The Ecology of Japanese Encephalitis Virus Infections in Chiangmai

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OBJECTIVE: To investigate the ecology of Japanese encephalitis virus (JEV) in the Chiangmai Valley, Northern Thailand, with particular reference to aspects contributory to infection in humans.

DESCRIPTION: The previous two Annual Reports explained the study methodology and results of work in Chiangmai Valley from November, 1969 to March, 1971. In summary, JEV was found to be widely disseminated throughout the Valley, causing infections in pigs and man in at least 9 consecutive months (April—December, 1970). Rapid buildup in virus transmission occurred in April at the start of the rainy season and most of the human and pig infections occurred in May, June and July; 84 of the 100 encephalitis cases for 1970 occurred in these 3 months. JEV was isolated from 3 *Culex* species—*C. tritaeniorhynchus*, *C. gelidus* and *C. fuscocephala* and all 3 species are believed to be acting as JEV vectors. Field and laboratory evidence suggested that small wild vertebrates and domestic animals (other than pigs) are not important variables of JEV transmission.

The specific areas of interest and their objectives in work that was pursued 1971 include the following:

(1) Epidemiology: Case ascertainment at the 3 valley hospitals was continued throughout 1971 as was collection of climatologic data. The sentinel pig study was continued through June, 1971, to both determine whether JEV transmission to pigs occurred in the dry, winter months and to see if a similar buildup in transmission occurred in 1971 after the start of the rainy season. The 4 village study areas were revisited once, in November, 1971; censusing of humans and animals was again performed and blood was obtained from the people in the random sample cohorts (selected in November, 1969) who were still remaining. Sera were also obtained from the Chiangmai City school cohort.

(2) Entomology: Studies on the ecology of the vectors of JEV in Chiangmai Valley were continued during 1971. Emphasis was directed at measurement of fluctuations in population density and physiological age, determination of host preferences, and flight range and dispersal characteristics. CDC light—trap collections were made twice weekly at three sites—villages A and B and in Chiangmai City (E). These sites are located in the northern, southern and central portions, respectively, of Chiangmai Valley. Material from light—trap collections was used for computing monthly indices of female mosquito densities as well as for determinations of physiological age and host preferences.

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(3) Virology: Studies on the role of small vertebrates and domestic animals as hosts of JEV in the Chiangmai Valley continued. The role of *Culex fuscocephala* as a vector of JEV was examined. The epidemiology of a Simbu group arbovirus, Ingwavuma virus, isolated in the Chiangmai Valley in 1970, was explored. Further studies on the specificity of IgM antibody to JEV and dengue viruses as a means of serologic diagnosis of specific Group B arbovirus infections were undertaken. These are detailed in subsequent sections of this Annual Report.

PROGRESS: Encephalitis occurred in 1971 in Chiangmai Valley at a remarkably similar incidence to that of 1970 (Table 1). The 99 Valley cases were admitted in the 10 consecutive months, from February to November. As in 1970, over 75% of the cases were admitted in May, June and July although the peak month (July) was both sharper and a month later in 1971. This may reflect the slower onset of rainfall in 1971. Paired sera were obtained from patients in McCormick Hospital only, but 81% of the adequately spaced serum pairs had diagnostic JEV titer rises, thus providing similar confidence in the 1971 case incidence data. Further evidence of both the widespread distribution of JEV in the Valley and the surprising agreement between the 2 years is shown in Table 2. Once again, cases occurred in each Valley district and at comparable incidence rates. One JEV isolate was obtained from the brain of a 9 year—old female admitted on 10 July 1971. Seventeen cases died in 1971, compared to 20 in 1970, and the case fatality rate was especially high (50%) under age 5 (Table 3). Both the age and sex distributions of the cases were similar in both years, although the 1971 cases were slightly younger on the average and had a smaller male preponderance. The type of HAI serologic response in the confirmed cases was also similar in both years, 72% in 1970 and 75% in 1971, having a primary response.

