STUDIES ON THE JAPANESE ENCEPHALITIS VECTORS IN AMPHOE MUANG, CHIANG MAI, NORTHERN THAILAND

PRADYA SOMBOON, WEJ CHOOCHOTE, CHIRASAK KHAMBOONRUANG, PRASERT KEHA, PRAMOTE SUWANPHANIT, KOM SUKONTASAN and PRASONG CHAIVONG

Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

INTRODUCTION

In 1969, the first large epidemic of Japanese encephalitis (JE) occurred in Chiang Mai area and other areas in northern Thailand (Yamada *et al*., 1971). At present, the disease has been reported every year and has become one of the leading causes of death and/or disability due to an infectious disease in Thailand. From studies on the first and following outbreaks, virus isolation with some other epidemiological studies were performed by many groups of investigators (Grossman *et al*., 1973a; b; c; Gould *et al*., 1974; Johnsen *et al*., 1974; Igarashi *et al*., 1983a; b; Uzuka *et al*., 1983; Fujita *et al*., 1983; Fukunaga *et al*., 1983; Ogata *et al*., 1983; Mori *et al*., 1983). It can be concluded that the epidemic occurs in early rainy season. Cases can be found throughout the year, but start to increase in June and reach a peak in July and then decline in August. The northern region is the area of highest incidence, and to a lesser extent the north-eastern, central, and southern regions, respectively. The etiological cause was JE virus, which is very similar to the strain which caused epidemics in Japan and Taiwan. Children under 15 years of age are the main victims. Three species of *Culex* mosquitoes, *Cx. tritaeniorhynchus*, *Cx. gelidus*, and *Cx. fuscocephala*, are the vectors of the disease. Pigs serve as major amplifying hosts.

At present there is no definite programme for JE control in Thailand. Health education, and instruction on insecticide spraying have been undertaken occasionally in places where frequent outbreaks have occurred. Some field trials of JE vaccine have been conducted in Chiang Mai (Fukunaga *et al*., 1974) with satisfactory results, but future expansion of a vaccination programme to cover the high risk population has not yet been undertaken.

Although much of epidemiological and virological data were obtained from the previous studies, ecological and biological data of JE vectors, both in laboratory and in field, are not so extensive. Moreover, the abundance of rice fields, the major breeding places of the vectors, present a difficult problem for the control of JE in Thailand.

The present study provided some basic ecological and biological information essential to understanding the JE vector status of Amphoe Muang, Chiang Mai, during February 1987 to January 1988. Attempts were made to learn their seasonal prevalence, population densities of the immature stages in rice fields at the various stages of rice cultivation, and daily survival rates of the adult females throughout the year.

MATERIALS AND METHODS

Description of study area

The study was undertaken in Amphoe Muang. Chiang Mai, northern Thailand. The area is situated between the Mae Ping River and the foot of Doi Suthep Mountain at latitude 18 ° 47′ N and longitude 98 ° 59′ E and is approximately 750 km far from Bangkok, Central Thailand. The mean height is about 312 metres above sea level and covers an area of about 172 km^2 (Fig. 1). The population (in 1986) was approximately 200,000 people. Rice fields were mostly in rural areas and a few were around suburban areas. Total area of rice fields was about 1920 ha during rainy season cultivation and about 530 ha during dry season cultivation. For the rainy season, cultivation of rice is carried out in the following stages: seeding in June; ploughing, usually in late June or early July; transplanting just after ploughing by late of July or early August; and harvesting in November to December. Irrigation begins 1−3 weeks before ploughing to soften the earth. Water supplied for cultivation comes from the rain and artificial canals. During the dry season cultivation, follows more or less the same pattern, but the period of rice growing is between January and May. Large numbers of buffalo, cattle and pigs are raised in rural areas and at a few suburban sites.

