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SEROEPIDEMIOLOGIC STUDY OF ARBOVIRUS INFECTIONS IN THE NORTH-EAST AND SOUTH OF THAILAND[1]

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SUMMARY Blood specimens were collected from villagers in Khon-Kaen province, in the north-eastern region, and in Songkhla province, in the southern region of Thailand in the pre- and post-epidemic seasons of hemorrhagic fever. The hemagglutination inhibition antibody titers of the specimens were measured against dengue virus type 2, type 4, Japanese encephalitis virus and chikungunya virus.

In general, Khon-Kaen villagers showed higher levels of HI antibodies against these arboviruses than Songkhla villagers and in both areas the antibody level against chikungunya virus, a group A arbovirus, was usually much lower than those against group B arboviruses. Specimens from Khon-Kaen province had such high antibody positive rates for group B arboviruses (80–100%) from an early age (3–4 years), in the pre-epidemic season, that scarcely any increase in the positive rate was observed in the post-epidemic season. The positive rate in specimens from Songkhla province was not so high in the pre-epidemic season and some increase was seen in the post-epidemic season, especially in lower age groups (3–4 to 7–9 years).

The patterns of HI antibody titers in different age groups in the Khon-Kaen and Songkhla provinces were also compared. The patterns changed with increase of

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ages and were also different with different viruses. The patterns for chikungunya virus were quite different from those for dengue type 2, type 4 and Japanese encephalitis virus.

These results suggest that people in Khon-Kaen are exposed to group B arbovirus infections more often in childhood and attain high antibody levels more rapidly than people in Songkhla.

INTRODUCTION

In Thailand, the most prevalent arbovirus disease is hemorrhagic fever, and large numbers of cases have been reported from almost all provinces to the Epidemiology Division of the Ministry of Public Health every year since 1971. Another prevalent arbovirus infection in this country is Japanese encephalitis and there were epidemics of this in the north of Thailand in 1969 and 1970 (Yamada et al., 1971). However, epidemics seem to be restricted to the northern region of the country, and there have been no epidemics in other regions.

Some information has been collected concerning various virus diseases in the central and northern regions of Thailand. However, little virological or serological information is available on the north-eastern and southern regions. Therefore, we studied the levels and distributions of antibodies against dengue viruses and chikungunya virus, which are considered to be the causative agents of hemorrhagic fever (Hammon et al., 1960; Halstead, 1966), and also against Japanese encephaitlis virus, in the north-eastern and southern regions of Thailand.

This paper describes results of a survey of the HI antibodies against dengue type 2 virus (DV-2), dengue type 4 virus (DV-4), Japanese encephalitis virus (JE-V) and chikungunya virus (chik-V) in villagers of the north-east and south in the pre- and post-epidemic seasons. Results on these four arboviruses in the two regions were compared. This paper also describes results on the rises of antibody titers observed with paired sera taken in the two seasons from both areas.

MATERIALS AND METHODS

1. *Description of areas where the blood specimens were collected*

1) The north-eastern region

The north-east of Thailand is largely a plateau and with the exception of certain well-irrigated valleys and rich alluvial lands on the right bank of the Mekong it appears rather unfertile and dusty in the dry season in contrast with the fertile lands of the north and the south of the country. Khon-Kaen city, which is the centre of the north-east, is located at latitude 16°22′N and longitude 102°57′E, about 500 km from Bangkok and about 170 km from Vientian, Laos. The villages chosen for the collection of blood specimens are about 10 km west of Khon-Kaen city.

Most of the villagers are peasants growing rice, jute and tapioca and rearing silk worms on a small scale. There is a great water shortage in the area, which makes the life of the villagers difficult. Very often only one or two wells can be used to supply water to the whole village, because the water is so salty in this region. Accordingly, villagers keep water or rain water in big jars all year round. These provide breeding sites for mosquitos such as *Aedes aegypti,* the major vector of dengue viruses and also of chikungunya virus in Thailand.

2) The southern region

The south is in general a richer region. The land produces tin, rubber, coconuts and many kinds of fruits, while various kinds of fishes, prawns and other sea food can be obtained from the sea on the west (Indian Ocean) and east (Gulf of Thailand) coasts of the Malay Peninsula.

In the south, Songkhla province which faces the Gulf of Thailand and is not far from the Malaysian border was selected for field work. Blood specimens were taken in several villages located about 10 km south-west of Haadyai district, the biggest district in the south, located at latitude 7°01′N and longitude 100°28′E, about 1,300 km from Bangkok.

The villagers grow rice, rubber trees, coconut trees and orchards of such fruits as durian, pine apple, rambutan and mangosteen.

2. *Blood samples*

Specimens were taken from the finger tip of subjects of more than six months of age by puncture, using a " readilance " lancet, which is a product of Clay-Adams, Inc., U.S.A. The blood which appeared after the prick was fully absorbed in the absorbing area (5 mm × 30 mm) and excess blood was allowed to spread out to the diffusion area of the strip type bleeding filter paper (a product of Toyo Roshi Co., Japan). The volume of blood absorbed in the absorbing area is 0.1 ml (0.04 ml as serum) according to Dr. K. Nobuto, who developed this method.

After absorption of the blood, the filter paper strips were not exposed to sunlight but air-dried in the shade. The finger tip was cleaned and disinfected before and after bleeding with 90% ethyl alcohol.

The numbers of blood specimens collected and the dates of bleeding in each area are summarized in Table 1.

June is usually the beginning of the epidemic season, not exactly the pre-epidemic season of hemorrhagic fever but we referred to it here as the pre-epidemic season because the peak of the epidemic of the disease is usually seen in July or August, as shown later in Fig. 5.

