The Ecology of Japanese Encephalitis Virus Infections in Chiengmai

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OBJECTIVE: To investigate the ecology of Japanese encephalitis virus (JEV) in the Chiengmai Valley, Northern Thailand, with particular reference to aspects contributory to infection in humans. Specific areas of interest and their objectives include the following:

1) Epidemiology: Assess the epidemiologic variables of human apparent and inapparent JEV infection; define human risk factors; determine animal reservoirs of JE virus; ascertain environmental variables of possible importance; study epidemiological interrelationships of multiple co-existing group B arboviruses in a discrete area; assess potential for JEV control measures.

2) Entomology: Determine the identity of mosquitoes involved in the transmission of JE virus and measure the seasonal density, host preferences, insecticide susceptibility status and flight dispersal characteristics of suspected vector species.

3) Neuropsychiatry: Define clinical patterns of acute neuropsychiatric defects and complications; follow mental status changes, EEG changes and neurological deficits in convalescent patients, assessing nature, duration and progression of such abnormalities; attempt to correlate antibody titer levels over time with nature of CNS changes in convalescent patients; assess the influence of home environment and school or job environment on the behavior of convalescent patients; determine if any JE patients present with acute psychosis at the major psychiatric hospital in Chiengmai.

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4) Virus Laboratory: Provide the virologic support for all of the above efforts including virus isolation, virus identification, and serology. Improve current virus isolation and identification systems; search for more specific serologic tests in group B arbovirus infections; characterize JEV isolates; attempt to improve current sentinel animal systems; examine role of small vertebrates in the ecology of JEV; perform mosquito transmission studies.

DESCRIPTION: The background and initial methods used were described in last year's report. To briefly summarize, a large epidemic of JE had occurred in the Chiengmai area in 1969. JE virus was isolated from a human brain, several suspect animal reservoirs had ample serologic evidence of previous JEV infection and two *Culex* species, known to be JEV vectors elsewhere, were found to be abundant. After the epidemic had ended in November, 1969, one urban school in Chiengmai City and 4 scattered villages in the Chiengmai Valley were selected as field study sites. Appropriate samples of people were selected at each site for intensive prospective surveillance. The village sampling procedure is shown in Table 1. In each village, the random samples proved to be quite representative of the respective village populations. The age distributions of these villages were all very similar (Figure 1).

The post-season (November, 1969) bleed of the cohorts in the 5 areas showed differences in group B arbovirus serological patterns between the areas, but the patterns could not be reliably interpreted from the HI test alone due to the large amount of real or cross-reactive dengue antibody present. Nevertheless, in each area the group B prevalence rose with age, the patterns being consistent with the belief that JEV, dengue (and possibly other group B agents) were indeed endemic in the Chiengmai Valley. Mosquito collections for virus isolation attempts were begun in October, 1969. No viruses were isolated from 1042 mosquito pools collected during the dry season from November, 1969, to March, 1970. Mosquito collections made between October, 1969, and February, 1970, indicated that mosquito breeding occurred during the winter season at a very slow rate. Males, nulliparous, and parous females and larvae of *Culex* species were collected during this period.

Over 80% of cattle, buffalo, dog and pig sera collected in November, 1969, were positive for JEV (HI) antibody. Starting in March, 1970, JEV antibody-free pigs (aged 3 months) were placed as sentinel hosts in each of the four study villages. They were bled monthly, and JEV serologic (HI) converters were promptly replaced. In addition, we twice attempted to use chickens as sentinel hosts, but on neither occasion did they show serological evidence of infection. We obtained blood samples from the human cohorts and available domestic indigenous animals three times in 1970: March-April (before the rains), July-August (during the rains) and November 1970 (after the rains). In addition, various wild animals, especially the house sparrow, *Passer montanus*, were caught and bled for serological and virological evidence of JEV infection and a possible role in the JE ecologic cycle.

The neuropsychiatric and clinical studies were established at the two major Chiengmai Hospitals (Suan Dork and McCormick), the Chiengmai psychiatric hospital (Suan Prung), and two adjacent provincial hospitals (Lamphun and Lampang). With this broad case detection network we believe that very few JE cases from Chiengmai Valley reaching a medical facility were missed. The accessible families of all cases from the Valley were visited and paired sera were collected. Confirmed JE cases are being followed monthly for one year after discharge with repeated physical examinations, (including E.E.G., mental status exam, and psychometric testing) and by interviews with teachers or employers of the patients.

PROGRESS: An encephalitis outbreak occurred again in 1970. Nearly 200 cases from Chiengmai, Chiengrai, Lamphun and Lampang provinces arrived at the 5 study hospitals. For most of the cases, JE was serologically confirmed. In addition, a US Peace Corps volunteer living in Phrae province, had serologically confirmed JE. There is thus good evidence for JEV endemicity in most of the major valley areas of Northern Thailand. Since the main purpose of this study was to define the ecology of JE in a relatively discrete area, the remainder of this report will present only results referable to the Chiengmai Valley which includes the two major cities, and parts of their provinces, Chiengmai and Lamphun.

The first rains of 1970 began in March and were followed by a steady increase in the population densities of suspected vector species of mosquitoes in the four village sites (Figure 2). Peaks in population densities of these species, as measured by weekly CDC light–trap collections, occurred between May and August, varying according to the village sampled. In two sites, Sanpatong and Saraphi, the population densities of the vector species remained at high levels for five months or longer, while in Sankamphaeng and Maerim population densities rose to a maximum in June and July and dropped off sharply thereafter. Biting collections from buffaloes, cattle and pigs indicated that both C. fuscocephala and C. tritaeniorhynchus fed actively all night long, but greatest biting activity by these two species occurred between 0100 and 0400 hours. Both biting collections and the results of precipitin tests of gut contents of engorged mosquitoes from light–traps and from vacuum sweep collections indicated that C. fuscocephala, C. gelidus and C. tritaeniorhynchus fed by preference on buffaloes and/or cattle and on pigs—in that order (Tables 2,3,4). All three of these species showed a reluctance to enter houses. Between 1 April, 1970, and 31 March 1971, a total of 391,108 mosquitoes were collected and processed for virus isolation attempts. These included 140,445 C. fuscocephala, 11,480 C. gelidus and 175,280 C. tritaeniorhynchus. A total of 13 JEV isolations were obtained from these mosquitoes (Table 5). The first isolate was recovered from a pool of C. tritaeniorhynchus collected in Saraphi on 27 April (Figure 3). During May, a total of 8 JEV strains were isolated—6 from C. tritaeniorhynchus pools and 2 from C. fuscocephala. From June through August, only 1 JEV mosquito isolate was obtained (ex. C. tritaeniorhynchus), although greater numbers of mosquitoes were tested during that period than during previous or succeeding months. During September, 3 strains of JEV were obtained from pools of C. gelidus collected from three separate sites. No JEV isolations were made from C. gelidus collected prior to September, although greater numbers of that mosquito had been collected and tested in previous months. Insecticide susceptibility tests made with C. fuscocephala, C. gelidus and C. tritaeniorhynchus from Chiengmai Valley according to standard WHO methods indicated that all three species were resistant to DDT but susceptible to malathion (Table 6).

Sentinel pigs placed in the four village sites in late March were bled at the end of April, and all were negative. However, between 27 April and the end of May, 30% of the sentinels in 3 of the 4 villages had developed antibodies to JEV (Table 7). The sentinel pig conversions paralleled the mosquito population indices, with a peak of 66% conversions in July, decreasing to 20% in December (Figure 3). In at least two of the four study villages sentinel pigs converted to positive during each of the eight successive months (May–Dec). It is estimated that the approximate date when 50% of the sentinel pigs were JEV positive was the second week of June, using data on either all sentinels or only those originally placed in March (Table 7). No conversions occurred in January or February, 1971. The sentinel chicken effort was negative, for none of 100 chickens set out in villages during July and September converted to JEV or had a detectable JE viremia. Over 1100 Passer montanus sera were collected in study villages from April, 1970, to March, 1971. Of 70 Passer sera tested so far by PRNT, four sera from birds collected in July showed significant plaque reduction at a dilution of 1:19. Serological and virological results on most of the sera from small wild vertebrates is pending.