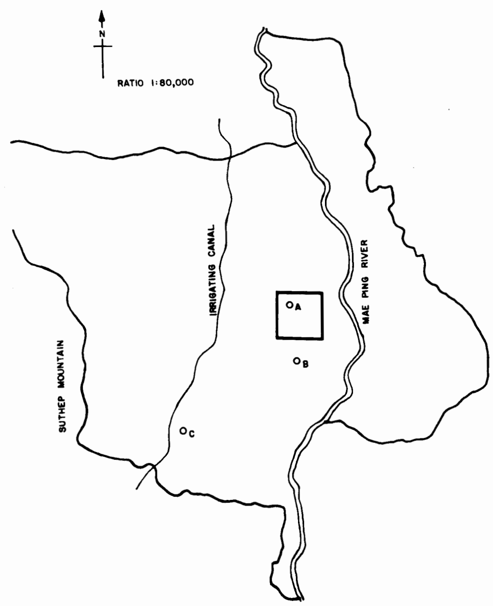

Fig. 1–Map of Amphoe Muang, Chiang Mai and locations of mosquito collections; A. urban, B. suburban and C. rural stations.

Mosquito studies:

Seasonal prevalence. Adult mosquitoes were collected outside the house four times a month from three fixed stations, in an urban (A), suburban (B), and rural area (C) (Fig. 1), by using UV-light traps and human-baited traps. In each location, light-traps were operated for two hours after dusk (usually 19.00−21.00 hours depending on photoperiod) and, simultaneously, two persons with bare legs sitting outdoors collected the mosquitoes as they attacked. The collected mosquitoes were killed by freezing and identified to species using the key of Bram (1967). Three species of JE vectors were sorted out and sampled in order to dissect them for parity determination as outlined by WHO (1975). The daily survival rates of adult females were estimated from the parous rates by the method proposed by Davidson (1954).

Population densities of the immature stages in rice fields. The study was carried out in the rural sites of Amphoe Muang, Chiang Mai. The total area of rice fields in the study station was about 8 ha. The number of included rice fields was about 150, therefore the average area of one rice field was 500 m^2. There were two pigsties beside the rice fields with the total number of pig of about 500. Mosquito larvae and pupae were collected with a dipper (15 cm diam and 3 cm depth) weekly from each rice field during July to November during each stage of rice cultivation. Only one collection was taken from each rice field. The collected specimens were preserved in 10% formalin solution except early instar larvae of *Culex* mosquitoes and pupae which were reared and identified later. Estimated densities of the larvae plus pupae per m^2 were calculated as described by Wada and Mogi (1974).

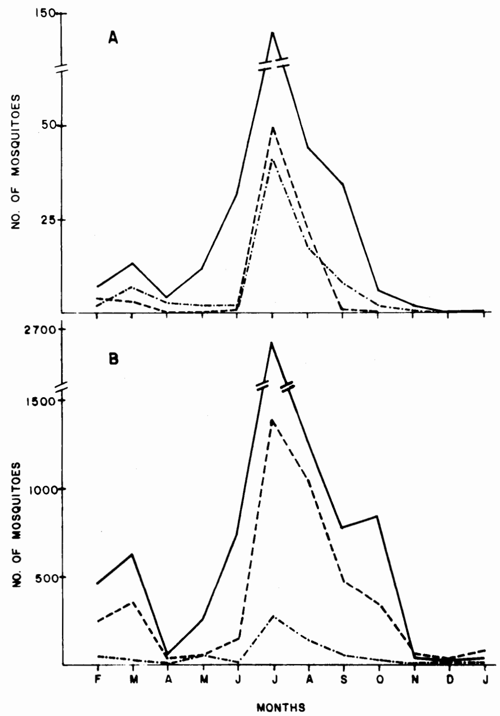

Fig. 2–Total number of female *Culex tritaeniorhynchus* (——), *Cx. gelidus* (– – – –) and *Cx. fuscocephala* (—·—) collected four times a month at rural station in Amphoe Muang, Chiang Mai between February 1987 to January 1988; A human-baited traps, B. UV-light traps.

