ECOLOGY OF MOSQUITOS (DIPTERA: CULICIDAE) AT A SITE ENDEMIC WITH JAPANESE ENCEPHALITIS ON LUZON, REPUBLIC OF THE PHILIPPINES

George W Schultz and Curtis G Hayes

US Naval Medical Research Unit No 2 Detachment, PAV-7 Sanhazaro Hospital Compound, Santa Cruz, 1003 Manila, Philippines

Abstract. A 2-year study of the ecology of mosquitos was conducted in a rice-growing area in the Philippines. Forty-four species in 8 genera were collected using 6 techniques, with *Culex vishnui* being most abundant. Of all the *Anopheles* collected, most species were exophagic, while most of the *Culex* species collected were endophagic. Anthropophilic or zoophilic feeding patterns were estimated using man-to-animal biting ratios. Peak periods of mosquito abundance occurred several months after the onset of both the rainy season and the rice irrigation period. Seasonal populations of the more abundant species revealed 3 distinct patterns: 1) populations are high during the rainy season and low during the irrigation period; 2) populations are equally strong during both periods; and 3) populations are highest during the irrigation period. Species' biting activity was grouped into 2 patterns: 1) those that feed primarily from 1800-0000; and 2) those that feed evenly throughout the night. Mosquito abundance and Japanese encephalitis virus activity were related to rainfall and rice-growing practices.

INTRODUCTION

Japanese encephalitis (JE), which occurs over a wide area of the Philippines, is an uncommon but serious disease (Hayes *et al*, 1986; Denning and Kaneko, 1987). Although no epidemics have occurred, over 100 confirmed cases have been diagnosed in man (Hayes, unpublished data). At least 26 species of mosquitos have been identified as potential JE vectors throughout its geographic distribution (Rosen, 1986; Olson *et al*, 1985). In the Philippines, JE virus has been isolated from pools of *Culex tritaeniorhynchus* Giles, *Cx. vishnui* Theobald (Trosper *et al*, 1980), *Cx. bitaeniorhynchus* Giles, and *Anopheles annularis* Van der Wulp (Ksiazek *et al*, 1980). In other countries, JE virus has also been isolated from *Cx. fuscocephala* Theobald, *Cx. gelidus* Theobald, *Cx. quinquefasciatus* Say, *Cx. pseudovishnui* Colless, *Cx. whitmorei* (Giles), *Aedes albopictus* (Skuse), *Ae. vexans* (Meigen), *Anopheles subpictus* Grassi, *An. vagus* Doenitz, *Armigeres subalbatus* (Coquillett), and *Mansonia uniformis* (Theobald), all of which are found in the Philippines (Apiwathnasorn, 1986).

The present study was conducted in an area where several cases of JE had previously been confirmed. The objectives were to: 1) determine species abundance and seasonal population changes using a variety of sampling techniques; 2) examine mosquito behavior; and 3) correlate potential environmental factors such as weather and rice-growing practices on mosquito abundance and JE virus activity.

The opinions and assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the US Navy Department or the Naval Service at large.

Reprint requests to the Publication Office, US Naval Medical Research Unit No. 2 Detachment, APO AP 96440 or PAV-7, San Lazaro Hospital Compound, Sta Cruz, 1003 Manila, Republic of the Philippines.

MATERIALS AND METHODS

Description of study area

The study site was located near the town of Sto Domingo, 105 km north of Manila in the Nueva Ecija Province. Collections were made in the purok (small community) of Cardenas, 7.5 km north of Sto Domingo in an area used almost exclusively for rice farming. The community surveyed in April 1986, consisted of 159 people living in 29 houses. The domestic animals within this purok consisted of 244 chickens, 38 ducks, 36 dogs, 30 goats, 23 turkeys, 20 pigeons, 19 carabao, 10 pigs, 3 cats, and 1 monkey. Mosquito netting was used by nearly every person during most of the year. The houses, made of split bamboo with thatched-grass roofs, were built at ground level with the sleeping quarters elevated about a meter above ground. Various domestic animals were often housed beneath the sleeping quarters. Carabao (water buffalo) were usually tied close to the owner's house each night. No periodic spraying with insecticides was done in any of the houses.

The climatic pattern within the Nueva Ecija Province consists of 3 basic seasons: a rainy season from May to October, a cool-dry season from November to January, and a hot-dry season from February to April. The average temperatures range from 25.0°C in January to 30.4°C in April.

Rice-growing practices play an important role in the ecology of musquitos at this site. Starting in May, the fields are dry and remain so until heavy rains begin in late May to early June. By the end of June the fields are flooded and a few seedling beds of rice are planted. By mid-July the rice is about 15 cm high and is transplanted to other fields, which remain flooded throughout August, when the rice reaches 45 cm height by month's end. By mid-September the rice reaches its maximum height of about 60 cm and the ground, while still moist, is no longer flooded. By mid-October the fields are dry and the rice seeds are hard. Harvesting begins in late October and finishes in early November, leaving the fields dry throughout November and December. In early January about 1/3 of the fields around the village are flooded by irrigation from wells. Seedling beds are started in early January and transplanted to the remaining flooded fields at month's end. The pumps run continuously throughout February and March, maintaining the flooded fields. By early April the pumps are stopped, and by mid-April the fields are dry and rice seeds are fully developed. The rice is harvested in late April, leaving dry, empty fields throughout May.