None of the sentinel pigs in the 4 study villages had a JEV infection in January or February, 1971 (Table 4) although this does not rule out the possibility that low level transmission was occurring. Pig conversions occurred in one village (B) between 9—31 March, in 2 villages in April, in 3 villages in May and in all 4 villages in June when all but one susceptible pig had a JEV infection. It is attractive to believe that the very high dissemination of JEV to pigs occurring in June is directly related to the sharp peak of human cases that occurred in July (the median human case was admitted on 10 July).

One possibility for dry season maintenance of JEV appeared to exist in low level transmission among the pig population. This possibility seemed very likely because serological evidence collected during 1970 showed that there were JEV susceptible pigs present all year along with JEV vector mosquitoes. In support of this theory, one JEV isolate was obtained from a group of 25 pigs near Udorn, Thailand, (see below) in December, 1972. Accordingly in January, 1972, blood samples taken from 479 pigs at the time they were butchered in Chiangmai and were inoculated intraperitoneally into weanling mice. Deaths before 14 days occurred in about 7% of the mice but viral isolates, none of them resembling JEV, were made in only three cases. Accordingly, there continues to be only indirect evidence supporting the maintenance of JEV through low level pig transmission during the dry season.

At the time the blood samples were collected from slaughter pigs, statistical information was collected concerning the number of pigs butchered and their origin. Such information was thought to be of potential usefulness as an indicator of changes in pig usage in Chiangmai that might, in turn, be related to the incidence of human JEV infections. Between 1961 and 1971 the number of pigs slaughtered annually varied between 28,000 to 46,000 (figures taken from official Chiangmai Municipal slaughterhouse records); 39,000 pigs were slaughtered in both 1970 and 1971. Pigs are usually slaughtered when they reach an acceptable size rather than age; this size is probably in excess of 100 kg. Smaller pigs are butchered but in significantly fewer numbers; they often are animals in poor health that have grown poorly. Ages of slaughter pigs usually vary from 7 months to more than a year, although a low percentage are older salvaged breeders. Younger pigs that reach slaughter size early have probably been fed a better ration. The greatest source of slaughter pigs for Chiangmai is Pitsanuloke, but animals are also purchased for slaughter anywhere within a 200 km. radius of Chiangmai and trucked in every day. Although the Chiangmai slaughterhouse is the only "official" one in the area, the animals killed there do not reflect the level of swine production in the valley because, in addition to a large number of imported pigs, there are many illegal slaughter-houses operating in the Valley. It therefore would seem that slaughterhouse statistics would be of very little use in helping to interpret local epidemiologic data in the Chiangmai Valley.

There was very little population change in the study villages' sample cohorts (Table 5). Only 7 people moved away and one person died between November 1970 and November 1971. Overall there was only a 2% decrease in the residents of the random sample houses over the 2 years of observation (446 to 436) and a loss of 56 people (12.6%) from the original random sample cohort. A total of 286 people in the 4 villages who were present and had been under serologic surveillance over the 2 years submitted another serum specimen in November 1971 (about 93% participation). Blood was also obtained from 125 of the Chiangmai City schoolchildren (study area E) still at school. The JEV and dengue prevalence data are presented in Table 6 for the 3 postepidemic specimens in November of 1969, 1970 and 1971. In those people not experiencing a group B infection, HI titers persisted well over the 2 years of observation and very few JEV titers reverted to negative despite the low proportion of infections that occurred in 3 of the 4 villages.

Two people each in Villages A, B and D had HI titer changes between 1970 and 1971 consistent with a JEV infection (2.8%). If more specimens had been collected during the year, it is probable that several more infections would have been found (only 60% of the 1969–1970 JEV infections would have been detectable if only those November sera had been collected). In Village C, a striking event occurred, as 28 villagers (39.4%) had a titer rise to one or more of the group B antigens employed (Table 7). For 16 of these infections, broadly cross–reactive titers to both JEV and dengue rose to $\geq$1:640, in all of which there was pre–existing antibody; i.e., typical secondary responses. Two people had obvious primary JEV infections with monospecific antibody rises to JEV antigen. There were no primary dengue responses. In 20 of the 28 infections the post–infection JEV titer was equal to or higher than the highest of the 4 dengue titers. None of these 28 people were hospitalized during the year. The overall sample titer changes are presented in Table 8.

