

Ecology of Japanese Encephalitis Virus.

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OBJECTIVE : To investigate the ecology of Japanese encephalitis virus in Thailand, with particular reference to aspects contributory to infection in humans. Specific objectives include the following :

1. to assess the extent of human infection, and its seasonal variation;
2. to observe clinical manifestations of human infection;
3. to determine which species of mosquitoes are transmitting human infection;
4. to investigate animal reservoirs of JE virus;
5. to ascertain environmental factors bearing on the incidence of human infection;
6. to attempt laboratory measurement of virus virulence or host defenses which bear on the nature of host-parasite relationships;
7. to study the inter-relationships of multiple co-existing group B arboviruses in a discrete area; whether competition, antagonism or synergism obtains between these agents, their mosquito vectors and the immune systems of their human hosts.

DESCRIPTION : Japanese encephalitis (JE) virus has been known to be endemic in Thailand for a number of years. Reports of outbreaks of clinical encephalitis have been received from the north and north-central plains area since at least 1962. In that year the existence of known JE virus mosquito vectors was documented, and subsequently group B arbovirus antibody was detected in sera of potential or known animal reservoirs of this agent. In 1964 an extensive outbreak of encephalitis at Pitsanuloke was serologically confirmed as JE, and the virus was isolated from a human case of encephalitis at Chiangmai.

Extensive ecologic studies of JE virus have been carried out by SMRL at Bang Phra, Southeastern Thailand. JE virus was isolated repeatedly from two species of mosquitoes, Culex gelidus and tritaeniorhynchus. Evidence was also accumulated suggesting that several species of domestic and wild vertebrates were involved in JE virus transmission cycles. Serologic evidence, however, indicated only low levels of JE virus infection in a nearby population of school children.

During the period June-September, 1969, an epidemic of encephalitis was reported in Chiangmai province that ultimately affected hundreds of children. Subsequent information indicated that the epidemic actually occurred throughout a wide area of the north and central plains area of Thailand. In the Chiangmai area alone 232 cases of encephalitis were reported, of whom 68 died. Paired sera were available from 55 children admitted to the McCormick and University Hospitals. Thirty-two of these showed evidence of recent infection with a group B arbovirus, and 15 monotypic HI antibody response to JE virus. JE virus was isolated from brain tissue of a patient dying of encephalitis. Initial serologic surveys of large domestic

animals (pig, dog, buffalo and cattle) in the area indicate a high incidence of past infection with group B arbovirus. Preliminary mosquito collections revealed the abundant existence of the potential vectors C. gelidus, C. tritaeniorhynchus, and the C. vishnui complex.

In contrast to other areas of the world where JE virus ecology has been studied (e.g. Japan and Taiwan), however, other members of the group B arbovirus complex are endemic in northern Thailand. The clinical existence of Thai hemorrhagic fever has been known for a number of years in this area and follows the same epidemiologic patterns as seen elsewhere in Thailand. Serological results of patients have been identical to those seen in dengue hemorrhagic fever and indicate the hyperendemicity of dengue virus in the area. Because infection(s) with dengue virus may result in broadly-reacting group B arbovirus antibody, which in at least some instances will protect the host to challenge with heterologous virus, the coexistence of JE and dengue viruses provides an opportunity to study this interaction in human populations.

In preparation of the anticipated increase in JE virus transmission to occur during the 1970 rainy season, study populations have been delineated. A comprehensive program to define mosquito populations in the area has been initiated. Light traps, bait-traps, and biting collections in several representative habitats have been collected. Pools of culicine mosquitoes have been tested for JE virus by mouse inoculation, and subsequently isolation systems will compare both mouse and LLC-MK₂ cell systems. Collections of Aedes will also be tested for dengue virus. Culicine larval populations have been surveyed by quantitative sampling methods. Simultaneous insecticide susceptibility tests have been established on colonized strains of vector species.

Serologic surveys are being carried out on potential reservoir hosts, based on data found both previously by SMRL at Bang Phra and in other countries. Antibody-free swine have been established in study areas as sentinel hosts. Studies in wild animals emphasize collection and analysis of suspect species in order to determine whether any are naturally infected to the same frequency as found previously in other areas of Thailand and whether the incidence of such infection relates to infection in humans.

Other aspects of this study are being reported separately in the section on Mosquitoes.

PROGRESS: Four villages in scattered locations of the Chiangmai Valley have been selected as study sites. Each has been completely censused and characterized, and approximately 20% sample of the population selected as demographically representative of the larger population. Initial bleeding was carried out in early November, the beginning of the dry season. In addition children from a school in the urban area of Chiangmai were bled.