TABLE 1. *Number of blood specimens tested*[a]

Age group (years)	Khon-Kaen		Songkhla	
	1st	2nd	1st	2nd
0–2	102	83	91	75
3–4	90	83	85	72
5–6	123	101	145	111
7–9	100	114	109	163
10–14	101	134	128	128
15–19	97	102	48	58
20–29	100	101	90	105
30–39	75	63	78	86
40–49	73	55	78	67
50–	59	112	75	112
Total	920	948	930	977

[a] Dates when taken, Khon-Kaen 1st=June 13–15, 2nd=Dec. 19–21, 1973 ; Songkhla 1st=June 28–30, 1973, 2nd=Jan. 7–9, 1974.

3. *Preliminary check of the filter paper method*

A preliminary study was carried out in 1971, on students of the School of Medical Technology of the Department of Medical Sciences with some of the authors of this report as subjects. Specimens were taken from 41 persons from both the cubital vein and finger tip. For the former a syringe was used and for the latter a bleeding lancet and the strip type filter paper were used.

The absorption area of specimens on filter paper was dried completely and then cut into 8–9 pieces with scissors at room temperature. The pieces were soaked in 0.4 ml of PBS (pH 7.2) in a serological tube. The tube was kept at 4 C overnight to allow extraction of the antibody components into the buffered saline. Then, 0.4 ml of 25% acid-washed kaolin (Fisher Co., U.S.A.) was added and the tube was kept at room temperature for 20 min. with frequent aggitation. The filter paper and kaolin were removed by centrifugation at 3,000 rpm for 20 min. and the supernatant was adsorbed with one drop of 50% one-day-old chicken red blood cells at 4 C, 1 hr with occasional shaking. The red blood cells were removed by centrifugation and the result-

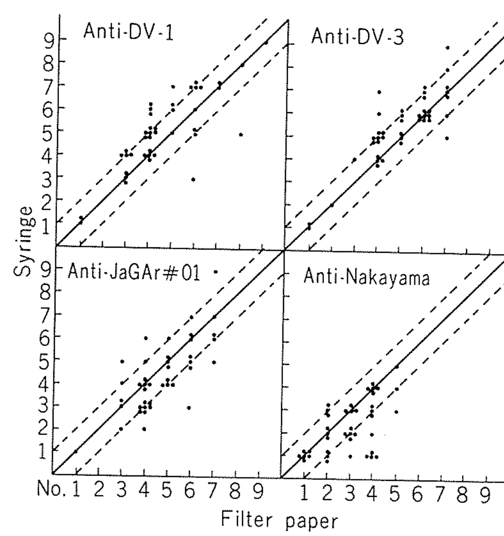


FIGURE 1. *Comparison of HI titers against 4 arboviruses obtained by syringe and filter paper method. No. 1=<20, 2=20, 3=40, 4=80, 5=160, 6=320, 7=640, 8=1280 and 9=2560 HI unit.*

ing supernatant was used for the HI test.

Specimens from the cubital vein were also adsorbed with kaolin and chicken red blood cells in a similar manner.

The HI antibodies against dengue virus type 1 (Hawaiian strain), type 3 (Philippine strain), and JaGAr#01 and Nakayama strain of JE-V, of both types of specimens, were titrated simultaneously by the microtiter method.

The HI titers obtained by the two methods, shown in Fig. 1, agreed well except in the case of anti Nakayama strain, for which the HI titers of some sera by the filter paper method were higher than those by the syringe method. As shown in the figure, the HI titers against the Nakayama strain appeares to be lower than those against dengue viruses and JaGAr#01 strain and a HI titer of less than 20 against Nakayama strain measured by the syringe method, which was taken as a standard, tended to be higher when estimated by the filter paper method.

Thus, the filter paper method sometimes gave unsatisfactory results with low HI titers. However we adopted this method for field work because it is difficult to obtain blood specimens by syringe, especially from younger children, because it is more convenient for taking large numbers of specimens and also because our main purpose was to compare the levels and distribution patterns of antibodies against arboviruses of the dengue group in the north-east and south of Thailand.

4. *HI antibody titration of specimens taken in the field*

The HI titers of blood specimens from Khon-Kaen and Songkhla were measured against DV-2 (New Guinea strain), DV-4 (H-241 strain), JE-V (JaGAr#01 strain) and chik-V (BaH 306 strain) antigens by the method of Clarke and Casals (1958) with slight modification for the microtiter technique. The antigens used were extracted from infected suckling mouse brains with acetone-ether or sucrose-acetone. The same treatment used for the sera described above was adopted to adsorb non-specific inhibitors and hemagglutinin(s) in the specimens.

To compare the antibody levels in the two areas more exactly, specimens of the same age groups from such area were titrated at the same time.