No encephalitis cases were admitted to any of the hospitals in the area between February and April. The first case (serologically confirmed) was admitted on 19 April, and several more cases appeared on 11 May. From 11 May to the end of 1970, 100 Chiengmai Valley residents were admitted with the diagnosis of encephalitis (Figure 3). The number of cases admitted per month was as follows: May (23), June (39), July (22), August (3), September (5), October (4), November (2) and December (2). The median week of admissions was the fourth week of June, about two weeks after 50% of the pigs are estimated to have developed JEV antibody. Although 4 of the first 5 cases in May came from one district (where 3 of the first 5 JEV mosquito isolates were obtained), there was no other evidence of spatial clustering of cases. The 100 cases came from each of the 11 districts and from 59 of the 113 subdistricts of the valley (Figure 4). Almost 2/3 of the cases were male, which was the sex ratio observed in 1969 and is the usual Japanese experience (Table 8). The overall case fatality rate was 19%, but females had an appreciably larger fatality rate (25%) than the males (15%). The median age (about 10 years) was strikingly similar for males, females, those that died and those with serologically confirmed JEV infections (Table 9). Ninety—three of the 100 cases had paired sera submitted and 70 (75.3%) were serologically confirmed by the HI test as JE. A small number of patients had confirmatory CF and PRNT tests performed. Convalescent patients are still under neuropsychiatric and serological surveillance and therefore followup data on these patients are not available at this time.

Serologic analysis of HI results on the 70 confirmed human cases revealed that 45 (64.3%) were definite or probable primary JEV infections while 21 (30.0%) gave cross—reactive, high—titer antibody response to dengue serotypes and are presumably recent JEV infections in individuals with previous group B infections (Table 9). The two confirmed cases over age 30 were females, age 39 (primary infection) and 47 (secondary infection). The criteria used for designating primary and secondary HI responses were similar to those used in dengue infections. In primary responses, the acute sera were negative for dengue antibody and the convalescent sera showed a fairly monospecific rise in JE titer. (The CF response was likewise monospecific.) In secondary HI responses, the convalescent sera showed a significant (≥4—fold) rise in JE titer to high levels (≥ 1:640) with marked cross—reactivity of several or all 4 dengue serotypes. Only 4 patients showed significant rises of JE titers between acute and convalescent sera which could not be classified as either primary or secondary infections by these criteria. These have been provisionally designated as intermediate responses. The median age was slightly lower for primary (9.2) versus secondary (12.5) responders. There was no difference in the serological patterns of males and females. Attempts are underway in the laboratory to identify the previous Group B arbovirus infections in patients with JE. Two or more biweekly serum specimens were collected from family members of 55 encephalitis cases. Of the 230 family members donating sera, 5 (2.2%) had significant JEV antibody rises by the HI and CF tests while 3 more people had rises only in the HI test. The total of 8 individuals (3.5%) thus represents the maximum estimate of concurrent familial inapparent JEV infection, when starting from index cases.

The village serum samples included over 90% of the cohorts randomly selected in November, 1969, for the 3 serum collections in 1970 (Tale 10). There was little overall human population change in these rural villages. There was a net decrease of only 11 people in the study households (445 to 434) between November 1969—November 1970, although there was an 11% decrease in the cohort population (445 to 397) over the year due to moving elsewhere plus one death (Table 11). There was a much larger turnover in the domestic animal populations of the four study villages, especially of the pigs (Table 12). The overall numbers of pigs were similar in November 1969 and November 1970, but virtually every non—breeder was eaten, sold or died, and was replaced by young pigs bought, or bred locally. This uninterrupted breeding pattern continued throughout the year. The bovine population was similarly stable, with a surprisingly high turnover rate of nearly 40%. That pigs, bovines, and other domestic animals are very abundant around most houses in these villages is shown in Table 13.

After the November, 1970, sera were collected, the study area samples were run simultaneously by the HI test, starting at a 1:10 serum dilution against 4—8 units of HA antigen. The November, 1969, sera had previously been run starting at a 1:20 dilution and those results were reported in last year's annual report. In village (A), blood was collected from cohort persons over age one, while in the other three villages only those between ages 1—39 were bled. The November, 1969, specimens have been re—analyzed, with the following results: Villages (A) and (B) had very little evidence of previous dengue exposure as all but a few (3%) positives (HI titer ≥ 1:20) had monospecific titers to JEV. For those few persons with a positive dengue titer, none had dengue titers higher than the JEV titers. The prevalence rose with age in both villages and the overall JEV antibody prevalence (ages 1—39 only) was 72.0% for (A) and 40.7% for (B) (Table 14). The geometric mean titers (GMT) also generally rose with age in both sexes (Table 15). Figures 5 and 6 show these age prevalence and GMT values for the total sample in each village.

Both the JEV prevalence and GMT are appreciably higher in (A) than in (B) up to age 30. Since 85% of the JEV infections occur in those under age 20 and since both villages show good evidence of endemic JEV transmission, it can be assumed that the yearly force of infection has been greater over the years for village (A) than for village (B). The data from 1969—70 would support this, as the incidence rate of JEV infections was almost three times higher in (A) than in (B) (though numbers are admittedly small) (See Table 22). The serological patterns in villages (C) and (D) and in the urban school (E) are still confusing due to the presence in most positive sera (including the 1–4 year–old age group) of elevated and broadly reactive antibody to both dengue (one or more serotypes) and JEV. The overall JEV antibody prevalence, ignoring the dengue titers, was 72.6% in (C) and 70.3% in (D). By considering only those JEV titers that were equal to, or greater than, the highest of the dengue titers, we can reduce the overall JEV prevalence to 54.8% in (C) and 52.7% in (D) (Table 16). Using these modified figures, the overall JEV prevalences in (B), (C) and (D) then become quite comparable and actually the 95% confidence limits of the overall JEV antibody prevalence in all four study villages then overlap (See Table 18). Thus, the modified prevalence figures for villages (C) and (D) may be closer to the true background prevalence of JEV. The overall village JEV prevalences are shown together in Table 17, and the modified overall prevalences in Table 18. No strong correlation was found in any village between the proportion of the family members positive for JEV in each house and the number and types of domestic animals present around the house.

Analysis of the 163 (6–8 yr. old) school children's sera obtained in March, 1970 (pre–season), revealed 84% positive for JEV, but 91% were positive for dengue (Table 19); 9% were positive for dengue alone (monospecific) and only 1.2% were positive for JEV alone. Interpretation of these results is difficult. Using the modified criteria of villages (C) and (D), the JEV antibody prevalence is reduced to 53% which is still much higher than any of the villages for 5–9 year olds (Table 20). Also, unlike any village, the overall GMT (calling negatives 1:5) was higher for dengue (mean of 114) than for JE (mean of 94.5) (Table 19). The highest titer of the four dengue serotypes was used in calculating the dengue GMT. Further attempts at clarification of these serological results are underway in the laboratory.

If JEV has indeed been fairly uniformly disseminated throughout the Valley (including "urban" Chiengmai) over the past few years, then these group B prevalence variations may mostly be reflecting the differences in degree of exposure to dengue viruses. Dengue hemorrhagic fever has been encountered for several years (before 1970) in "urban" Chiengmai children such as our schoolchildren. Moreover, villages (C) and (D) are both closer to and have more frequent social contacts with Chiengmai City, than villages (A) and (B). The Aedes aegypti house index data in the five areas add further evidence for this contention. The index was highest in Chiengmai City, intermediate in villages (C) and (D) and low (almost zero) in villages (A) and (B).