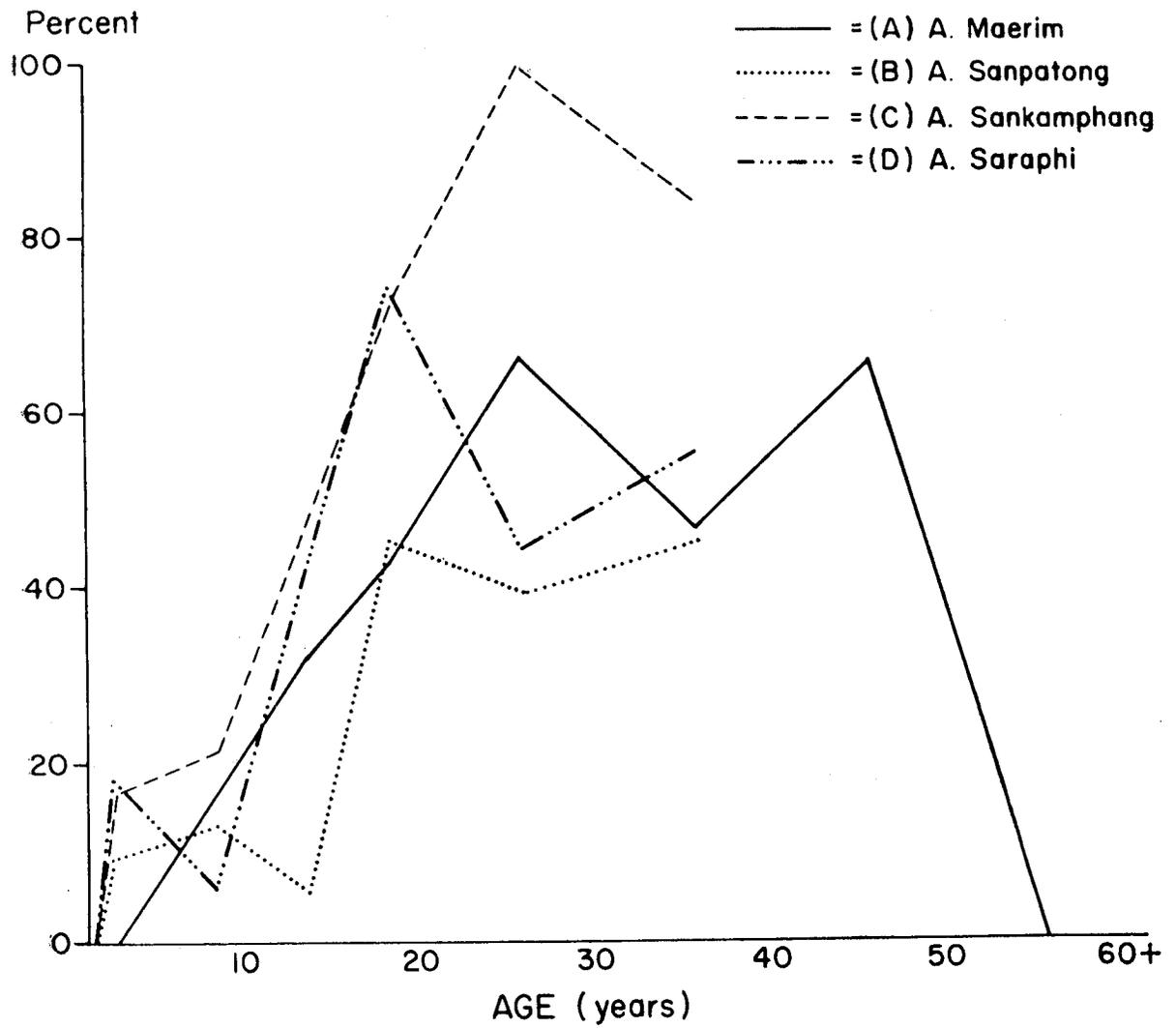
Table 1 presents the age prevalence distribution for each area. Because of the cross-reactivity, interpretation is provisional. It seems clear, though, that most of the antibody prevalence in villages (A) and (B) is due to JE virus, while results in (C) and (D) probably represent both JE and dengue antibody. The city school (E) more clearly represents dengue prevalence. This is suggested not only by the prevalence difference between the two viruses but also by the level of geometric mean titers (Table II) for each area. Only in the city school are the dengue titers greater than the JE titers. Villages A and B are farther away and more rural and isolated from Chiangmai city than are villages C and D. Although only 6-8 year olds were sampled in Chiangmai city the picture looks like that of Bangkok for dengue prevalence and it is probable that virtually 100% of the population has been infected by group B virus(es) by early adulthood.