RESULTS

1. *Comparison of HI antibody positive rates*

In this paper specimens with less than 20

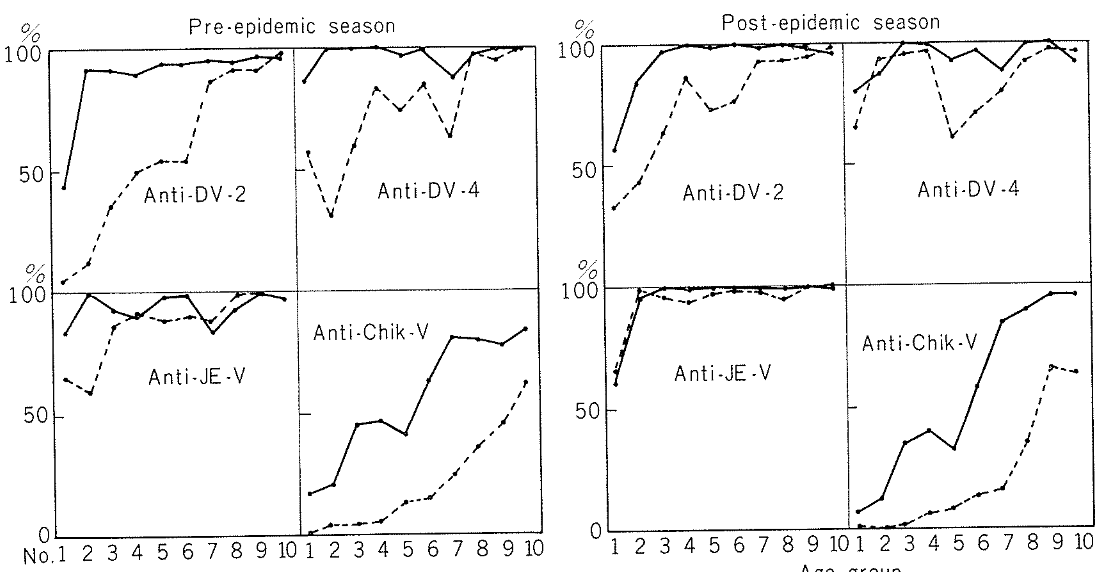


FIGURE 2. *HI antibody positive rates in age groups.* ●——●, *Khon-Kaen;* ●- - - -●, *Songkhla. No. 1=0–2 yr, 2=3–4 yr, 3=5–6 yr, 4=7–9 yr, 5=10–14 yr, 6=15–19* yr, *7=20–29 yr, 8=30–39 yr, 9=40–49* yr, *and 10=50-yr.*

HI units are referred to as HI negative.

(1) Fig. 2 shows the HI antibody positive rates against DV-2, DV-4, JE-V and chik-V in the various age groups of Khon-Kaen and Songkhla villagers in the pre- and post-epidemic seasons.

Khon-Kaen villagers show such high positive rates (80–100%) against DV-2, DV-4 and JE-V even at 3–4 years of age in the pre-epidemic season. The antibody positive rate to chik-V appears to be rather lower than those to group B arboviruses (DV-2, DV-4 and JE-V) and it reaches about 80% at 20–29 years of age in both seasons. Slight rises of the rate for chik-V in the post-epidemic season can be seen in higher age groups.

Songkhla villagers generally showed lower HI antibody positive rates than Khon-Kaen people, especially against DV-2 and DV-4 in the pre-epidemic season and against chik-V in both seasons. Moreover, increase of the positive rates for DV-2 and DV-4 in the post-epidemic season is apparent in all age groups in this area.

(2) High positive rates against group B arboviruses were observed in pre-epidemic sera, especially in those from the Khon-Kaen area (Fig. 2). This suggests the existence of high cross-reactivity among the group B arboviruses employed. To investigate this possibility, 96 blood specimens were taken in July, 1973 from school children (10–13 years old) in a Japanese Language School in Bangkok. These children had only recently arrived in Thailand from Japan and so seemed likely to have antibody against JE-V only, because only this group B arbovirus is prevalent in Japan.

The sera were titrated for HI antibodies against DV-2, DV-4, JE-V and chik-V by the method described above. However, the results were not serviceable for the comparison, as the Japanese children showed much lower HI antibody positive rates against all four viruses, including JE-V, than those of Khon-Kaen and Songkhla school children, as shown in Table 2. However, results showed that the method adopted did not produce much deviation in results.

2. *Comparison of the average HI antibody titers*

Fig. 3 shows the average HI titers of each age group in the pre- and post-epidemic seasons. Generally, the average HI titers of younger age groups were lower in Songkhla province but in groups of 20–29 years and over the average HI titer levels were similar except with anti chik-V.

In Khon-Kaen, especially in post-epidemic specimens peaks in the titers were observed in the 5–6 and 7–9 year age groups against DV-2 and DV-4, and in the 5–6, 7–9 and 10–14 year age groups against JE-V. The average HI titers with anti chik-V also showed a peak, although it was small.

The presence of the peak, at 5 to 9 years of age, appears to coincide with the peak of age-incidence of hemorragic fever in Thailand, but the significance of the relationship between the two phenomena is uncertain.

A marked increase in the HI titer against JE-V, in the post-epidemic period was seen in Khon-Kaen province.

TABLE 2. *Comparison of HI antibody positive rates of children of the Japanese Language School (Bangkok) and Khon-Kaen and Songkhla schools*

	Khon-Kaen	Songkhla	Japanese School (Bangkok)
Anti DV-2	96/101 (95%)	68/128 (54%)	6/96 (6%)
Anti DV-4	98/101 (97%)	85/128 (74%)	7/96 (7%)
Anti JE-V	99/101 (98%)	114/128 (89%)	35/97 (36%)
Anti chick-V	40/101 (40%)	17/128 (13%)	0/96 (0%)