Over the 12 months of observation, inapparent JEV infections were serologically detected in 5 to 8 family members of JE cases, 7 to 22 villagers and 6 to 8 schoolchildren in Chiengmai (Table 21). As stated, these ranges reflect the minimum (CF confirmed) and maximum (CF unconfirmed) numbers of inapparent infections that can be serologically diagnosed at present. Four of the 22 village infections occurred between November, 1969, and April, 1970. Since the time of infection cannot be pinpointed closer we cannot determine whether active JE transmission (to humans) occurred before the first rain fell in late March. Most (over 60%) of the village and school infections occurred between April and July, 1970, during which time 85% of the encephalitis cases were admitted. The inapparent infection rates ranged from 2.4% in village (B) to 11.0% in village (C) (Table 22). These differences, although suggestive, are not statistically significant due to the small number of infections in these villages. The sex ratio was quite similar for each group of inapparent infections, using either the minimum or maximum numbers. In fact, the sex ratio for inapparent infections was similar to the ratio for the clinical cases, where over 60% were males. The constancy of this ratio suggests that, despite an equal sex ratio in the overall JEV antibody prevalence in each village in 1970, the males were more at risk for both apparent as well as inapparent JEV infection. The age range and median age of JEV infections were also similar in the villagers, in the family members, and in the clinical cases of JE. These similarities between the three different types of human populations provides further evidence of the representativeness, validity, and presumed accuracy of the sampling procedures and serological results.

A basic requirement for interpreting serologic cross–sectional prevalence data in terms of past exposure to an agent is that antibody titers to that agent remain positive for an extended period. Our one–year collections provide good evidence that the HI test can probably be used for such interpretation in Chiengmai Valley (Table 23). Overall prevalence was virtually unchanged in each village and only 10% of the people had a 4—fold drop in titer, despite the fact that only 6.5% had an inapparent JEV infection during the 12 months. Even in village (B), with the lowest overall JEV prevalence and the lowest infection rate (2.4%), neither the prevalence nor the overall GMT changed appreciably in one year. Only rarely did a November, 1969, JEV titer of 1:20 or 1:40 become negative over the year, and no titer $\geq$1:80 in November, 1969, became negative. We plan to obtain a 2 year followup collection in November, 1971.

Incidence rates per 10,000 population were calculated for both apparent (JE cases) and inapparent infections and estimates obtained for inapparent to apparent (I/A) JE ratios. The case incidence (100 cases) in Chiengmai Valley was 1.5/10,000 using an estimated total population at risk of 680,000 (Table 24). When the five cases admitted from the subdistricts of the four study villages (total population 27,000) are used for the same calculation, a similar case incidence rate of 1.8/10,000 is obtained (Table 25). This provides further evidence of the representativeness of the study areas and further justifies extrapolation of study area results to the entire valley area.

The incidence rates of inapparent infections (HI results) of the villagers, schoolchildren and case families were likewise similar. They were in the range of 350—650 infections/10,000 population. These results suggest that: 1) family members of JE cases are probably at no greater risk of acquiring JEV infection than the general Valley population; 2) children in the urban Chiengmai environment are being exposed to JEV infection similarly to those living in more rural areas; and 3) there was widespread and uniform exposure, presumably to infected vectors, throughout urban and rural areas of the Valley, with a random rather than cluster distribution of factors leading to human infection.

The I/A ratios were 232 to 1 for case families, 335 to 1 for schoolchildren and 431 to 1 for villagers (Table 26). These values are consistent with various previously reported estimates from other JEV areas in Asia and the S.W. Pacific. Since the sex ratios were approximately 60:40 male to female for both apparent and inapparent infections, these I/A estimates are likewise the same for both sexes. It should be understood that the family members of cases is a highly artificial and biased sample, having been identified by the index cases and followed only for a 2—4 week period. This sample is thus not strictly comparable to the schoolchildren and village cohorts in the above estimates and the true incidence rate and I/A ratio is probably better estimated from these prospectively identified cohorts.

There is some evidence that those villagers and schoolchildren acquiring JEV infection might have had a mild illness as a consequence. Only 16% of both the villagers and the schoolchildren had a history of FUO or URI, as documented by the nurses on their weekly visits between April and November, 1970. However, 25% of the villagers and 50% of the schoolchildren developing JE infection had such a medical history during the 4—month period between bleedings in which the infection occurred. Since the time of infection and illness cannot be better dated than within a 3—4 months period, these results remain merely suggestive.

An important question is whether the presence of pre—existing Group B antibody protects against, or modifies the course of subsequent JEV infection and/or illness. The following two points argue against the presence of serum Group B arbovirus antibody alone being important: (1) About 1/3 (21 out of 64) of the confirmed cases under age 40 had a typical secondary Group B antibody response; 8 (or 42%) of the 19 villagers under age 40 developing inapparent infections had pre—existing Group B (HI) antibody. In addition, the same percentage (about 90%) of both the primary and secondary responses occurred in those under age 20 for both cases and inapparent village infections. (2) Based on the village antibody prevalence data obtained before the start of the 1970 outbreak, it can be presumed that at least 50% of the entire Valley population, or about 340,000 people, had Group B serum antibody; yet an outbreak occurred with at least and an estimated 23,000 to 43,000 inapparent infections. Assuming the presence of preexisting JEV, dengue 100 cases or other Group B antibody in about 40% of those with inapparent infection, about 9,000 to 17,000 inapparent JEV infections occurred in people having had previous Group B infection. Obviously there must be some host factor, other than previous Group B experience, that accounts for the majority of infections (inapparent and apparent) occurring in the young, since our data strongly suggest a fairly uniform exposure of the 1—39 years age groups to infection.

FIGURE I.