Since dengue hemorrhagic fever (which was not diagnosed in 1970) did occur in Chiangmai in 1971, it is possible that this high infection incidence reflects both JEV and dengue infections having occurred in the same village at the same general time, but it is impossible to verify this. The most unique feature of these infections is their occurrence at about the same rate in all ages from 1–39 (Table 7). This is not typical of either JEV or dengue infections. Of course, the possibility also exists that another closely related group B virus is involved. Village D, which had a similar background prevalence of JEV and dengue, and is even closer to Chiangmai City than Village C did not experience a similar phenomenon. The schoolchildren in the City (E), however, also experienced a high infection rate (17.8%). As in Village C, the large majority of these infections were secondary and the post–infection JEV titers were mostly equal to or higher than the highest dengue titer. Although there was no clear primary JEV infection, there was one monospecific primary dengue 3 titer rise.

The first rains of 1971 began in March and, as observed during 1970, were followed by a steady rise in population densities of the three vector species at Village A and Chiangmai City (see Page 15). There was an unexplained decline in the C. fuscocephala and C. tritaeniorhynchus populations in village B during April, but densities of both populations at that site rose in the following months. Peak densities for C. fuscocephala and C. tritaeniorhynchus were measured during June in Village A and Chiangmai City, while in Village B the peak for these two species occurred in July. As in 1970 the fluctuations in populations of C. fuscocephala and C. tritaeniorhynchus during 1971 were closely parallel. The patterns observed for C. gelidus populations in Villages A and B during 1971 were almost identical to those seen in 1970. In Village A the numbers of C. gelidus rose in May to reach a peak in July and declined rapidly thereafter, while in village B the C. gelidus population increased during May and June and remained elevated through

September. The numbers of all three species dropped off sharply in the dry season (November—March), but the most pronounced decline occurred in the C. gelidus population. Breeding continued during the dry season, although at greatly reduced levels; newly emerged, gravid and old females were collected throughout this period.

From April through July mosquitoes collected in CDC light—traps and resting outdoors during daylight hours were compared with respect to sex—ratio and the physiologic age and insemination rates in females. Resting mosquitoes were collected from vegetation in the study sites by means of a battery—powered vacuum collector. Males and females were present in almost equal proportions in the samples from the resting populations, while the light—trap collections consisted almost entirely of females. Since the light—traps were set near animal shelters the higher proportions of females in these collections was not surprising. Several other important differences were observed in the mosquitoes obtained by the two collection methods (Tables 9 & 10). There was a larger proportion of newly emerged females in the resting population, as indicated by the higher nulliparous rates and lower insemination rates (Table 9). On the other hand, a higher proportion of gravid females was also present in the resting population (Table 10). Between July 1971 and March 1972 ovaries of vector species were examined in an effort to determine if seasonal variations in the number of gonotrophic cycles could be detected (Table 11). For most mosquito species, the number of gonotrophic cycles a female mosquito has undergone is directly related to the number of blood meals she has taken. Ovarian dissections are thus useful in estimating the proportion of older females (i.e., theoretically, those which have had greatest opportunity to become infected) present in the population. A decline in the proportion of parous females was observed for all three species between January and March. The proportions of multiparous C. fuscocephala and C. tritaeniorhynchus were higher during July—the month of highest incidence of encephalitis cases—than during the next four months. The largest proportions of multiparous C. gelidus were observed in September and in December. This is of special interest because in 1970 the only isolations of JEV obtained from C. gelidus were made during September, although greater numbers had been tested during previous months.

During July and August, flight range and dispersal experiments were conducted with the three vector species. Blood—fed females were collected from buffalo, cattle, and pigs, marked with Helecon luminescent pigments and released. A total of 8,831 mosquitoes, of which approximately 53% were C. fuscocephala, 20% C. tritaeniorhynchus 9% C. gelidus, and 18% other species, was released. Four marked C. fuscocephala and two C. tritaeniorhynchus were recaptured. Five of the recaptured were made within three days of the time of release at distances varying from 10 to 500 meters from the release point. One C. fuscocephala was recaptured nine days after release but only 50 meters from the release site.