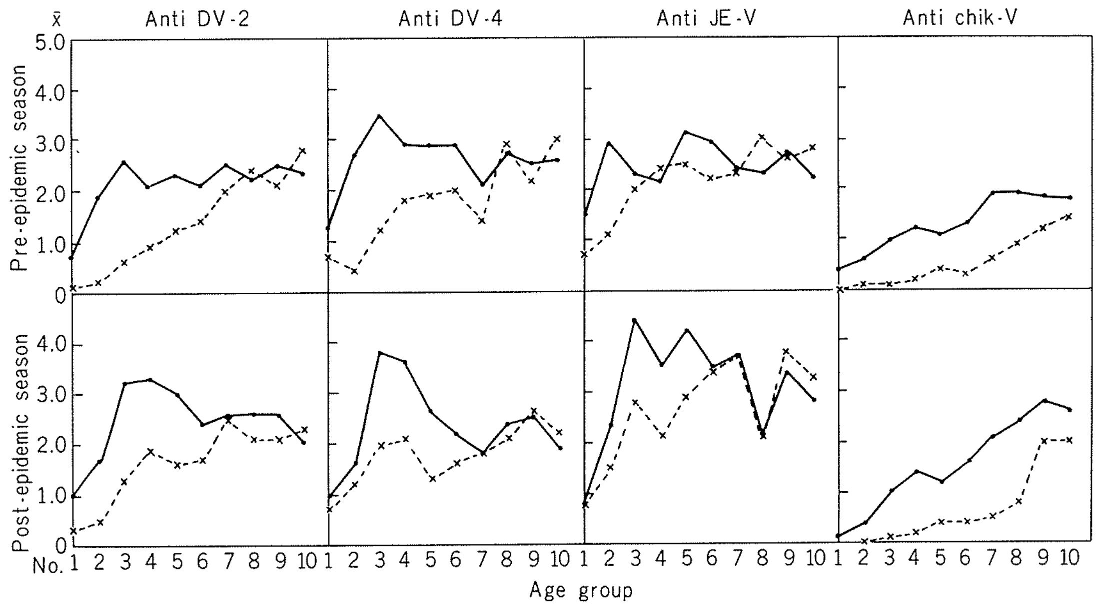


FIGURE 3. *Average HI antibody titers against 4 arboviruses in age groups, expressed as* $2^{\bar{x}} \times 10$ *HI unit.* ●——●, *Khon-Kaen;* ×-----×, *Songkhla. Age groups are same as in Fig. 2.*

3. *Distribution of HI antibody titers*

The distributions of the HI antibody titers for the four arboviruses in the different age groups, expressed as the per cent distributions of HI titers in these groups, were compared. The patterns are shown in Figs. 4a and 4b. Mathematical analysis showed that these distribution curves did not conform to a normal distribution curve, Poisson's distribution curve, or Polia-Eggenberger's distribution curve and further mathematical analysis was impossible, possibly because the numbers of samples were too small.

1) Anti DV-2 titers

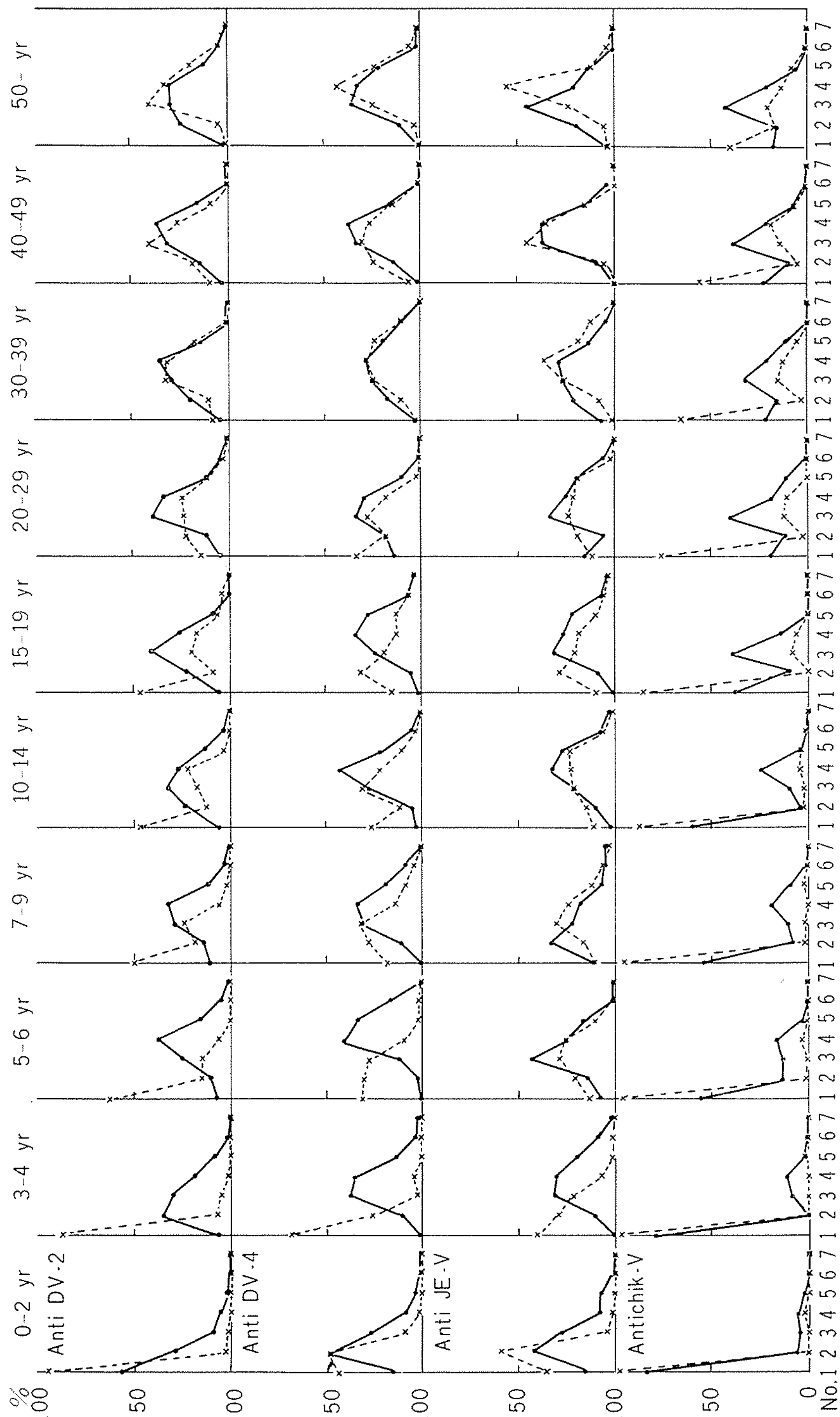
In the pre-epidemic season, a hyperbolic distribution was observed only in the 0–2 year age group in Khon-Kaen, while in Songkhla, this pattern was observed for the 0–2, 3–4 and 5–6 year age groups.