RESULTS

Seasonal prevalence

More than 30 species of mosquitoes were identified from light trap collections made in three locations in Amphoe Muang, Chiang Mai during the study. However, only about one third of these were species collected by human-baited traps. Appreciably greater numbers of mosquitoes were collected in the rural station than in suburban and urban stations. Among the three vectors of JE, *Cx. tritaeniorhynchus*, *Cx. gelidus* and *Cx. fuscocephala*, the former was the predominant species caught by light-traps and human-baited traps in rural site (Fig. 2). In contrast, *Cx. gelidus* was collected in greater numbers than the last two species both in suburban and urban sites as measured by light-trap collections but by human-baited traps *Cx. tritaeniorhynchus* was most collected in suburban while in urban site *Cx. tritaeniorhynchus* as many as *Cx. gelidus* were collected (Fig. 3,4).

Total monthly rain falls and temperature from January 1987 to January 1988 are shown in Fig. 5. All meteorological data were obtained from Northern Region Weather Forecast Centre, Chiang Mai. Total precipitation was rather small during April to July. A marked increase of precipitation occurred in August.

By May or June the population of these three vector species started to increase. In

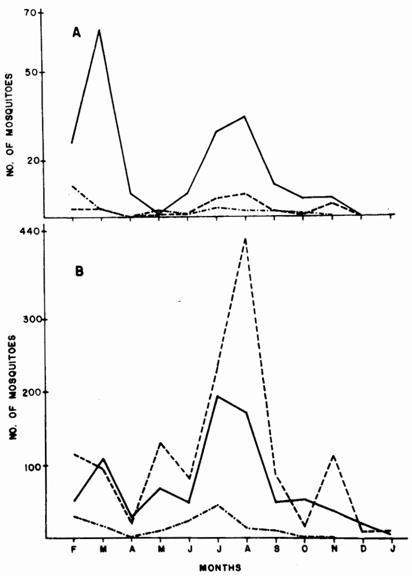

Fig. 3—Total number of female *Culex tritaeniorhynchus* (———), *Cx. gelidus* (– – – –) and *Cx. fuscocephala* (–·–·–) collected four times a month at suburban station in Amphoe Muang, Chiang Mai between February 1987 to January 1988; A. human-baited traps, B. UV-light traps.

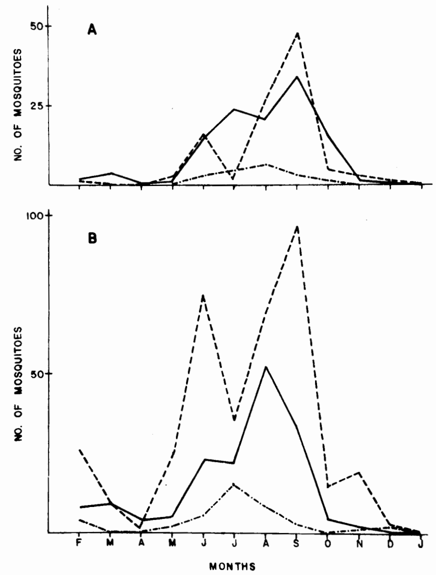

Fig. 4—Total number of female *Culex tritaeniorhynchus* (———), *Cx. gelidus* (– – – –) and *Cx. fuscocephala* (–·–·–) collected four times a month at urban station in Amphoe Muang, Chiang Mai between February 1987 to January 1988; A. human-baited traps, B. UV-light traps.

rural station there was a sharp rise in the populations of the JE vectors in July when most of the rice fields were ploughed and a marked decline in mosquito population densities occurred after transplanting in August when the fields were flooded. During dry season cultivation, however, the population densities of the mosquitoes were not high. Fewer numbers of *Cx. fuscocephala* than *Cx. gelidus* were found in light-traps placed in rural station, the proportions of these two species represented in human-baited traps were not so different. In suburban station, peaks in the population of the mosquitoes occurred from July to August. In addition, high numbers of *Cx. tritaeniorhynchus* were caught by human-baited traps in February and the highest number in March. Consider-