Cumulative Percentage Age Distributions

Chiengmai Valley Study Villages – November 1969

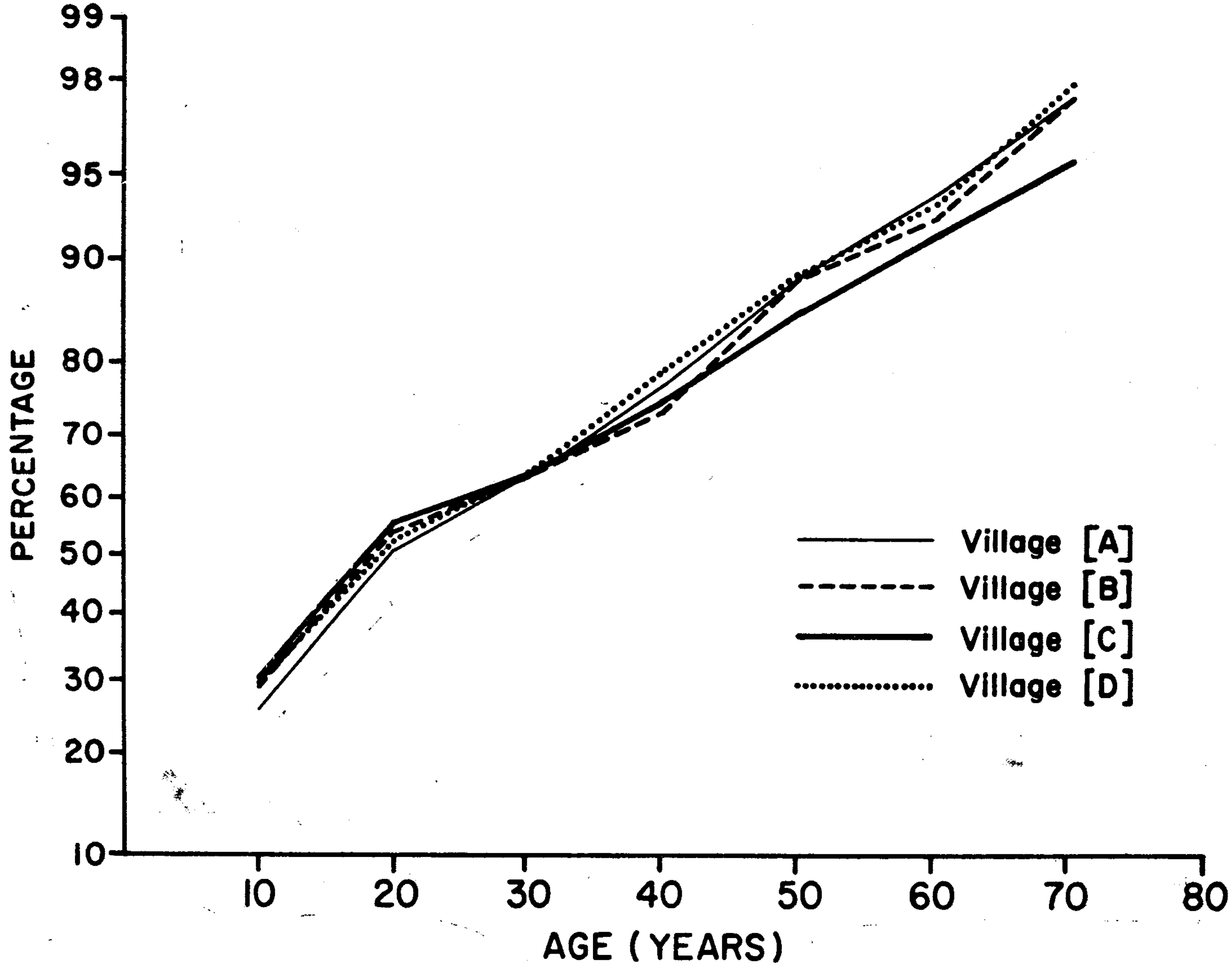

FIGURE 2.
NUMBER FEMALE MOSQUITOES COLLECTED PER CDC TRAP-NIGHT

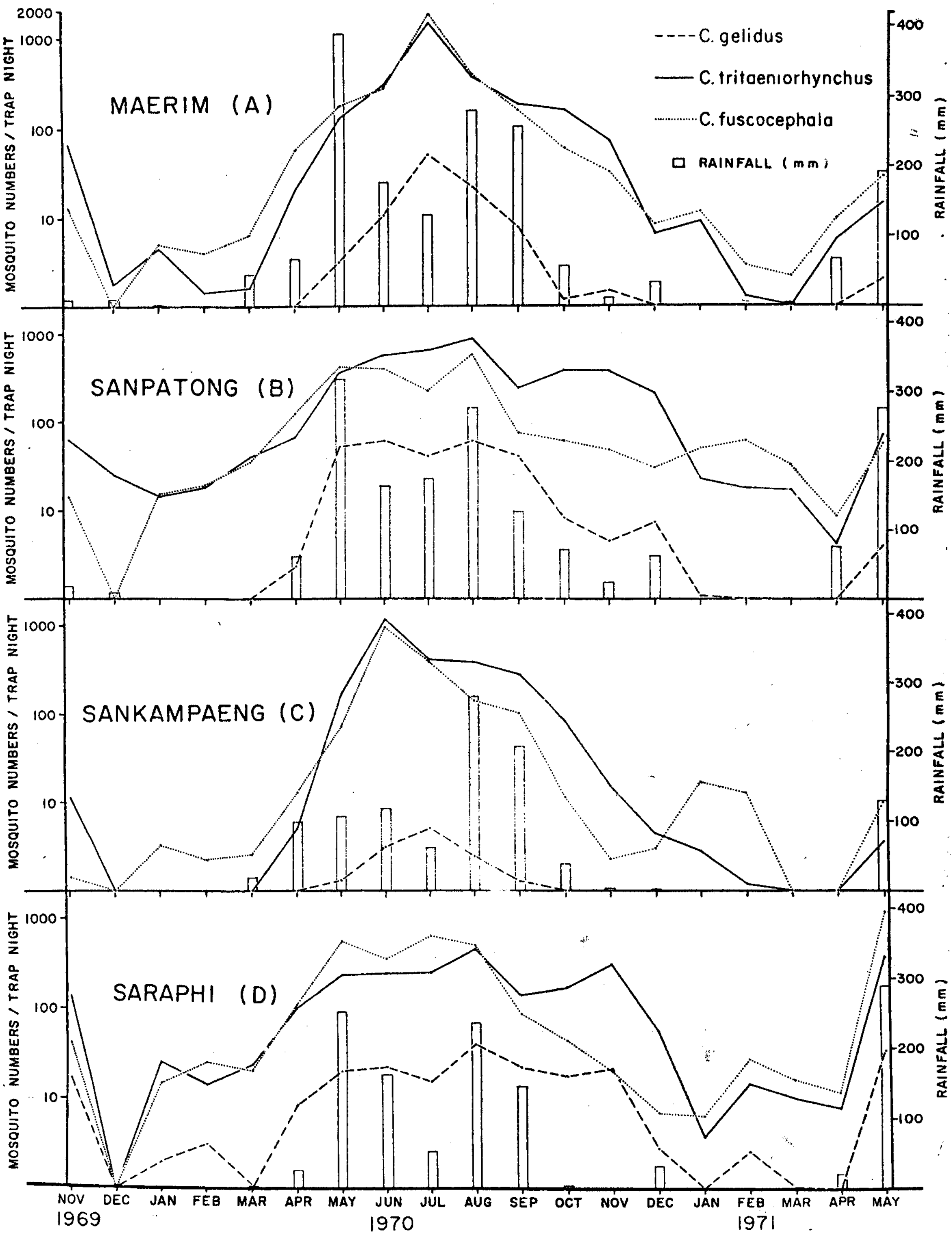

FIGURE 3.

JE Virus Infection in Man, Sentinel Pigs and Mosquitoes in Chiengmai Valley, 1970