Table 1.
Monthly Rainfall and Encephalitis
Admissions from Chiangmai Valley
in 1970 and 1971

Month	Rainfall (cm.)		No. Cases	
	1970	1971	1970	1971
Jan	0.0	0.0	0	0
Feb	0.2	0.0	0	1
Mar	9.2	1.7	0	2
Apr	7.0	3.3	1*	1
May	35.2	24.0	23	16
Jun	24.5	17.3	38	14
Jul	17.9	30.0	22	47
Aug	34.9	32.5	3	12
Sep	19.3	19.5	5	4
Oct	3.8	13.4	4	1
Nov	0.7	2.4	3	1
Dec	3.5	1.2	2	0
TOTAL	156.2	145.3	101	99

* Case not included elsewhere as valley address not verified.

Table 2.
Incidence Rates of Encephalitis Cases in Chiangmai Valley, by District, for 1970 and 1971

District	Estimated Population	No. Cases		Incidence Rate per 100,000	
		1970	1971	1970	1971
Muang	120,000	12	17	10.0	14.2
Maerim	25,000	5	7	20.0	28.0
Sansai	45,000	8	7	17.8	15.6
Doi Saket	40,000	9	5	22.5	12.5
Sankamphaeng	50,000	7	7	14.0	14.0
Saraphi	60,000	7	8	11.7	13.3
Hang Dong	40,000	8	13	20.0	32.5
Sanpatong	80,000	10	11	12.5	13.8
Jomthong	20,000	7	7	35.0	35.0
Muang*	125,000	18	9	14.4	7.2
Pasang*	75,000	9	8	12.0	10.7
TOTAL	680,000	100	99	14.7	14.6

* Lamphun Province.

Table 3.

Age and Sex Distributions of 1970 and 1971 Encephalitis Admissions and Fatalities

Age	Male				Female				Total					
	No. Cases		No. Died		No. Cases		No. Died		No. Cases		No. Died		CFR*	
	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971	1970	1971
0–4	9	8	1	3	4	6	1	4	13	14	2	7	15.4	50.0
5–9	18	24	4	4	12	14	3	2	30	38	7	6	23.3	15.8
10–14	16	17	4	0	11	14	3	0	27	31	7	0	25.9	0.0
15–19	9	4	0	2	5	2	0	0	14	6	0	2	0.0	33.3
20–29	9	2	1	0	1	3	1	0	10	5	2	0	20.0	0.0
30–	3	2	1	1	3	3	1	1	6	5	2	2	33.3	40.0
TOTAL	64	57	11	10	36	42	9	7	100	99	20	17	20.0	17.2
Median	11.6	9.3	10.6	7.5	10.9	10.4	10.8	4.4	11.3	9.7	10.7	6.2	—	—

* Case Fatality Rate (%)

Table 4.

JEV Infections in Sentinel Pigs, January — June 1971

Dates Between Bleedings	No. Pigs at Risk	JEV Infections		JEV Infections by Study Area %			
		No.	%	A	B	C	D
1—26 Jan	18	0	0.0	0.0	0.0	0.0	0.0
27 Jan — 8 Mar	28	0	0.0	0.0	0.0	0.0	0.0
9—31 Mar	18	2	11.1	0.0	33.3	0.0	0.0
1—26 Apr	13	3	23.1	0.0	66.7	0.0	20.0
27 Apr — 26 May	22	8	36.4	0.0	40.0	16.7	83.3
27 May — 29 Jun	15	14	93.3	100.0	66.7	100.0	100.0
TOTAL	114	27	23.7	21.4	28.6	21.4	23.3
				N=28	N=28	N=28	N=30

Table 5.

Population Changes in Random Sample Households of Chiangmai Valley Study Villages, Nov 69 — Nov 71

Village	No. in Sample Houses		Pop. Change (%)	Losses		Additions	
	Nov 69	Nov 71		Moved Away	Died	Moved In	Born
A	89	93	+4.5	6	0	6	4
B	113	108	−4.4	18	0	8	5
C	112	117	+4.5	8	0	12	1
D	132	118	−10.6	22	2	5	5
TOTAL	446	436	−2.2	54	2	31	15

Table 6.