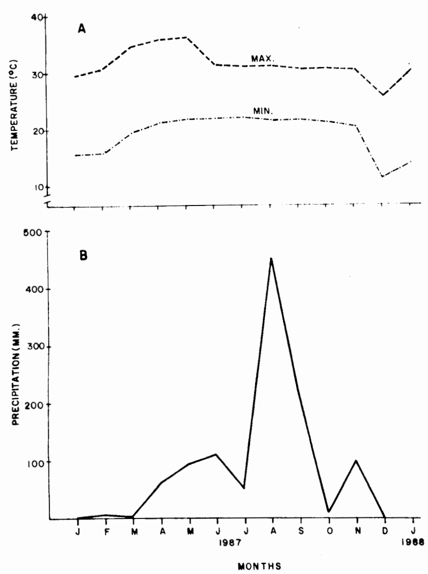

Fig. 5—Summary of temperature (A) and rainfall (B) recorded in Amphoe Muang, Chiang Mai from January 1987 to January 1988.

ably greater numbers of *Cx. gelidus* were caught by light-traps, since not many of these mosquitoes attack man. In urban station, most of JE vectors appeared in the rainy season (June to Octorber), while in other seasons densities were very low. *Cx. tritaeniorhynchus* and *Cx. gelidus*, rather than *Cx. fuscocephala*, were frequently collected in this area.

Daily survival rates of the adult females.

Table 1. shows the parous rates and calculated daily survival rates of the females throughout the year. The daily survival rates of these three species were mostly as high as 0.7.

Population densities of the immature stages in rice fields.

Population densities of larvae plus pupae per m^2 (based on the unweighted average per dip) is shown in Fig. 6. The densities of three vector species in rice fields during the wet season was highest in July when the fields were ploughed. The highest average densities of *Cx. tritaeniorhynchus* was 3162

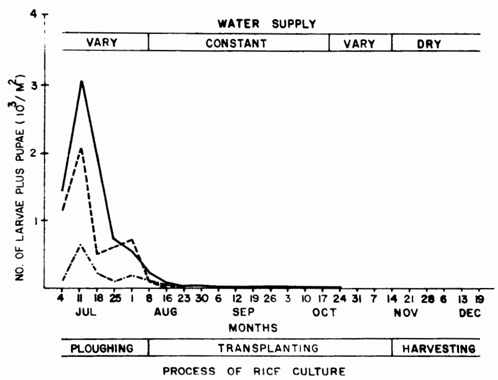

Fig. 6—Densities of larvae plus pupae of *Culex tritaeniorhynchus* (——), *Cx. gelidus* (----) and *Cx. fuscocephala* (-·-·-) in rice fields at rural areas in Amphoe Muang, Chiang Mai, 1987.

Table 1

Parous rates (p) and daily survival rates (P)* of *Culex tritaeniorhynchus* (C.t.), *Culex gelidus* (C.g.) and *Culex fuscocephala* (C.f.) collected by light-traps between February 1987 to January 1988

Month	C.t.	p	P	C.g.	p	P	C.f.	p	P
February	45	.311	.747	92	.250	.707	19	.211	.678
March	75	.733	.925	50	.600	.880	6	.677	.907
April	44	.682	.909	13	.308	.745	5	.400	.795
May	131	.573	.870	120	.383	.787	28	.571	.869
June	170	.447	.818	115	.443	.816	32	.565	.900
July	224	.433	.811	178	.393	.792	44	.568	.868
August	157	.389	.790	133	.322	.753	73	.479	.832
September	168	.524	.851	123	.293	.736	11	.545	.859
October	120	.575	.871	44	.545	.859	7	.571	.869
November	45	.689	.911	66	.348	.768	2	.500	.841
December	48	.687	.910	20	.700	.915	0	–	–
January	22	.364	.776	26	.269	.720	8	.500	.841

*** assuming the gonotrophic cycle is 4 days.**

per m^2 (17.0 per dip), *Cx. gelidus* was 2092 per m^2 (11.5 per dip) and *Cx. fuscocephala* was 651 per m^2 (3.5 per dip) on July 11. The two former species were found abundantly both before and after the fields were ploughed, while the latter was mostly found in ploughed fields. After transplanting, which begins in August, such densities show a marked decrease and both larvae and pupae of the JE vectors were rarely found until rice plants were harvested.