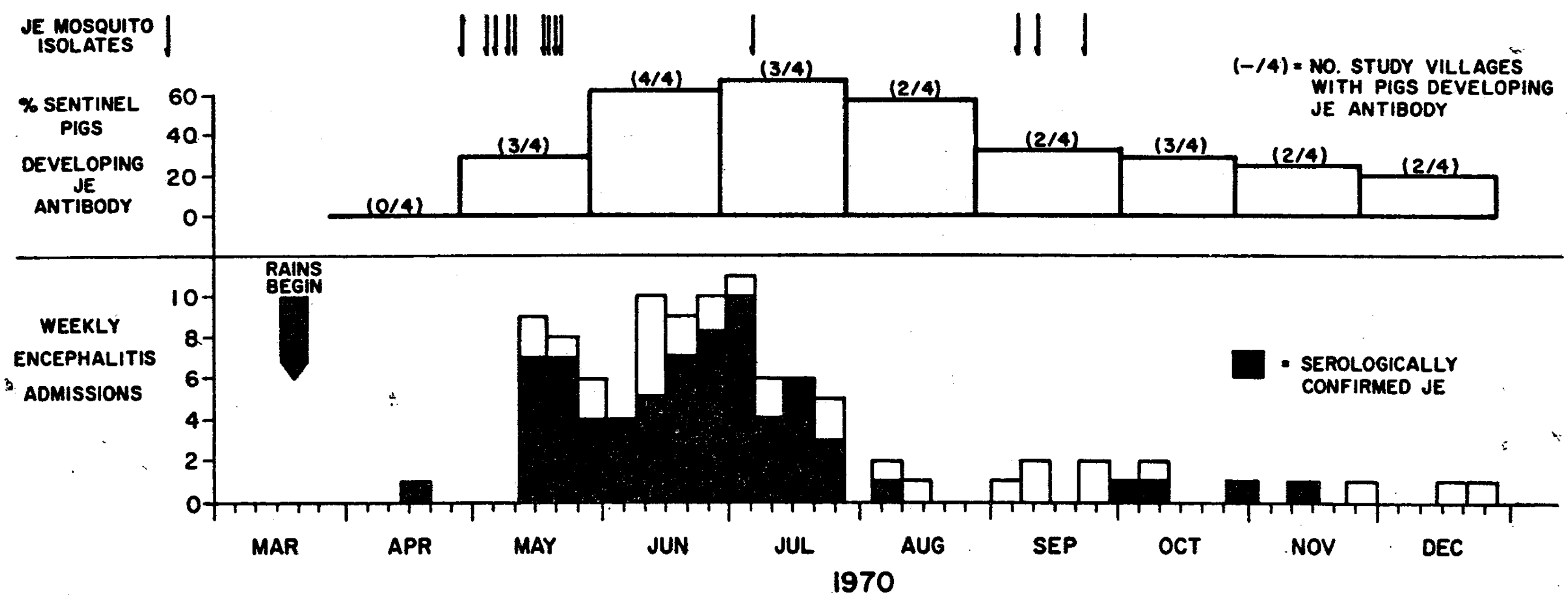

FIGURE 4.

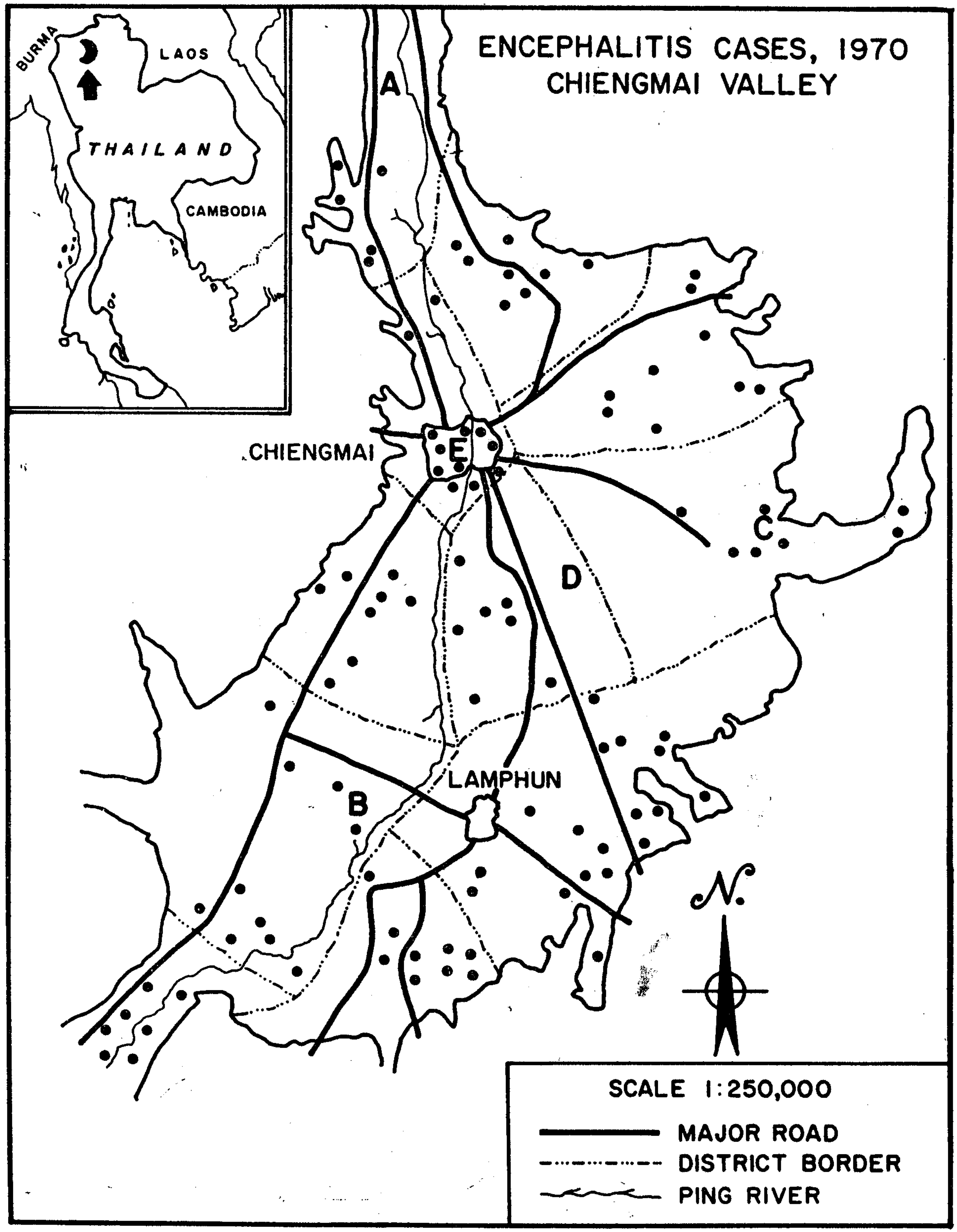
ENCEPHALITIS CASES, 1970
CHIENGMAI VALLEY
BURMA
LAOS
THAILAND
CAMBODIA
A
B
C
D
E
CHIENGMAI
LAMPHUN
N.
SCALE 1:250,000
MAJOR ROAD
DISTRICT BORDER
PING RIVER

FIGURE 5.

Age Prevalence and Geometric mean Titer of JE Antibody (HI) - Village [A] Chiengmai Valley November 1969