In Khon-Kaen groups of more than 3–4 years old showed a single peak in the pattern of titer distribution. In contrast, in Songkhla the patterns in the 7–9, 10–14 and 15–19 year age groups showed two peaks. This type of distribution seems to be intermediate between the hyperbolic distribution and the single peak distribution. Groups of more than 20–29 years gave patterns with a single peak in Songkhla also and the distribution curves almost coincide with those for Khon-Kaen.

The curves for the post-epidemic season show no significant difference from those for the pre-epidemic season.

2) Anti DV-4 titers

In the Khon-Kaen area all age groups gave a single peak pattern in the pre-epidemic season. On the contrary, the patterns in the Songkhla area were not uniform: the 0–2 and 3–4 year age groups gave hyperbolic patterns, the 5–6 year to 20–29 year groups gave variable patterns, and in groups of over 30–39 years the patterns showed single peaks, like those of specimens from the Khon-Kaen area. The patterns of post-epidemic specimens were similar to those of pre-epidemic specimens in Khon-Kaen. In Songkhla, however, the


0-2 yr
3-4 yr
5-6 yr
7-9 yr
10-14 yr
15-19 yr
20-29 yr
30-39 yr
40-49 yr
50- yr
Anti DV-2
Anti DV-4
Anti JE-V
Antichik-V
%
100
50
0
No. 1 2 3 4 5 6 7

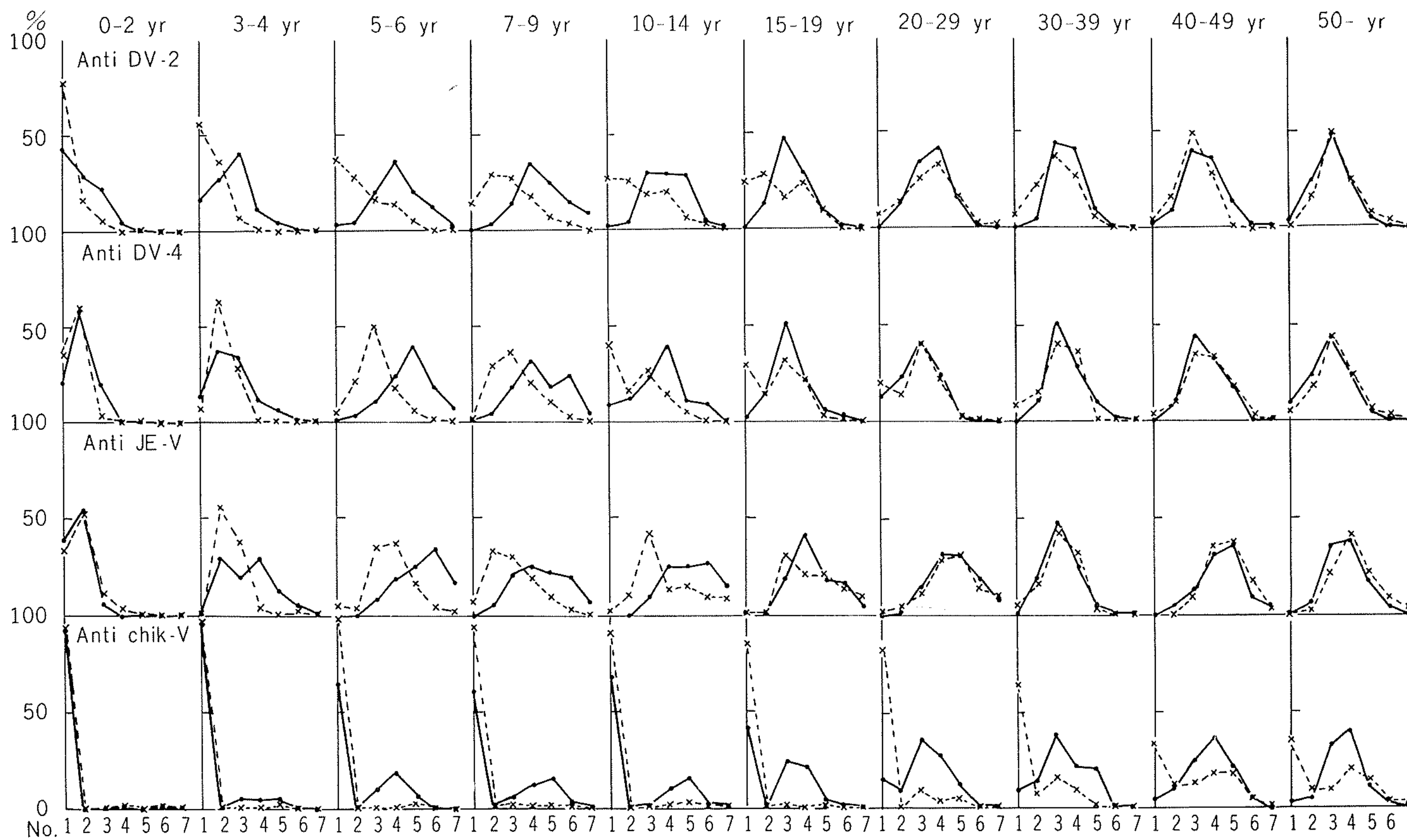


FIGURE 4. *Distribution curves of HI antibody titers against 4 arboviruses by age groups in pre- (4a) and postepidemic season (4b).* ●——●, *Khon-Kaen*; ×- - - -×, *Songkhla*. *No.* $1 = <20$, $2 = 20$, $3 = 40$, $4 = 80$, $5 = 160$, $6 = 320$, $7 = \geqq 640$ *HI unit.*

0–2 and 3–4 year age groups gave single peaks instead of the hyperbolic patterns seen in the pre-epidemic season. The patterns of the groups of more than 20–29 years were similar to those of specimens from the Khon-Kaen area.