DISCUSSION

The dissemination of JE vectors in urban, suburban and rural areas of Amphoe Muang, Chiang Mai is obviously related to the climatic conditions and the process of rice cultivation in the fields. It is already seen that the densities of JE vectors are highest in rural sites. Thus people who live in these areas are frequently attacked by the vectors. The appearence of the vectors in urban sites, where their breeding places are rarely found, is believed due to the dispersal of the mosquitoes from suburban sites where they had previously generated in many breeding places. The greater numbers of *Cx. gelidus* than *Cx. tritaeniorhynchus* found in light-traps placed in urban and suburban sites may be explained by the many sources of polluted water where *Cx. gelidus* prefers to breed. It is frequently found that this species, together with *Cx. quinquefasciatus*, an urban mosquito species, breed in the same places. Polluted water, especially those with grasses, seem to be more suitable breeding places of *Cx. gelidus* than *Cx. tritaeniorhynchus* and *Cx. fuscocephala*. However, *Cx. tritaeniorhynchus* as well as *Cx. gelidus* is found to bite man in urban sites while appreciably greater numbers of the former attack man in suburban sites. This phenomenon may be attributed to the absence of large animals eg., buffaloes, cows and pigs in urban sites. Thus humans become more available blood sources. In contrast to the suburban sites, there are still large animals to provide blood meals. It is possible that. *Cx. gelidus* may be more strongly zoophilic than *Cx. tritaeniorhynchus*.

There is no doubt that ploughed fields, rather than transplanted fields, provide a more suitable breeding place for these mosquitoes. It is observed that before ploughing, most rice fields retain some water for about 1−3 weeks irrespective of rainfall and maintain vegetation tolerable to wet ground. The immature stages of the mosquitoes are firstly found in rice fields during that period. We believe that such rice fields provide an important breeding place where females can oviposit at least 2−3 times prior to ploughing. When the fields are ploughed, the vector population immediately shows a marked increase. Water in ploughed fields is enriched with organic matter and micro-organisms as demonstrated by the characteristic odour due to the fermentation of rice-straw, grass and mud. In addition, rainfall during that period is not abundant and water is stagnant and a stable source of food for a period of time. Water temperature at 3 cm under the surface measured in afternoon is about 35−39 °C which is optimum for the growth of bacteria and the stimulation of fermentation. These water qualities are very attractive for oviposition of these three species. After rice transplanting, there are many ecological changes in rice fields. Water in rice fields becomes cleaner and fermentation ceases which may be due to the effect of heavy rain, herbicides (butachlor +2, 4−D isobutyl esters) and some spontaneous biochemical changes in the water. The amount and mode of precipitation influence immature mosquito populations both directly and indirectly as examined by Mogi and Wada (1973) and Mogi (1979). Water currents directly influence migration rates and, indirectly, egg density and survival rates through their effects on the quality of water, since the attractiveness of water to gravid females and the amount of food for larvae are both functions of water quality. Therefore, it is not surprising that despite an increase of water surface in rice fields during transplanting, the number of vectors decreases. Because of high daily survival rates as measured throughout the year, some female mosquitoes which emerged in July can survive until at least late August. The small number of the females caught in September is due to the appreciably small number of newly emerged adults in mid or late August and also in September. Therefore, the concept that the more water surface in rice fields the more breeding places of JE mosquito vectors in Thailand is probably not true. We have shown in our work that during ploughing rice fields are the principal breeding places of JE vectors. Because farmers in the north usually plough their rice fields in late June or early July, depending on the precipitation, the vector population in this region is believed simultaneously to reach a peak in this period. Again, as reviewed by Thongcharoen (1985), it is already seen that in nearly all of the previous epidemics of JE between 1969 to 1984, the majority of the patients were found in July and a few throughout the year. This relation is likely caused by the fact that the densities of the vectors are maintained in certain levels throughout the year during which time only a few cases will appear. When these densities increase during the period of ploughing in late June or early July, epidemics are more likely to occur.

