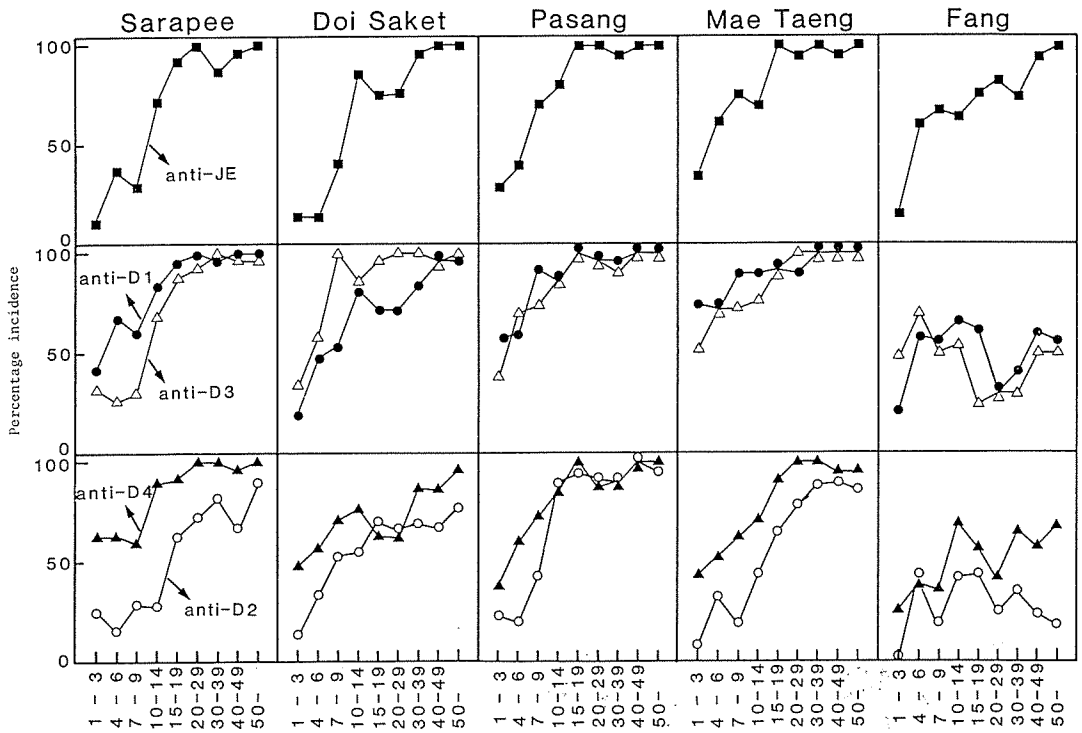


FIGURE 2. Percentage incidences of N antibodies to JE and DEN viruses in different age groups.

Mai area. The percentage incidences of N antibodies to DEN type 1 and 3 viruses increased with age in the Chiang Mai Valley, reaching 67–90% at 10–14 years old. In Fang, the percentage incidences of antibodies to DEN type 1 and 3 did not rise with age as a whole, though they reached 65% and 54%, respectively, in the 10–14 age group, and marked low values of both were seen in the 20–29 age group. In the Chiang Mai Valley, the patterns of change in the incidence of N antibody to DEN type 4 virus appeared to be similar to those to DEN type 1 and 3 viruses, while the patterns of change in the incidences of antibody to DEN type 2 were lower than those to DEN type 1 and 3 viruses in the Sarapee, Doi Saket and Mae Taeng districts, although they increased with age in all four districts. In the Fang district, the incidences of N antibodies to DEN type 2 and 4 viruses did not increase as a whole, and were especially low in the 20–29 age group. Thus the transmission patterns of DEN type 1–4 viruses in the Fang district appeared to be markedly different from those in the Chiang Mai Valley.

2. Relations of incidences of N antibodies to JE and DEN viruses

Serum specimens were classified into 4

groups with respect to N antibodies to JE and DEN viruses: (1) Antibodies to JE and either type of DEN viruses. (2) Antibodies to either type of DEN viruses but not to JE virus. (3) Antibody to JE virus but not to DEN viruses. (4) No antibodies to either JE or DEN viruses. The percentages of samples in the 4 groups in each age group are shown in Fig. 3. In the Chiang Mai Valley, the percentage of the group with N antibodies to JE and either type of DEN viruses increased with age, reaching 76–100% in the 20–29 age group. In the Fang district, however, the percentage of this group did not rise so much after 4–6 years, and was decreased in the 20–29 age group. In the same age group (20–29 years), the percentage with antibody to JE but not to DEN viruses was 42% (10/24), which was equivalent to the percentage with antibody to JE and to either type of DEN viruses. Furthermore, an appreciable percentage of persons in each age group of more than 20–29 years had N antibody to JE virus but not to DEN viruses, unlike in districts in the Chiang Mai Valley. Furthermore appreciable percentages of persons in older age groups in the Fang district had no N antibody to either JE or DEN viruses. The results suggest that in the Fang district 20–30 years