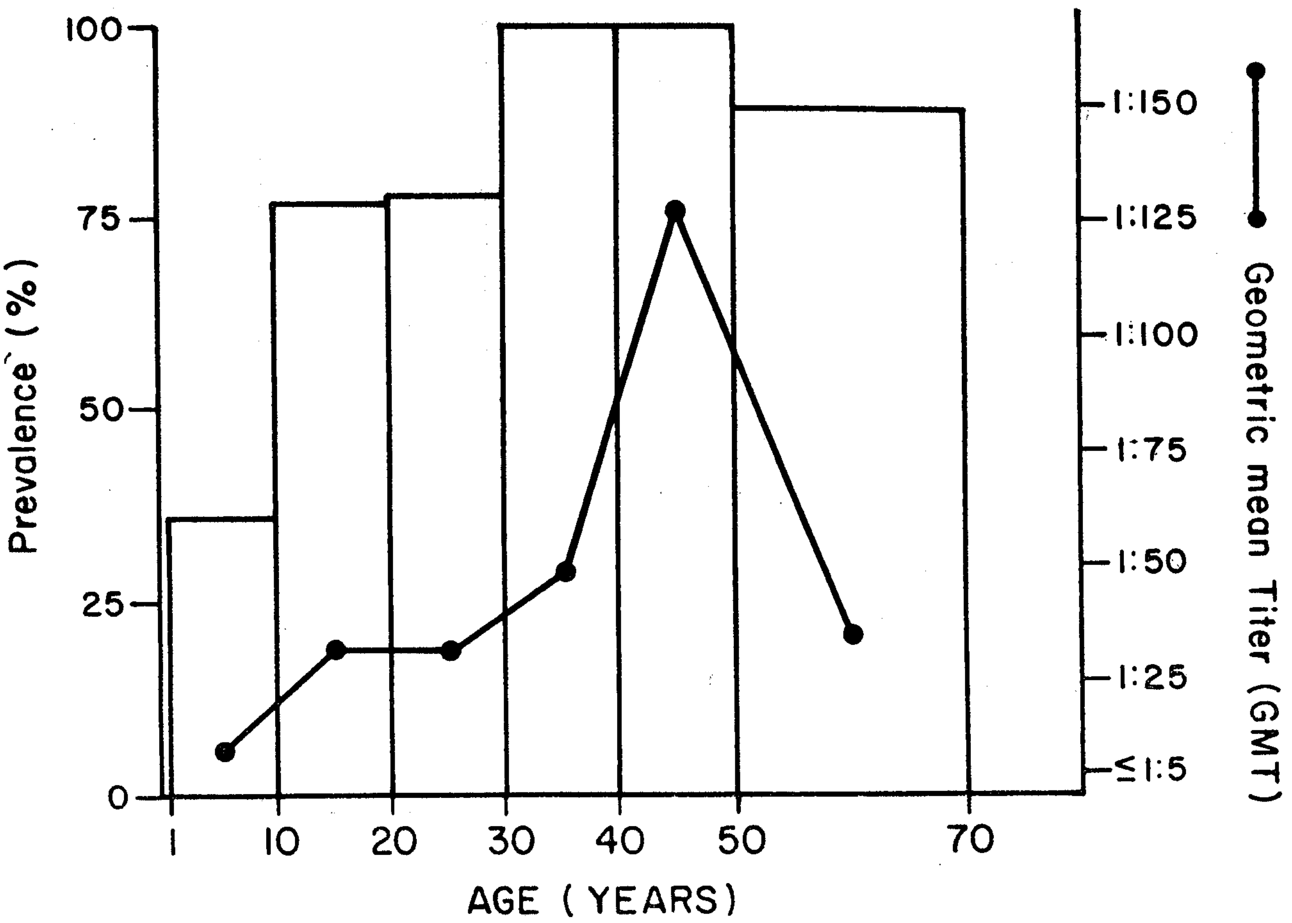

FIGURE 6.

Age Prevalence and Geometric mean Titer of JE Antibody (HI)-Village [B] Chiengmai Valley November 1969

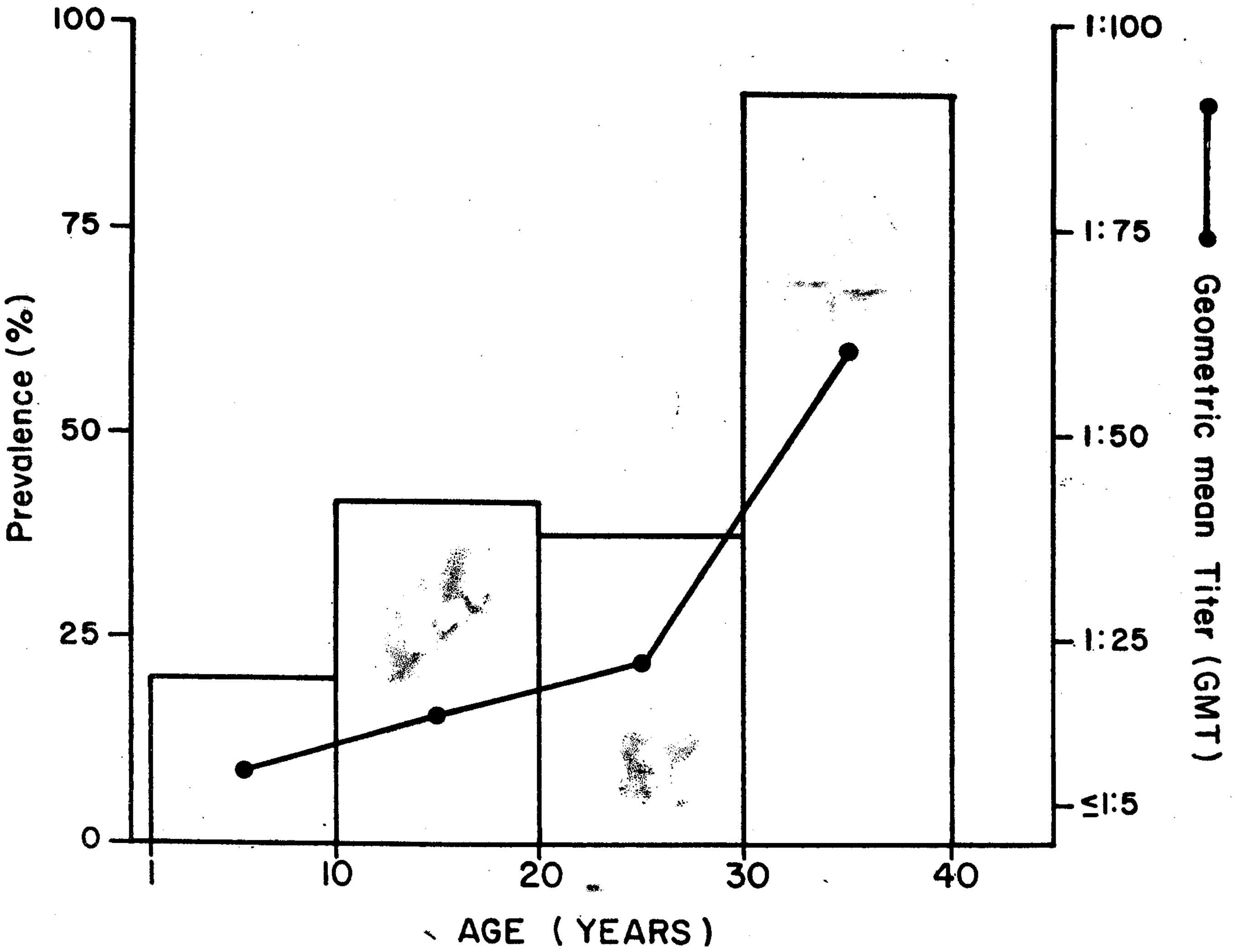

Table 1.

Selection of Random Samples in Chiengmai Valley Study Villages — Nov 1969

Village	District	No. Houses		Sampling Fraction	Population		Average No. Occupants/House	
		Total	RS*	*f*	Total	RS	Total	RS
(A)	Maerim	135	20	.15	635	89	4.7	4.4
(B)	Sanpatong	80	20	.25	420	113	5.2	5.6
(C)	Sankamphaeng	92	20	.22	505	111	5.5	5.6
(D)	Saraphi	69	25	.36	372	132	5.4	5.3
	TOTAL	376	85	—	1932	445	—	—

* RS = Cluster random sample (of houses). Only houses with > 2 occupants were used for sampling in villages (B), (C) and (D).

Table 2.

Results of Blood Meal Identifications for Mosquitoes Captured with Light Traps in Houses in Chiengmai, Nov. 1969 — Oct. 1970

Species	Reacting antisera								
	Primate	Bovine	Dog	Pig	Gecko	Tree Shrew	Bovine Pig	Bovine Horse	Neg.
C. tritaeniorhynchus	0	81	0	1	0	0	0	0	3
C. fuscocephala	2	79	0	1	0	0	0	1	9
C. gelidus	0	5	0	0	0	0	0	0	1
C. vishnui subgroup	3	22	1	4	0	1	1	0	5

Table 3.

Results of Blood Meal Identifications for JE Vector Mosquitoes Captured in Light Traps Set Near Bovines and Pigs in Chiengmai, Nov. 69 – Oct. 70

Species	Light traps location	Reacting antisera				
		Primate	Bovine	Pig	Misc.	Neg.
C. tritaeniorhynchus	near bovines	0	1343	27	28	87
" "	near pigs	0	60	112	5	28
" "	near bovines + pigs	0	68	9	1	8
C. fuscocephala	near bovines	0	1768	30	21	83
" "	near pigs	0	104	79	0	25
" "	near bovines + pigs	0	78	3	0	4
C. gelidus	near bovines	0	369	1	8	9
" "	near pigs	1	8	22	3	4
" "	near bovines + pigs	0	43	4	0	1

Table 4.