Other evidence supporting JE transmission during on epidemic are thought to be the high daily survival rate of the vectors and their ability to maintain the virus until they become infective mosquitoes. The daily survival rates estimated by the present study for Chiang Mai *Cx. tritaeniorhynchus* did not differ so much from those of 0.682 estimated by Mori *et al.*, (1983). In Japan, the daily survival rate of *Cx. tritaeniorhynchus* was 0.42–0.74 as estimated by Buei and Ito (1982).

Control of JE should focus on rural areas and may be successful by the two following methods: firstly, by mass vaccination of the children and, secondly, by control of the mosquito vectors. For vector control, the method used should start just after the first precipitation, especially from May to the period of ploughing in July. Insecticides, such as Abate, should be used for control of the immature stages in rice fields and other breeding places such as grass land flooded with water, in addition to, the spraying of some insecticides in order to kill the adult mosquitoes, especially on vegetations, pigsties and livestockpens. Control of the immature stages in rice fields during transplanting is unneccessary, but control of the adults should be continued at least one month after rice plant have been transplanted.

SUMMARY

From February 1987 to January 1988, biological and ecological studies were made to obtain the basic knowledge of Japanese encephalitis vectors, *Culex tritaeniorhynchus*, *Cx. gelidus*, and *Cx. fuscocephala*, in Amphoe Muang, Chiang Mai, northern Thailand. The following results were found. Peaks in the population densities of the vectors as measured four times a month, by

UV-light trap and human-baited trap collections, occurred during rainy season. The JE vectors in rural areas showed a sharp rise in the population in July when most of the rice fields were ploughed and a marked decline in mosquito population densities occurred after transplanting in August when the fields were flooded. The average mumber of larvae plus pupae per m^2 in rice fields was highest in July when the fields were ploughed, but in the period from transplanting to harvesting (August to November), the densities were very low. Daily survival rates of the adult females, as estimated from parous rates, were mostly as high as 0.7 throughout the year.

ACKNOWLEDGEMENTS

The authors wish to thank The National Research Council of Thailand for research support; Mr. Peter Lange for manuscript correction; Mr. Chai Kiriyaporn, Miss Srivan Thasee, Mrs. Suprathra Pugpon and Miss Lavan Intaraj for technical assistance.

REFERENCES

BRAM, R.A., (1967). The genus *Culex* in Thailand (Diptera: Culicidae). *Contr. Am. Ent. Inst., 2* : 225.

BUEI, K. and ITO, S., (1982). The age-composition of field populations and the survival rates in *Culex tritaeniorhynchus* Giles. *Jap. J. Sanit. Zool., 33* : 21.

DAVIDSON, G., (1954). Estimation of the survival-rate of anopheline mosquitoes in nature. *Nature, 174* : 792.

FUJITA, N., IGARASHI, A., BUNDO, K., OGATA, T., PUPAWADEE, J., PEERAKOME, S., LEECHANACHAI, P., PANASAMPOL, K., CHANYASANHA, C. and CHATIYANONDA, K., (1983). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. IV. Serological examination on hospitalized patients. *Trop. Med., 25* : 155.

FUKUNAGA, T., IGARASHI, A., OGATA, T., FUJITA, N., CHAROENSOOK, O., JATANASEN, S., CHANYASANHA, C., CHATIYANONDA, K., PEERAKOME, S., SUPAWADEE, J. and PANASAMPOL, K., (1983). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. V. Seroepidemilogical survey on humans. *Trop. Med., 25* : 165.

FUKUNAGA, T., ROJANASUPHOT, S., WUNGKORBKIAT, S., THAMMANICHANON, A., KAN, T., OTSU, K., TUCHINDA, P., JETANASEN, S. and CHIOWANICH, P., (1974). Japanese encephalitis vaccination in Thailand. *Biken J., 17* : 21.

GOULD, D.J., EDELMAN, R., GROSSMAN, R.A., NISALAK, A. and SULLIVAN, M.F., (1974). Study of Japanese encephalitis virus in Chiangmai vally, Thailand, IV. Vector studies. *Am. J. Epidemiol., 100* : 49.