3) Anti JE-V titers

In the pre-epidemic season, all groups gave a single peak except the 20–29 year group, in Khon-Kaen. In Songkhla, the 0–2 and 3–4 year groups showed hyperbolic distributions but the patterns for older groups were similar to those for the Khon-Kaen area.

In the post-epidemic season, the 3-4 year group showed two peaks while the 0–2 year group showed a hyperbolic distribution, in Khon-Kaen. The groups from 5–6 years to 15–19 years seemed to be more or less heterogeneous in the Khon-Kaen area; double- or multi-peak patterns were seen in the 10–14 and 15–19 year age groups, while the 7–9 year age group seems to be skewed towards lower titers. In groups of more than 20–29 years the distributions were similar in the two areas.

4) Anti chik-V titers

In the pre-epidemic season, in Khon-Kaen, a gradual shift of the pattern was observed. A typical hyperbolic distribution was observed in the 0–2 year group, a double-peak looking like a mixture of a hyperbolic pattern and a single peak pattern (for the 10–14 and 15–19 year groups), and then a single peak pattern in the 50- year group. A similar shift was observed in the Songkhla series but it was delayed and even in the 50- year age group the shift was not complete and a composite distribution pattern was seen.

In the post-epidemic period similar shifts were observed in both Khon-Kaen and Songkhla but especially in Khon-Kaen, they were bigger than in the pre-epidemic season. The patterns for the 30–39, 40–49 and 50– year age groups showed single peaks in Khon-Kaen.

4. *Rises of HI antibody titers in post-epidemic sera*

In all 197 paired sera were collected in Khon-Kaen and 278 in Songkhla. The frequency distribution of the differences in individual HI titers between paired, pre- and post-epidemic sera are shown in Table 3.

A decrease of 1/4 in post-epidemic sera was observed in 2% of the post-epidemic sera in Khon-Kaen and 3% in Songkhla, while four fold or more increase was observed in 21% of the sera in Khon-Kaen and 9% in Songkhla. This suggests that people in Khon-Kaen were exposed to arbovirus infections more than

TABLE 3. *Distribution of differences in HI titers of paired sera*

	$\times 2^{-2}$	$\times 2^{-1}$	$\times 2^{0}$	$\times 2^{1}$	$\times 2^{2}$
Khon-Kaen					
Anti DV-2	3 (2%)	7 (4%)	56 (28%)	77 (39%)	54 (27%)
Anti DV-4	11 (6%)	41 (21%)	58 (29%)	67 (34%)	20 (10%)
Anti JE-V	2 (1%)	11 (6%)	41 (21%)	63 (32%)	80 (41%)
Anti chik-V	1 (1%)	16 (8%)	137 (70%)	32 (16%)	11 (6%)
Total	17 (2%)	75 (10%)	292 (37%)	239 (30%)	165 (21%)
Songkhla					
Anti DV-2	3 (1%)	21 (8%)	121 (44%)	101 (36%)	32 (12%)
Anti DV-4	20 (7%)	64 (23%)	97 (35%)	72 (26%)	25 (9%)
Anti JE-V	12 (4%)	59 (21%)	87 (31%)	82 (29%)	38 (14%)
Anti chik-V	1 (0.4%)	8 (3%)	253 (91%)	12 (4%)	4 (1%)
Total	36 (3%)	152 (14%)	558 (50%)	267 (24%)	99 (9%)

people in Songkhla during this six month period. As shown in the table, however, the frequency of four fold or more increase in post-epidemic sera was highest with anti JE-V HI antibody in both Khon-Kaen and Songkhla and the frequency decreased in the order, anti DV-2, anti DV-4 and anti chik-V, in both areas.

TABLE 4. *Frequency of rises of 4-fold or more in post-epidemic HI titers of paired sera*

	×4	×8	×16	×32	×64	Total
Khon-Kaen						
Anti DV-2	39 (72%)	13 (24%)	1 (2%)	0 (0%)	1 (2%)	54
Anti DV-4	18 (90%)	1 (5%)	0 (0%)	1 (5%)	0 (0%)	20
Anti JE-V	40 (50%)	27 (33%)	9 (11%)	3 (4%)	1 (1%)	80
Anti chik-V	10 (91%)	0 (0%)	0 (0%)	1 (9%)	0 (0%)	11
Total	107 (65%)	41 (25%)	10 (6%)	5 (3%)	2 (1%)	165
Songkhla						
Anti DV-2	26 (81%)	2 (6%)	3 (9%)	1 (3%)	0 (0%)	32
Anti DV-4	22 (88%)	2 (8%)	1 (4%)	0 (0%)	0 (0%)	20
Anti JE-V	29 (76%)	4 (11%)	2 (5%)	2 (5%)	1 (3%)	38
Anti chick-V	3 (75%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	4
Total	80 (81%)	8 (8%)	6 (6%)	4 (4%)	1 (1%)	99