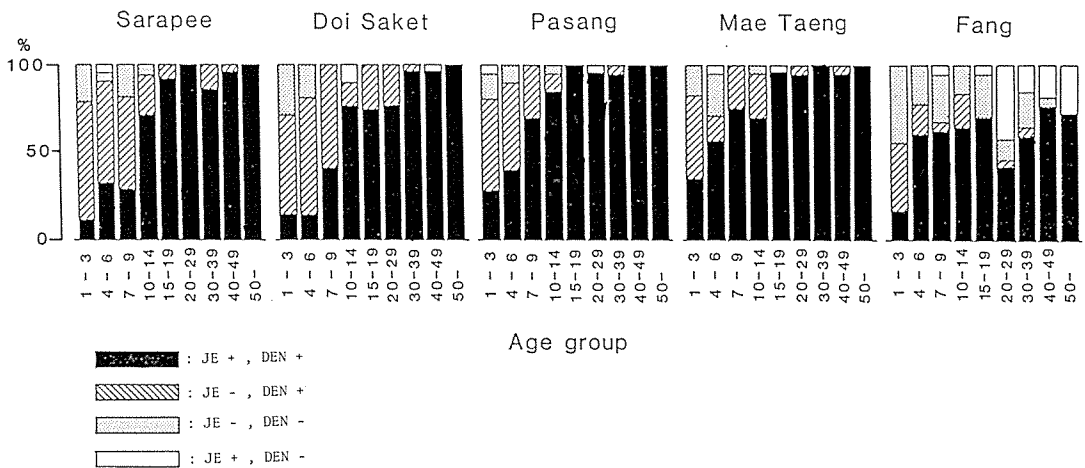


FIGURE 3. Percentages of serum specimens with N antibodies to JE and, or DEN viruses.

ago, transmissions of DEN viruses had not yet been established, but JE virus was already prevalent. On the other hand, in the youngest age group (1-3 years), no serum specimen had N antibody to JE virus but not to DEN viruses and 39% of the specimens (9/23) had antibodies to either type of DEN viruses but not to JE virus; this was more than twice the percentage of those with antibodies to JE and either type of DEN viruses. These results suggest that at present, most infants in the Fang district are exposed to DEN viruses first and infected with JE virus later. In the Chiang Mai Valley, it also seems likely that infants are exposed to DEN viruses first and to JE later, because in all districts studied in the 1-3 year age group the percentages of

specimens with N antibodies to either type of DEN viruses but not to JE virus were the largest.

3. Percentages of serum specimens with no N antibodies and, monotype and multitype antibodies to DEN viruses

The specimens examined were divided into 3 groups with respect to their N antibodies to DEN viruses; namely those with (1) no antibodies to either type, (2) monotype antibodies and (3) multitype antibodies (to two or more types). The results are shown in Fig. 4. In the 4 districts in the Chiang Mai Valley, the percentage of the group with multitype antibodies rose with age to 100% in the 15-19 age group, indicating that almost all people in the

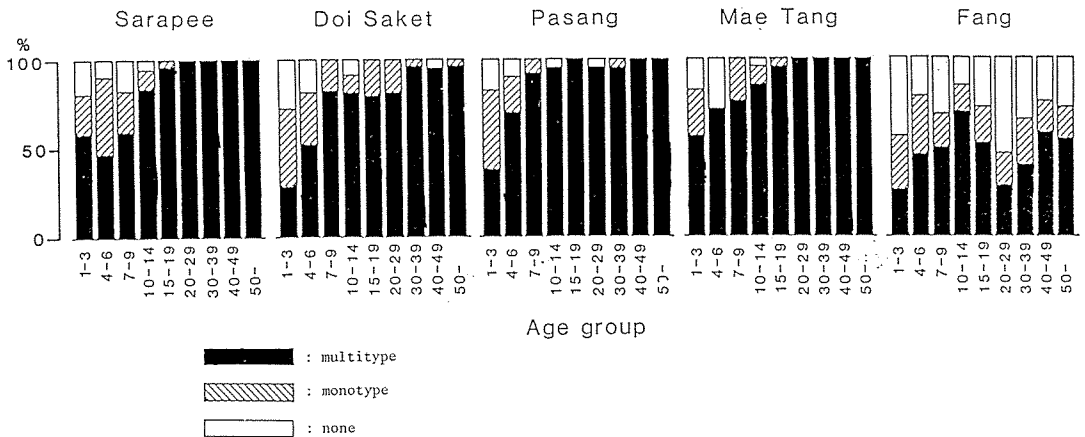


FIGURE 4. Percentages of serum specimens with no N antibodies and, monotype and multitype N antibodies to DEN viruses.