GROSSMAN, R.A., EDELMAN, R., CHIEWANICH, P., VOODHIKUL, P. and SIRIWAN, C., (1973a). Study of Japanese encephalitis virus in Chiangmai valley, Thailand. II. Human Clinical infections. *Am. J. Epidemiol., 98* : 121.

GROSSMAN, R.A., EDELMAN, R., WILLHIGHT, M., PANTUWATANA, S. and UDOMSAKDI, S., (1973b). Study of Japanese encephalitis virus in Chiangmai valley, Thailand. III. Human seroepideminology and inapparent infections. *Am. J. Epidemiol., 98* : 133.

GROSSMAN, R.A., GOULD, D.J., SMITH, T.J., JOHNSEN, O.D. and PANTUWATANA, S., (1973c). Study of Japanese encephalitis virus in Chiangmai valley, Thailand, I. Introduction and study design. *Am. J. Epidemiol., 98* : 111.

IGARASHI, A., SRISUKRIT, A. and TUCHINDA, P., (1983a). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. I. Study design and conclusion. *Trop. Med., 25* : 129.

IGARASHI, A., CHIOWANICH, P., LEECHANACHAI, P. and SUPAWADEE, J., (1983b). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. III. Virus isolation from clinical materials. *Trop. Med., 25* : 149.

JOHNSEN, D.O., EDELMAN, R., GROSSMAN, R.A., MUANGMAN, D., POMSDHIT, J. and Gould, D.J., (1974). Study of Japanese encephalitis virus in Chiangmai valley, Thailand. V. Animal Infections. *Am. J. Epidemiol., 100* : 57.

MOGI, M., (1979). Dispersal of *Culex tritaeniorhynchus* larvae (Diptera, Culicidae) by water currents in rice fields. *Trop. Med., 21* : 115.

MOGI, M. and WADA, Y., (1973). Spatial distribution of larvae of the mosquito *Culex tritaeniorhynchus summorosus* in a rice field area. *Trop. Med., 15* : 69.

MORI, A., IGARASHI, A., CHAROENSOOK, O., KHAMBOONRUANG, C., LEECHANACHAI, P. and SUPAWADEE, J., (1983). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. VII. Mosquito collection and virus isolation. *Trop. Med., 25* : 189.

OGATA, T., IGARASHI, A., FUJITA, N., CHANYASANHA, C., PEERAKOME, S. and SUPAWADEE, J., (1983). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. VI. Antibody survey on animals. *Trop. Med., 25* : 183.

THONGCHAROEN, P. (1985). Japanese encephalitis in Thailand. *J. Med. Assoc. Thai., 68* : 534.

UZUKA, Y., IGARASHI, A., CHIOWANICH, P., SATHAPANAKUL, C., SUPAWADEE, J., GUYER, J.J. and JATANASEN, S., (1983). Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. II. Hospitalized patients. *Trop. Med., 25* : 139.

WADA, Y. and MOGI, M., (1974). Efficiency of the dipper in collecting immature stage of *Culex tritaeniorhynchus summorous. Trop. Med., 16* : 35.

WHO, (1975). Manual on practical entomology in malaria. Part II. Method and Techniques. Geneva. p191.

YAMADA, T., ROJANASUPHOT, S., TAKAGI, M., WUNGKORBKIAT, S., HIROTA, T., YAMASHITA, T., AHANDARIK, S., PISUTHIPORNKUL, S., SAWASDIKOSOL,S., SANGKAWIBHA, N., TUCHINDA, P., WACHAROTHAL, S., JETANASEN, S., HIRANNIRAMON, S., LAOSUTHIBONGSE, V., CHIOWANICH, P., ROBERTS, C.E., OESAWADI, P., BUKKAVESA, S., GAEW-IM, M., SHIMIZU, A. and KITAOKA, M., (1971). Studies on an epidemic of Japanese encephalitis in the northern region of Thailand in 1969 and 1970. *Biken J., 14* : 267.