Results of Blood Meal Identifications for Mosquitoes Captured in Vegetation by Vacuum Sweep Traps in Chiengmai, Aug – Oct 70

Species	Reacting antisera							
	Primate	Bovine	Pig	Chicken	Dog	Bovine + Chicken	Bovine + Dog	Neg.
C. tritaeniorhynchus	0	117	2	0	0	0	0	35
C. fuscocephala	0	197	0	0	0	1	1	18
C. gelidus	0	13	0	0	0	0	0	12
C. vishnui subgroup	0	93	7	4	4	0	0	47

Table 5.

Summary of Results of JE Virus Isolation Attempts Made from C. fuscocephala, C. gelidus and C. tritaeniorhynchus Collected in Chiengmai Valley, 1970 – 1971

Month	C. fuscocephala			C. gelidus			C. tritaeniorhynchus		
	No. Pools	No. Mosq.	No. JE Isol.	No. Pools	No. Mosq.	No. JE Isol.	No. Pools	No. Mosq.	No. JE Isol.
April 1970	111	7875	0	4	100	0	107	8375	1
May	256	20580	2	28	1365	0	260	21130	6
June	318	27470	0	40	1660	0	368	31915	0
July	502	44130	0	69	2150	0	448	38960	1
August	293	23095	0	79	2300	0	354	29065	0
September	147	8310	0	54	1650	3	207	14535	0
October	87	2590	0	40	1035	0	218	12315	0
November	55	1500	0	31	695	0	175	11980	0
December	56	875	0	26	490	0	114	5340	0
January 1971	53	1565	0	—	—	0	32	610	0
February	54	1610	0	4	35	0	26	560	0
March	36	845	0	—	—	0	26	495	0
Total	1968	140,445	2	375	11,480	3	2335	175,280	8

Table 6.
Toxicity of DDT and malathion to *Culex tritaeniorhynchus*, *Culex gelidus* and *Culex fuscocephala* from Chiengmai, 1970

Species	Stage	Insecticide	LC_{50}*	LC_{90}*	Interpretation
C. tritaeniorhynchus	larva	malathion	0.016 ppm at 24 hr	0.026 ppm at 24 hr	susceptible
" "	adult	malathion	<0.4% at 1 hr	<0.4% at 1 hr	susceptible
" "	adult	DDT	2.2% at 2 hr >4% at 1 hr	>4% at 2 hr	resistant
C. gelidus	larva	malathion	0.022 ppm at 24 hr	0.031 ppm at 24 hr	susceptible
" "	adult	malathion	<0.4% at 1 hr	0.7% at 1 hr	susceptible
" "	adult	DDT	2.8% at 2 hr >4% at 1 hr	>4% at 2 hr	resistant
C. fuscocephala	larva	malathion	0.035 ppm at 24 hr	0.05 ppm at 24 hr	susceptible
" "	adult	malathion	<0.4% at 1 hr	<0.4% at 1 hr	susceptible
" "	adult	malathion	3.8% at 2 hr >4% at 1 hr	>4% at 2 hr	resistant

* LC_{50} & LC_{90}: Concentrations of an insecticide required to kill 50% and 90%, respectively, of the exposed population in a specified period of time.

Table 7.
JE Infections (HI) in Sentinel Pigs—Chiengmai Valley, 1970

Months	No. Pigs at Risk	JE Inf. %	JE Infections by Area %				JE Infections in Pigs Placed in March 1970		
			(A)	(B)	(C)	(D)	No. at Risk	No. Inf.	Cumul % Inf.
April	25	0.0	0.0	0.0	0.0	0.0	25	0	—
May	23*	30.4	0.0	33.3	20.0	57.1	23*	7	0.0
June	16	62.5	20.0	100.0	75.0	66.7	16	10	30.4
Jul—Aug	16	62.5	59.1	33.3	100.0	75.0	6	5	73.9
Sept—Dec	58	25.9	0.0	46.2	20.0	37.5	1	0	95.7
TOTAL	138	30.4	13.5	40.6	28.1	40.5	—	22	95.7

* 2 Pigs died in May

Table 8.
Encephalitis Admissions—Chiengmai Valley, May — Dec 1970

Month	No. Cases	% Male	% Died	% JE Confirmed*
May	23	65	30	86
June	39	64	13	95
July	22	59	18	85
Aug—Dec	16	69	19	33
TOTAL	100	64	19	75

* For the 93 cases with acute and convalescent sera.

Table 9.
Encephalitis Admissions — Chiengmai Valley (1970)
Case Fatality Rates and Serologic Results by Age

Age	No. Cases	% Died	Confirmed JE Cases—Serologic Response (HI)			
			Primary	Secondary	Intermediate	Total
1—4	13	15.4	8	0	0	8
5—9	31	22.6	17	8	1	26
10—14	26	23.1	9	5	1	15
15—19	14	0.0	6	5	1	12
20—29	10	20.0	4	2	1	7
30—	6	33.3	1	1	0	2
Total	100	19.0	45	21	4	70
Median Age	10.5	10.4	9.2	12.5	—	10.3

Table 10.
Participation of Random Sample Villagers in Giving
Blood Specimens, Chiengmai Valley, 1969 — 1970

Village	Age Range	No. Present Nov 69—Nov 70	% Giving blood Specimens*		
			I, IV	I, II, IV	I, II, III, IV
(A)	1—69	82	97.6	97.6	96.3
(B)	1—39	78	92.3	92.3	92.3
(C)	1—39	76	96.1	93.4	88.2
(D)	1—39	89	92.1	91.0	89.9
Total	—	325	94.5	93.5	91.7

* I: Nov 1969 III: Jul 1970
II: Apr 1970 IV: Nov 1970

Table 11.
Population Stability Chiengmai Valley, 1969—1970

Village	No. in Sample Cohort Nov 69	Moved Away	Died	No. Left in Sample Cohort Nov 70	Additions to Sample Houses		No. in Sample Houses Nov 70
					Born	Moved In	
(A)	89	5	0	84	3	1	88
(B)	113	10	0	103	3	8	114
(C)	111	14	0	97	0	16	113
(D)	132	18	0	113	3	3	119
Total	445	47	1	397	9	28	434

Table 12.
Pig and Bovine Population in Chiengmai Valley Study Villages
November 1969 — November 1970

Village	No. Pigs Present		No. Pigs Added*	No. Pigs Lost	No. Bovines Present		No. Bovines Added	No. Bovines Lost
	Nov 69	Nov 70*			Nov 69	Nov 70		
(A)	178	189	175	164	191	190	64	65
(B)	159	191	249	217	96	79	33	50
(C)	136	112	83	107	194	225	86	55
(D)	69	71	67	65	65	62	24	27
Total	542	563	574	553	546	556	207	197

* Does not include sentinel pigs.

Table 13.
Domestic Animals in Chiengmai Valley Study Villages
by House Ownership — Chiengmai Valley, Nov 69

Village	Pigs		Bovines		Dogs		Chickens	
	No.	%*	No.	%	No.	%	No.	%
(A)	178	71	191	44	104	54	1268	83
(B)	159	82	96	48	99	63	522	86
(C)	136	79	194	48	115	63	1229	80
(D)	69	54	65	33	131	68	1219	88
Total	542	72	546	44	449	61	4238	84

* % of houses owning ≥ 1 animal.
Others (Total): 8 Horses; 67 Cats; 275 Ducks; 39 Birds.