TABLE 5. *Seroconversion rates against 4 arboviruses in different age group*

Age group (years)	Khon-Kaen					Songkhla				
	Anti DV-2	Anti DV-4	Anti JE-V	Anti chick-V	Total	Anti DV-2	Anti DV-4	Anti JE-V	Anti chick-V	Total
0–2	6/11 (55%)	4/4	3/3	0/16	13/34 (38%)	4/13 (31%)	7/8 (88%)	5/5	0/13	16/39 (41%)
3–4	0/0	0/0	0/0	0/7	0/7	2/10 (20%)	6/7 (86%)	7/7	0/10	15/34 (44%)
5–6	6/7 (86%)	0/0	8/8	1/23 (4%)	15/38 (39%)	22/37 (59%)	16/18 (89%)	6/9 (67%)	0/52	44/116 (38%)
7–9	3/3	0/0	3/3	0/8	6/14 (43%)	19/29 (66%)	6/7 (86%)	2/3 (67%)	0/54	27/93 (29%)
10–14	4/4	1/3 (33%)	2/2	0/30	7/39 (18%)	20/40 (50%)	6/21 (29%)	5/6 (83%)	0/82	31/149 (21%)
15–19	2/2	1/1	1/1	1/19 (5%)	5/23 (22%)	0/4	0/1	1/1	0/6	1/12 (8%)
20–29	0/0	0/0	0/0	0/1	0/1	2/2	3/5 (60%)	1/1	1/9 (11%)	7/17 (41%)
30–39	0/0	0/0	0/0	2/3 (67%)	2/3 (67%)	0/0	0/0	0/0	0/6	0/6
40–49	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2/6 (33%)	2/6 (33%)
50–	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/3	0/3
Total	21/27 (78%)	6/8 (75%)	17/17 (100%)	4/107 (4%)	48/159 (30%)	69/135 (51%)	44/67 (66%)	27/32 (84%)	3/241 (1%)	143/475 (30%)

Results of analyses of cases with a rise of four fold or more in post-epidemic HI titers of paired sera are shown in Table 4. Most cases from both areas showed a 4-fold rise with the exception of the case for anti JE-V in Khon-Kaen.

Pre-epidemic HI negative sera were compared individually with the corresponding post-epidemic HI titers. The results are summarized in Table 5.

In the Khon-Kaen area there were fewer pre-epidemic HI negative cases of DV-2, DV-4 and JE-V in almost all age groups than in the Songkhla area where negative cases were found especially in young age groups (less than 14 years). Thus it was difficult to compare the seroconversion rates in the two areas, which indicates the density of incidence of the viruses. Nevertheless, the seroconversion rates in the Khon-Kaen area for the three viruses mentioned above, seem to be higher than those in the Songkhla area.

It was a little easier to compare data on chik-V in the two areas. In both areas most of the pre-epidemic specimens with a negative titer were in younger age groups. Seroconversion was observed in the Khon-Kaen area in the 5–6, 15–19 and 30–39 year age groups, while in the Songkhla area it was only seen in the 20–29 and 40–49 year age groups. The Khon-Kaen area seemed to have a slightly higher seroconversion rate (4/107=4%) than the Songkhla area (3/241=1%).

DISCUSSION

Judging from reports to the Epidemiology Division of the Ministry of Public Health of Thailand, cases of hemorrhagic fever (HF) seem to be increasing all over the country.

In the 1960's, most of the cases reported were from the central region, where the Bangkok-Thonburi Metropolitan area is located, but during the period from 1971 to 1973, many cases of HF were reported not only from the central region but also from the north-east, as shown in Table 6, and the number of cases in the Khon-Kaen province in the north-east, for instance, exceeded those in the Bangkok-Thonburi area.

TABLE 6. *Reported cases of hemorrhagic fever in Thailand during 1971–1973*

	1971	1972	1973	Average	Population
Central region (25 provinces)	4,976[a] (43)[b] 39[c] (0.9%)[d]	7,788 (126) 62 (1.6%)	3,693 (69) 29 (1.9%)	5,489 (92) 43 (1.7%)	12,472,098 (33%)
Northern region (16 provinces)	2,570 (45) 31 (1.8%)	3,895 (68) 48 (1.6%)	1,146 (36) 14 (3.1%)	2,537 (48) 31 (1.9%)	8,113,509 (21%)
North-eastern (16 provinces)	3,824 (203) 29 (5.3%)	10,236 (445) 78 (4.3%)	3,035 (189) 23 (6.2%)	5,698 (279) 43 (4.9%)	12,990,872 (34%)
Southern region (14 provinces)	170 (8) 3 (4.7%)	1,863 (51) 38 (2.7%)	406 (21) 8 (5.2%)	813 (27) 16 (3.3%)	4,782,529 (12%)
Total (71 provinces)	11,540 (299) 30 (2.6%)	23,782 (685) 61 (2.9%)	8,280 (315) 21 (3.8%)	14,534 (533) 37 (3.7%)	38,359,008
Bankok-Thonburi (central region)	1,092 (1) 28 (0.1%)	2,295 (6) 60 (0.3%)	1,509 (22) 39 (1.5%)	1,632 (10) 43 (0.6%)	3,793,763
Chiang Mai (northern region)	13 (0) 1 (0%)	186 (1) 17 (0.5%)	416 (5) 39 (1.2%)	205 (2) 19 (0.02%)	1,049,802
Khon-Kaen (north-eastern)	397 (21) 33 (5.3%)	1,996 (63) 167 (3.2%)	566 (23) 47 (4.1%)	986 (36) 82 (3.7%)	1,192,898
Songkhla (southern region)	21 (2) 3 (9.5%)	615 (15) 90 (2.4%)	38 (6) 5 (15.8%)	225 (8) 33 (3.6%)	680,293

[a] Reported cases. [b] Deaths. [c] Cases per 100,000 population. [d] Mortality rate.

Differences were seen in the mortality rates in the different regions of the country and also in the four provinces as shown in the table. Namely the central and northern regions, and the Bangkok-Thonburi area and Chiang Mai province have low mortality rates while the north-east and south, and Khon-Kaen and Songkhla provinces have high ones. Similarly Halstead et al. (1969) reported that the mortality rate in the Bangkok-Thonburi area was lower than that in the north-east during an epidemic of HF in 1964.