Changes in Prevalence of JEV and Dengue HAI Antibodies*
Over Two Years (Nov 69 – Nov 71)

Study Area	No. People Followed**	JEV Prevalence (%)			Dengue Prevalence (%)			% Converting From Negative to Positive Nov 70 – Nov 71		% Reverting From Positive to Negative Nov 70 – Nov 71	
		Nov 69	Nov 70	Nov 71	Nov 69	Nov 70	Nov 71	JEV	DEN	JEV	DEN
A	69	71	74	71	19	19	17	0.0	4.3	2.9	5.8
B	63	38	32	35	19	16	16	4.8	3.2	1.6	3.2
C	71	68	72	90	77	75	83	18.3	9.8	0.0	1.4
D	83	69	65	63	61	51	53	4.8	3.6	4.8	1.2
A–D	286	62	62	65	46	41	44	7.0	4.9	2.4	2.8
E	125	78	77	85	94	96	98	10.4	3.2	2.4	1.6

* Titer ≥ 1:20.
** Ages 1–39 for B,C,D; everyone over age 1 for A; ages 6–8 for school E.

Table 7.

Incidence of Group B Infections in Study Village (C) Between Nov 70 — Nov 71

Age	Male			Female			Total			People Lacking JEV and Dengue Antibody in Nov 70	
	No. People	Titer Rises*		No. People	Titer Rises		No. People	Titer Rises			
		No.	%		No.	%		No.	%	No.	No. Infections
1—9	16	5	31.2	13	5	38.5	29	10	34.5	9	5
10—19	12	6	50.0	8	4	50.0	20	10	50.0	2	1
20—39	11	4	36.4	11	4	36.4	22	8	36.4	1	1
TOTAL	39	15	38.4	32	13	40.6	71	28	39.4	12	7

* 4—Fold rise in JEV and/or dengue antibody titer between Nov 70 and Nov 71.

Table 8.

Geometric Mean Titers of JEV and Dengue HAI Antibodies, by age, for Village (C)

Age	Geometric Mean Titer*					
	JEV			Dengue**		
	1969	1970	1972	1969	1970	1971
1—9	15	19	53	30	27	56
10—19	59	40	197	102	51	190
20—39	363	146	387	291	110	310
TOTAL	60	44	142	86	50	134

* Negative sera (1:10) called 1:5.
** Highest dengue 1—4 titer used.

Table 9.

Proportions of nulliparous and inseminated females in resting and light—trap collections, Chiangmai, April – July 1971

Species	Resting			Light—trap		
	Total No. Dissected	Nulliparous	Inseminated	Total No. Dissected	Nulliparous	Inseminated
C. fuscocephala	761	.555	.652	1347	.366	.939
C. gelidus	140	.717	.384	714	.432	.947
C. tritaeniorynchus	422	.623	.551	1275	.328	.963

Table 10.

Proportions of gravid females in resting and light—trap collections, Chiangmai, April – July 1971.