Table 14.
Prevalence of JE Antibody (HI)* By Age and Sex
for Study Villages (A) and (B) Chiengmai Valley — Nov 1969

Age	Prevalence (%)					
	Village (A)			Village (B)		
	Male	Female	Total	Male	Female	Total
1–9	25.0	33.3	31.2	25.0	14.3	20.0
10–19	88.9	70.6	76.9	46.2	38.9	41.9
20–29	75.0	80.6	77.8	50.0	25.0	37.5
30–39	100.0	100.0	100.0	100.0	80.0	91.7
Total	82.1	65.0	72.0	47.5	34.1	40.7
Sample Size	28	40	68	40	41	81

* HI Titer ≥ 1:20

Table 15.

Geometric Mean Titers (GMT)* of JE Antibody (HI) by Age and Sex for Study Villages (A) and (B) Chiengmai Valley — November 1969

Age	Village (A)			Village (B)		
	Male	Female	Total	Male	Female	Total
1–9	10.0	10.0	10.0	8.4	8.2	8.3
10–19	37.4	28.9	31.5	12.8	17.8	15.5
20–29	23.8	38.2	31.0	40.0	11.9	21.8
30–39	54.8	37.0	47.7	88.3	34.8	59.9
Total	43.1	25.8	32.2	18.0	14.3	16.0

* HI negatives (< 1:20) called 1:5 for calculating GMT

Table 16.

Overall and Modified Prevalence of JE Antibody (HI) by Age for Study Villages (C) and (D) Chiengmai Valley — November 1969

Age	Village (C)		Village (D)	
	Overall* Prev. (%)	Modified** Prev. (%)	Overall Prev. (%)	Modified Prev. (%)
1–9	46.7	30.0	44.8	27.6
10–19	79.3	48.3	72.7	45.4
20–29	100.0	100.0	85.7	71.4
30–39	94.7	89.5	95.4	90.9
Total	72.6	54.8	70.3	52.7
Sample Size	84		91	

* HI Titer ≥ 1:20
** HI (JE) Titer ≥ 1:20 and ≥ Highest Dengue 1–4 Titer

Table 17.

Overall Prevalence of JE Antibody (HI)*
Chiengmai Valley Study Villages November 1969

Study Village	District	Prevalence %	95% Confidence Interval	Geometric Mean Titer △
(A) +	Maerim	72.0	59.4—84.6	32.2
(B)	Sanpatong	40.7	29.8—51.6	16.0
(C)	Sankamphaeng	72.6	60.4—82.8	70.0
(D)	Saraphi	70.3	60.9—79.7	34.9

* HI Titer ≥ 1:20
△ Titer of 1:5 used for HI Negatives
\+ Ages 1—39 only

Table 18.

Modified Overall Prevalence of JE Antibody (HI)*
Chiengmai Valley Study Villages November 1969

Study Village	District	Prevalence %	95% Confidence Interval	Age—Adjusted Prevalence (%)
(A) +	Maerim	72.0	59.4—84.6	67.9
(B)	Sanpatong	40.7	29.8—51.6	43.6
(C)	Sankamphaeng	54.8	44.8—64.8	55.7
(D)	Saraphi	52.7	43.2—62.2	52.0

* HI (JE) Titer ≥ 1:20 and JE Titer ≥ Highest Dengue 1—4 Titer
\+ Ages 1—39 only

Table 19.

Prevalence of JE and Dengue Antibodies (HI)*
in Urban Chiengmai Schoolchildren — March 1970

	Sample Size	JE		DENGUE	
		Prevalence (%)	GMT△	Prevalence (%)	GMT+△
Male	82	86.6	98	92.7	127
Female	81	81.2	91	90.0	101
Total	163	84.0	94	91.4	114

* HI Titer ≥ 1:20

△ Geometric mean titer (GMT), HI negatives (< 1:20) Called 1:5

\+ Highest Dengue 1—4 titer used.

Table 20.

Comparison of JE and Dengue HI Responses in Urban Schoolchildren
(Age 6—8) and Village Children (Age 5—9) — Chiengmai Valley

	Urban Schoolchildren (E) March 1970	Villages (A) + (B) Nov. 1969	Villages (C) + (D) Nov. 1969
Sample Size	163	24	42
JE Prevalence (%)	84.0	29.2	52.4
JE Titer ≥ Dengue (%)	52.8	29.2	31.0
Dengue Prevalence (%)	92.0	0.0	47.6
Dengue Titer ≥ JE (%)	73.6	0:0	33.3
JE GMT	1:94	1:10	1:18
Dengue GMT*	1:114	≤ 1.5	1:18

* Highest dengue 1—4 titer used.

Table 21.
Summary of Inapparent JE Infections (HI) in Family Members of Cases and Study Areas Chiengmai Valley 1970

Population Studied	No. Studied	JE Infections (HI)				
		No.	% Male	Age Range	Median Age	Incidence Rate (%)
Village (A) — (D)	339	22	55	2—51	11.5	6.5
Urban						
Schoolchildren (E)	159	8	50	6—8	—	5.0
Family Members of Cases	230	8	75	6—36	10.0	3.5

Table 22.
Inapparent JE Infections (HI) in Study Villages and Urban Schoolchildren Chiengmai Valley 1970

Study Area	No. Studied	No. JE Infections	Incidence Rate (%)
Village (A)	85	6	7.1
Village (B)	83	2	2.4
Village (C)	82	9	11.0
Village (D)	89	5	5.6
Urban			
School (E)	159	8	5.0
TOTAL	498	30	6.0

Table 23.

Persistence of JE Antibody Titers (HI) Over One Year (Nov 69 — Nov 70) — Chiengmai Valley

Study Area	No. Serum Pairs	JE Prevalence* (%)		≥ 4—Fold Titer Change (%)		GMT+	
		Nov 69	Nov 70	Rise	Fall	Nov 69	Nov 70
(A)	79	80	75	1	5	1:36	1:29
(B)	71	39	41	1	10	1:16	1:14
(C)	73	77	81	8	21	1:92	1:74
(D)	82	66	65	5	5	1:35	1:30
Total	305	66	66	4	10	—	—

* HI Titers ≥ 1:20

\+ Geometric Mean Titer

Table 24.

Incidence Rates of Encephalitis Cases in Chiengmai Valley (1970) By District

District	Province	Estimated Valley Population	Population Density (People/Km^2)	No. Cases	Estimated Incidence Rate Per 10,000 Pop.
Muang	Chiengmai	120,000	800	12	1.0
Maerim	"	25,000	250	5	2.0
Sansai	"	45,000	225	8	1.8
Doi Saket	"	40,000	400	9	2.2
Sankamphaeng	"	50,000	333	7	1.4
Sardphi	"	60,000	460	7	1.2
Hang Dong	"	40,000	400	8	2.0
Sanpatong	"	80,000	533	10	1.2
Jomthong	"	20,000	200	7	3.5
Muang	Lamphun	125,000	550	18	1.4
Pasang	Lamphun	75,000	600	9	1.2
TOTAL		680,000	450	100	1.5

Table 25.

Incidence Rates of Encephalitis Cases (1970) in Subdistricts of Chiengmai Valley Study Villages

Subdistrict	District	Population at Risk (1969)	No. Cases	Incidence Rate/100,000 Pop.
(A) Khiilek	Maerim	6,000	0	0.0
(B) Makhamluang	Sanpatong	11,200	2	1.8
(C) Rongwuadaeng	Sankamphaeng	4,600	2	4.4
(D) Chomphu	Saraphi	5,200	1	1.9
TOTAL		27,000	5	1.8

Table 26.

Estimate of Inapparent to Apparent (I/A) JE Infection Ratios—Chiengmai Valley 1970

Population Studied	No. JE Infections (HI)	Incidence Rate/10,000	I/A Ratio*
Villages (A)—(D)	22	647	431 to 1
Urban Schoolchildren (E)	8	503	335 to 1
Family Members of Cases	8	348	232 to 1
TOTAL	38	521	347 to 1

* Based on Overall Valley Incidence Rate of 1.5/10,000