Table III shows the overall village prevalences and their 95% confidence limits for JE. Only (B) and (C) do not overlap but it appears that these villages appear similar in overall prevalence. The age prevalence distributions are likewise fairly similar (Figure 1) except for ≥ 20 year population of village (C).

Chikungunya activity has evidently been absent in villages (A), (B), and (C), while present in village (D) and Chiangmai city. This is interesting and may deserve further entomologic and sero-epidemiologic investigation.

FIGURE I

Percentage Age Distributions of Prevalence of JE Antibody (HI)
Chiengmai Villages - Nov. 1969



The similar prevalence of JE in males and females (Table IV) is surprising in view of the marked male preponderance of overt hospitalized encephalitis cases. There are several possible explanations, of course, and this certainly deserves thorough investigation in the coming season. It does suggest equal male-female exposure (and for each age group) to infection. From Figure 1 note the curve for village (A) in Amphur Maerim. Assuming this represents true JE prevalence the picture is as expected from the known epidemiology. Exposure to infected mosquitoes (presumed to be Culex) is most likely out in the nearby fields that are farmed by almost every family. Only the elderly and very young usually do not go out to the fields and the low prevalence, < 5 and < 50 years, is shown.

The picture in Figure 1 is also compatible with that seen in a population exposed endemically with epidemics superimposed (assuming that HI positivity either remains > 1 year or periodic exposures keep boosting HI levels). This can be analyzed by fitting a catalytic model to the prevalence data. This was done for Area (C) and appears reasonable (Table V). Thus, the estimated force of infection of 74 effective contacts per 1000 population per year for village (C) is quite high. Again this may be a combined Dengue-JE picture in village (C), which would be of interest epidemiologically and virologically.

The findings of marked differences in A. aegypti indices during the same month (November) as this survey and in the same villages suggested that there would also be differences in Dengue prevalence (Table VI). Indeed there is virtually perfect correlation, highly significant despite having only 5 data pairs to compare. Table VII presents data on incidence of group B arbovirus HI antibody as found in sera of domestic animals in the Chiengmal area. It is apparent that all species of domestic animals show a very high rate of exposure to group B arbovirus, and most probably this is JE virus. Preliminary neutralization test data confirms this but is not yet complete. Further sera collections of these animals are being made, as well as a wide variety of wild animal vertebrate species, in an effort to define which may be involved in the maintenance of group B arboviruses in nature.

It is recognized that to date unresolved difficulties in interpretation of serological data limit the ability to differentiate previous infection with JE and dengue viruses. Experiments are in progress to try to improve laboratory methods in this regard.

Continuing mosquito collections are being carried out. During the period October 1969 to March 1970, 1042 pools of Culex species have been tested for virus isolation. No viruses were isolated.

Table I. Age Prevalence of JE, DENGUE and CHIKUNGUNYA HI Antibodies Chiangmai (Nov 1969)

Age	(A) Mearim		(B) Sanpatong		(C) Sankamphang		(D) Saraphi			(E) Chiangmai City			
	JE	D	JE	D	JE	D	JE	D	CHIK*	AGE	JE	D	CHIK
1-4	0	14.3	9.1	0	16.7	16.7	18.2	9.1	9.1				
5-9	16.7	16.7	13.3	13.3	21.7	34.8	5.9	35.3	5.9	6	75.0	87.4	25.0
10-14	31.6	10.5	5.5	5.5	47.0	64.7	36.4	50.0	4.5	7	64.0	81.3	12.0
15-19	42.8	28.6	45.4	18.2	72.7	63.6	75.0	75.0	12.5	8	55.5	85.2	0
20-29	66.7	33.3	40.0	30.0	100.0	100.0	44.0	66.7	11.1				
30-39	47.0	5.9	45.4	9.1	84.2	78.9	55.0	60.0	15.0				
40+	33.3	16.7	—	—	—	—	—	—	—				
Total	36.6	15.8	23.7	11.8	53.1	58.0	36.8	48.3	9.2	—	62.8	82.7	10.0
Sample Size	82		96		81		87			110			

+ Titers \geq 1:40

* CHIK completely Negative in the villages (A), (B), and (C).