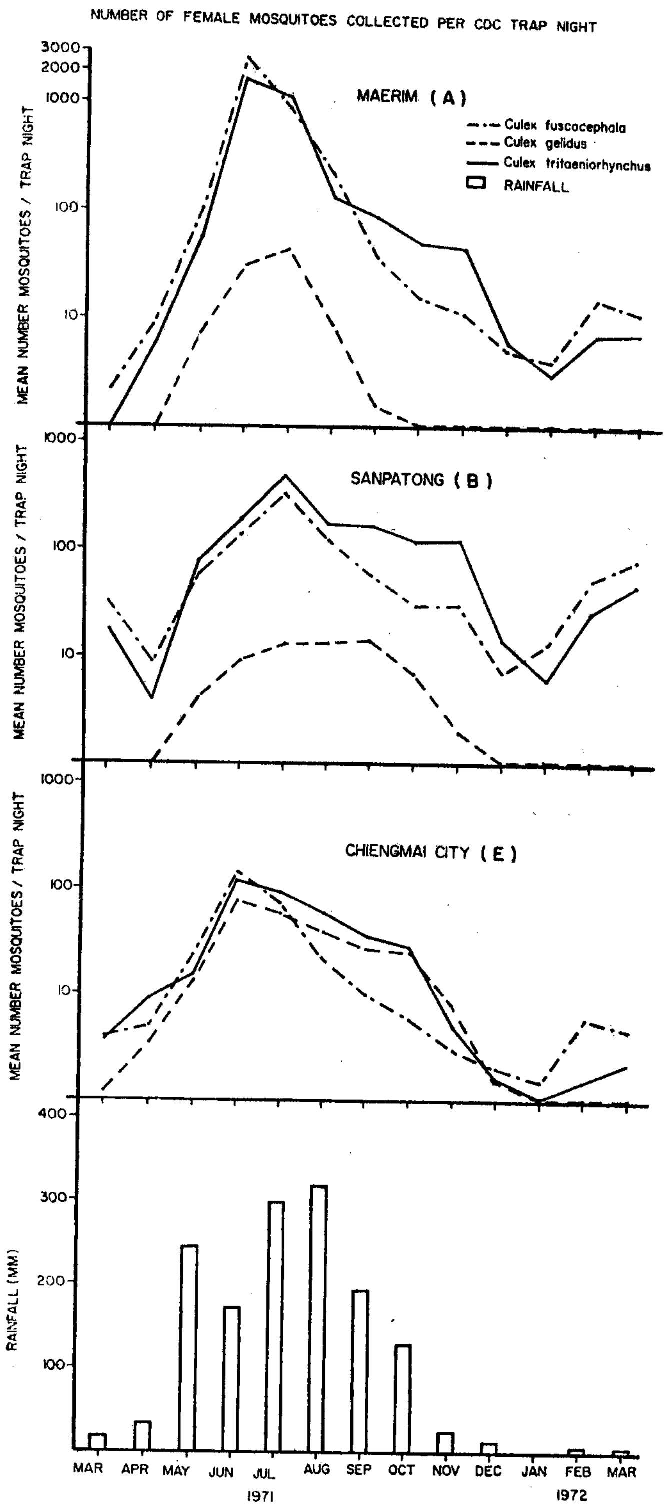
Species	Resting		Light—trap	
	Number Examined	Gravid	Number Examined	Gravid
C. fuscocephala	761	.364	16728	.083
C. gelidus	140	.244	2206	.124
C. tritaeniorhynchus	422	.176	18672	.037

Table 11.

Proportions of parous, uniparous and multiparous Culex fuscocephala, gelidus and tritaeniorhynchus from light trap collections Chiangmai, July 1971 — March 1972

Month	Total No. Dissected	Total Parous	Proportions uni—and multiparous: Recent Oviposition*	Uniparous	Multiparous
C. fuscocephala					
July	379	.612	.017	.927	.056
August	0	—	—	—	—
September	129	.566	.068	.918	.014
October	127	.512	.138	.862	.000
November	241	.518	.114	.846	.040
December	495	.612	.076	.875	.049
January	842	.568	.077	.868	.055
February	1419	.428	.118	.837	.045
March	1048	.429	.077	.873	.050
C. gelidus					
July	323	.502	.012	.907	.081
August	613	.574	.134	.847	.019
September	977	.481	.134	.743	.123
October	715	.508	.339	.634	.027
November	266	.436	.371	.586	.043
December	39	.487	.000	.895	.105
January	29	.483	.071	.929	.000
February	19	.315	.000	1.000	.000
March	24	.291	.142	.857	.001
C. tritaeniorhynchus					
July	553	.653	.008	.898	.094
August	1909	.754	.134	.808	.058
September	2889	.577	.100	.854	.046
October	3327	.619	.129	.818	.053
November	2238	.613	.134	.829	.037
December	1304	.696	.095	.798	.107
January	426	.617	.091	.852	.057
February	805	.477	.101	.854	.045
March	784	.429	.112	.857	.031

* Females in which the number of gonotrophic cycles was not determined because ovarian tubules were stretched by recent oviposition.


NUMBER OF FEMALE MOSQUITOES COLLECTED PER CDC TRAP NIGHT
MAERIM (A)
Culex fuscocephala
Culex gelidus
Culex tritaeniorhynchus
RAINFALL
SANPATONG (B)
CHIENGMAI CITY (E)
MEAN NUMBER MOSQUITOES / TRAP NIGHT
RAINFALL (MM)
3000
2000
1000
100
10
400
300
200
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
1971
1972