Of the four provinces, Chiang Mai shows the lowest incidence and mortality rate of HF, but JE epidemics occurred in Chiang Mai and other provinces in the north in 1969 and 1970. The antibody positive rates against DV-2, DV-4 and JE-V (JaGAr#01) were found to be 35%(33/95), 41%(39/95) and 38%(37/97), respectively in school children (6–13 years) in Chiang Mai in 1972 (Fukunaga et al., 1974). These positive rates appear to be lower than those in the north-east and south, as shown in Fig. 2, although blood specimens were collected in a different way in Chiang Mai field work (syringe method). Halstead et al. (1969) also reported that the positive rates of dengue type 1 HI antibody in young men in the Khon-Kaen and Chiang Mai provinces were 95.7% (111/116) and 36.1%(193/534), respectively.

These observations suggest that JE epidemics take place where the antibody level is low, while HF epidemics occur where the antibody level is high.

The antibody level against chik-V was found to be much lower than those against DV-2 and DV-4 in both the Khon-Kaen and Songkhla areas, and HF cases due to chik-V were also rare (2–5%), according to results of serologic diagnoses of HF obtained in Virus Research Institute, Thailand during the period of 1970 to 1972, as shown in Table 7 (Annual Report of Virus Research Institute, Thailand, 1970, 1971 and 1972).

In 1970 and 1971, paired serum specimens of suspected cases of HF in Burma were sent to this institute for serologic examination. Results are also shown in Table 7. Judging from

TABLE 7. *Serologic diagnosis of hemorrhagic fever in 1970, 1971 and 1972*

	Dengue infection	Chikungunya infection	Double infection	Subtotal	Inconclusive	Negative	Total
Thailand 1970	287 (86%) (59%)	16 (5%) (3%)	32 (10%) (7%)	335 (100%) (69%)	40 (8%)	112 (23%)	487 (100%)
Burma 1970	17 (21%) (16%)	35 (43%) (32%)	30 (36%) (28%)	82 (100%) (75%)	5 (5%)	22 (20%)	109 (100%)
Thailand 1971	312 (90%) (73%)	8 (2%) (2%)	26 (8%) (6%)	346 (100%) (81%)	31 (7%)	49 (12%)	426 (100%)
Burma 1971	15 (24%) (16%)	41 (66%) (45%)	6 (10%) (7%)	62 (100%) (68%)	12 (13%)	17 (19%)	91 (100%)
Thailand 1972	579 (85%) (47%)	18 (3%) (1%)	80 (12%) (7%)	677 (100%) (55%)	206 (17%)	336 (28%)	1219 (100%)

these specimens, HF cases due to chik-V are more common than those due to dengue viruses in Burma, and some severe cases of HF were due to chik-V (Sompop Ahndarik of Virus Research Institute, Thailand, personal communication). These findings are strange and interesting and require further study. During studies of HF in Thailand, Halstead et al. (1967, 1970) observed that a severe form of HF of children older than 1 year of age is associated with a secondary type of antibody response and not with primary infections of dengue viruses or chik-V.

In both Khon-Kaen and Songkhla, the antibody level against JE-V was high but no JE epidemics have been reported from either area. The high level of antibody to JE-V may partly reflect serologic cross-reactivity of the JaGAr#01 strain with dengue viruses, because it is known that the strain shows higher cross-reactivity than the Nakayama strain or other newer strains of JE-V (Oya, A., NIH, Japan, personal communication; Fukunaga et al., 1974). Another possible reason is the slight enhancement of the HI titer using the filter paper method for blood collection, as discussed in MATERIALS AND METHODS, although the Japanese children in Bangkok were found to have a rather low antibody level against JE-V using this method. Considering the high antibody level to JE-V in Khon-Kaen and Songkhla, attempts should be made to isolate dengue and JE viruses from mosquitos in these areas.

The rainy season in the south of Thailand usually begins one or two months later than in other regions of the country. According to records of the meteorological stations in both provinces, the total rainfall in Khon-Kaen and Songkhla provinces in 1971 were 1,200 mm and 2,415 mm, respectively; that is, the rainfall in Songkhla is twice that in Khon-Kaen province. The monthly rainfall exceeded 100 mm in May, June, July, August and September in Khon-Kaen in 1971 and in Songkhla province in August, October, November and December. The rainfall in these months were 83% and 81% of the totals for the year, respectively.

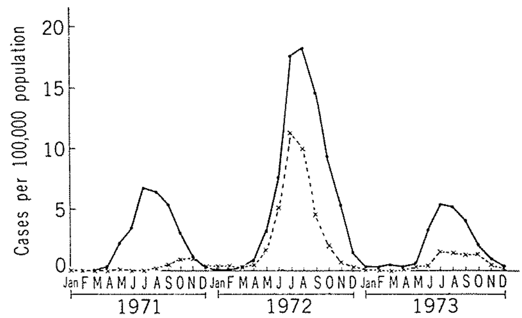


FIGURE 5. *Monthly incidence of hemorrhagic fever per 100,000 head of population in Khon-Kaen and Songkhla provinces in 1971–1973.*

Fig. 5 shows the monthly incidences of HF per 100,000 head of population in Khon-Kaen and Songkhla provinces during 1971 to 1973. The curves for the monthly incidences in the Chiang Mai and Bangkok-Thonburi areas appeared to be similar to those in Khon-Kaen (not shown here). In this three-year period, the HF epidemic was biggest in 1972 and the peak was seen in July-August in both Khon-Kaen and Songkhla, even though the rainy season started later in the Songkhla area. This is strange but is probably explained as follows. In the south there are two types of monsoon. One is the south-west monsoon which causes rain from June to September. The other is the north-east monsoon which causes rain from October to December. The latter causes much heavier rain and often floods, which would wash away the larvae of *Aedes* mosquitos. Consequently, in the south HF epidemics occur during the milder rainy season of June to September. This is a simple explanation based on the rainfall in the south. Other unknown factors, especially ecological factors related to dengue viruses, must also be involved in this phenomenon.

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