TABLE 2. Specimens with monotype N antibody to DEN viruses

District	Number	Anti-DEN-1	Anti-DEN-2	Anti-DEN-3	Anti-DEN-4	Number tested
Sarapee	19	4 (21%)	2 (11%)	3 (16%)	10 (53%)	186
Doi Saket	31	1 (3%)	3 (10%)	19 (61%)	8 (26%)	197
Pasang	16	6 (38%)	2 (13%)	3 (19%)	5 (31%)	188
Mae Taeng	13	9 (69%)	1 (8%)	1 (8%)	2 (15%)	195
Fang	39	5 (13%)	3 (8%)	13 (33%)	18 (46%)	203
Total	118	25 (21%)	11 (9%)	39 (33%)	45 (36%)	969

Chiang Mai Valley were infected with at least two serotypes of DEN virus by the age of 20. In the Fang district, however, the pattern was different: The percentage of persons with multitype antibodies increased with age to 10–14 years and then decreased, being low at 20–29 years. This pattern supports the idea deduced from Fig. 3 that transmission of DEN viruses was not established until 20–29 years ago in the Fang district. The presence of specimens with monotype antibody in older groups only in the Fang district, suggests that the prevalences of two or more serotypes of DEN viruses were much lower in Fang than in the Chiang Mai Valley in the past.

4. *Serotypes of specimens with monotypic N antibodies to DEN viruses*

The frequencies of specimens with monotypic N antibodies to DEN viruses are summarized in Table 2. Monotypic N antibodies to all 4 serotypes were found in all districts, including the Fang district, indicating that all 4 serotypes of DEN viruses were transmitted in the entire Chiang Mai area. The total frequency of DEN type 2 virus was the lowest (9%), suggesting that its prevalence was lowest in the Chiang Mai area.

DISCUSSION

Previously, the filter paper method was used instead of vein puncture for collection of blood specimens for seroepidemiological study in Thailand, but the specimens collected had been examined by the hemagglutination inhibition (HI) test, not by the neutralization (N) test (Fukunaga et al., 1974), because the filter paper was not sterile. Recently, successful use of blood specimens collected by the filter paper method was reported (Halstead et al., 1983) and we employed this method for the N test in the present study. This method for seroepidemiological studies seems to be useful, since results obtained were sufficiently accurate to allow deduction of seroepidemiological conclusions on JE and DEN virus

transmissions in the Chiang Mai area as shown above.

In the present study, four districts in the Chiang Mai Valley were selected for collection of blood specimens. No remarkable differences in JE or DEN virus transmission were found in these 4 districts; the percentage incidences of N antibodies to JE and DEN viruses increased with age in all 4 districts, though minor differences were observed in the patterns of increase. Grossman et al. (1973) reported that DEN virus transmission had occurred previously in Chiang Mai City and in two villages (C and D) near the city, but not in two other villages (A and B) that were 28 and 32 km, respectively, from Chiang Mai City. The Mae Taeng district studied in this work is 40 km from Chiang Mai City, but the patterns of percentage incidences of N antibodies to DEN viruses in different age groups appeared similar to those in other districts in the Valley. Thus, DEN virus transmission seemed to have expanded in the Chiang Mai Valley since 1970 when Grossman et al. collected specimens, possibly due to urbanization of the Valley.

On the other hand, marked differences in DEN virus transmission were found in the Fang district which is in another valley. In Fang, the percentage incidences of N antibodies to all four serotypes of DEN viruses did not rise with age and was especially low in the 20–29 year age group. Moreover, a considerable percentage of the specimens in groups of 20–29 years or more had N antibody to only JE virus. These patterns suggested that none of the four serotypes of DEN virus were prevalent to any appreciable extent in the Fang Valley 20–29 years ago. Thus it seems certain that in the Fang Valley JE virus was prevalent before DEN virus transmissions were established. The fact that the Valley borders the Shan State, Burma, where JE outbreaks occurred in the 1970's (Than Swe et al., 1979), is interesting in relation to the genetical similarity between the JE strains in the Shan State and Fang. However, in the

youngest age group in Fang, the percentage of specimens with N antibodies to either type of DEN virus but not to JE virus was much higher than that of specimens with N antibodies to both JE and DEN viruses, and no specimen was found with N antibody to only JE virus, indicating that recently DEN viruses have been more prevalent than JE virus even in the Fang district.

The percentage incidences of N antibody to JE virus at ages of 3, 4 and 5 years in the entire Chiang Mai area were 21%(7/33), 44%(14/32) and 53%(16/30), respectively. This suggests that initial doses of JE vaccine, if applicable, should be administered to children of under 4 years old, followed by booster doses in subsequent years.

In the Chiang Mai area, DEN type 2 virus appeared to be less prevalent than the other 3 serotypes. According to the statistics of the Ministry of Public Health, Thailand, the incidence of dengue hemorrhagic fever (DHF) has been lower in the Northern Region than

in the Central or North-eastern Region. Recently, Burke et al. (1983) reported that pre-existing flavivirus immunity was an important factor in the pathogenesis of DHF due to DEN type 2 and 4 viruses, but less so for DEN type 3 virus, and little if at all for DEN type 1 virus. Thus the fact that DEN type 2 virus has the lowest prevalence of the 4 serotypes might be related to the lower incidence of DHF in the Chiang Mai area.

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