Table II. Geometric Mean Titers of those with HI Antibody* Present—Chiangmai (Nov 1969)

	(A)	(B)	(C)	(D)	(E)
JE	56.6	105	185	132	117
Dengue**	44.5	74.1	142	127	129

* Titers \geq 1:40

** Highest D1—D4 Titer used

Table III. Overall Prevalences (%) of JE Antibody (HI)* and 95% Confidence Limits Chiangmai (Nov 1969)

Area	Prevalence (%)	Sp**	95% Confidence Limits
(A) Maerim	36.6	.0442	27.4 – 45.8
(B) Sanpatong	23.7	.0501	13.2 – 34.2
(C) Sankamphang	53.1	.0386	45.0 – 61.2
(D) Saraphi	36.8	.0595	26.3 – 47.3

* Titers \geq 1:40

** S_p = Standard error of sample % positive

Table IV. Sex Prevalence of JE, DENGUE and CHIKUNGUNYA HI Antibodies Chiangmai (Nov 1969)

Area	PERCENTAGE POSITIVE*							
	No. Tests		JE		DENGUE		CHIK	
	Male	Female	Male	Female	Male	Female	Male	Female
(A) Maerim	36	46	41.7	32.6	16.7	15.2	0	0
(B) Sanpatong	36	40	22.2	25.0	11.1	12.5	0	0
(C) Sankamphang	49	32	55.1	50.0	51.0	68.7	0	0
(D) Saraphi	43	44	34.9	38.6	51.2	45.4	7.0	11.4
Total (A)–(D)	164	162	33.5	35.8	34.8	33.3	—	—
(E) Chiangmai City	57	53	61.4	64.1	82.4	83.0	10.5	9.4
TOTAL	221	215	—	—	—	—	—	—

*Titers \geq 1:40

Table V. Fitting a Simple Catalytic Model to Area (C)—JE (HI) Prevalence Data.

Age	t*	y	w	A	0.074 t	$e^{-0.074t}$	y
1-4	2.0	.167	4	.668	.148	.8624	.1376
5-9	6.5	.217	5	1.085	.481	.6132	.3868
10-14	11.5	.470	5	2.350	.851	.4270	.5730
15-19	16.5	.727	5	3.635	1.219	.2955	.7045
20-29	24.0	1,000	10	10.000	1.776	.1693	.8307
30-39	34.0	.842	10	8.420	2.516	.0808	.9192
—	—	—	A = 26.158		—	—	—

*t = Age-1 year. Thus the t scale has been shifted to begin at age 1 instead of 0, the value of t on the scale is therefore always 1 year less than the population age at that point, and the total space covered by the data, from ages 1-40, is 39 years wide.

$$A = 26.158 \times \frac{100}{39} = 67.1$$

$$r' = .029$$

$r = \frac{.029}{.39} = .074$ or estimated force of infection produces 74 effective contacts per 1000 pop. per year.

The fraction-positive pop. proceeds at rather less than the average rate for the years until about age 15 when the reverse is largely the case. This can be rationalized as the effect of extra exposure in the rice fields by the young and middle-aged population that does not attend school. The basic hypothesis here, of course, is that JE in this area is an endemic-epidemic disease and the curve tends to uphold this.

Table VI. Correlation of Aedes aegypti House Index and Prevalence of Dengue Antibody (HI) Chiengmai (Nov 1969)

Area	X = <u>A. aegypti</u> Index	Y = Dengue HI Prevalence
(A)	.019	.155
(B)	.001*	.117
(C)	.300	.580
(D)	.156	.461
(F)	.570	.827
\bar{X}_2	1.046	2.140
\bar{X}_2	0.4396	1.2706
$(\bar{X})/N$	0.2188	0.9159
C...	0.2208	0.3547
$\bar{X}XY = .7204$ $\bar{X}\bar{X}\bar{Y}/N = .4477$ $C_{X,Y} = .2727$		

$$r = \frac{.2727}{\sqrt{(.2208)(.3547)}}$$

$$r = 0.974$$

$$p < .01$$

*Actual index was zero (.000). Correction used for analysis purposes.

Table VII. Incidence of HI Antibody in Sera of Domestic Animals in Chiengmai

Animal	Per Cent with Antibody to:			
	Chikungunya	Dengue-1	JE (Nak)*	JE (CM)**
Cattle	17	7	84	96
Pig	29	27	73	81
Dog	10	7	86	86
Buffalo	11	2	80	95

* Nakayama prototype strain

** Chiengmai strain