Survey techniques

Every 2 weeks mosquitos at the field site were sampled using 6 techniques:

1. **Man-biting:** During the first year 2 people collected for 10 minutes indoors and 10 minutes outdoors every 2 hours from 1800-0600 hours. During the second year 8 people collected for 15 minutes indoors and 15 minutes outdoors every 2 hours from 1800-0600. The men collected mosquitos from their own exposed legs using red-filtered flashlights and oral aspirators. Mosquitos were placed into carton and later identified.

2. **Carabao-baited trap:** This trap consisted of a fine mesh tent 3.9 m by 3.9 m and 1.8 m high. The mesh size was 8 holes by 5 holes per cm^2. The tent had 1 door measuring 1.5 m wide by 1.8 m high, which could be sealed. The tent was protected from rain by a tarp 6.9 m by 6.9 m which was elevated over the tent. One carabao was secured in the center of the tent. The door was open from 1830-1930, 2000-2130, 2200-2330, 0000-0130, 0200-0330, and 0400-0500. During the 30-minute intervals, collectors entered the tent, and with the door closed, used mechanical aspirators to capture all of the mosquitos.

3. **Pig-baited trap:** This technique employed the same procedure as the carabao-baited trap except that 2 pigs were confined in the center of the tent.

4. **Light trap:** Each night 5 CDC light traps, baited with dry ice, were placed near the edge of the village. Collections were made from 1800-0600, and collecting bags were changed every 2 hours.

5. **Indoor resting:** Between 0600-0700, 3-4 houses were sampled for mosquitos resting indoors. Mosquitos were collected using mechanical aspirators and placed in carton containers.

6. **Larval survey:** From 0730-0930 any potential breeding habitat within a 100 m radius of the houses was sampled. Larvae and pupae were collected with dippers and returned to the laboratory for rearing and identification.

Seasonal transmission of JE virus was monitored with sentinel pigs. Two pigs, seronegative for JE virus, were placed in the village and bled weekly from ear veins. The blood was tested for JE virus antibody using a microtiter hemagglutination-inhibition test (Clark and Casals, 1958; Sever 1962). Each pig that seroconverted was replaced as soon as possible.

Human cases of JE in the area were monitored in a hospital located in Cabanatuan City about 19 km south of the study village. This hospital was the major medical facility serving the entire region. All clinically suspect cases of viral encephalitis admitted to the hospital were tested using an antibody capture enzyme linked immunosorbent assay (ELISA) to detect the presence of JE virus IgM in cerebrospinal fluid (Burke *et al*, 1985).

Table 1

Comparison of species and percentages of mosquitos using 6 collecting techniques.

Species*	Man-biting %	Carabao-baited trap %	Pig trap %	Light trap %	Indoor resting %	Larval survey %
Total collected	15,933	495,234	147,074	701,350	12,395	3,353
Culex vishnui	35.56	45.51	50.07	55.37	7.82	16.49
Cx. pseudovishnui	13.98	2.20	2.99	1.23	1.75	1.37
Cx. quinquefasciatus	8.40	0.14	0.65	0.12	14.40	8.95
Cx. fuscocephala	5.03	24.79	23.59	14.07	9.95	53.59
Cx. tritaeniorhynchus	2.35	2.93	3.73	7.66	0.33	0.15
Cx. bitaeniorhynchus	2.00	0.40	0.21	0.89	0.17	0.98
Cx. sinensis	0.68	0.14	0.20	0.11	0.03	-**
Cx. annulirostris	0.51	0.06	0.13	0.02	-	-
Cx. gelidus	0.41	1.23	2.17	1.16	0.15	2.18
Cx. whitei	0.22	0.00***	-	-	0.10	-
Cx. sitiens	0.14	0.07	0.04	0.02	0.37	0.09
Cx. whitmorei	0.08	0.04	0.03	0.13	0.02	-
Cx. fuscanus	0.01	0.00	0.02	0.04	0.06	1.01
Anopheles peditaeniatus	22.61	7.54	4.07	12.68	1.20	0.66
An. annularis	1.49	2.27	1.73	2.46	0.61	0.30
An. subpictus	1.37	5.17	7.51	1.54	23.40	2.54
An. vagus	0.17	1.77	1.79	0.10	29.56	7.63
An. indefinitus	0.14	0.35	0.24	0.02	9.79	0.84
An. tesselatus	0.06	0.26	0.10	0.58	0.02	0.60
An. flavirostris	0.04	0.00	0.00	0.00	-	-
An. franciscoi	0.01	0.01	0.00	0.00	-	-
An. pseudobarbirostris	0.01	-	0.00	0.00	0.01	0.06
An. filipinae	0.01	-	-	0.00	-	-
An. ludlowae	-	0.00	-	0.00	-	0.03
An. mangyanus	-	-	-	-	0.01	-
Mansonia uniformis	4.14	0.53	0.51	1.21	0.18	-
Aedes lineatopennis	0.36	0.48	0.17	0.50	0.06	2.36
Ae. vexans	0.09	0.10	0.03	0.07	-	-
Ae. albopictus	0.05	0.01	0.00	0.01	0.02	0.03
Ae. luzonensis	0.04	0.00	0.00	0.00	0.01	-
Ae. aegypti	0.01	-	-	-	0.08	0.12
Ae. flavipennis	-	0.00	0.01	0.01	-	0.03
Mimomyia chamberlaini	-	0.01	0.00	0.01	-	-

* Species collected very rarely were: *An. karwari, An. lesteri, Ae. desmotes, Ae. gardnerii, Ae. longirostris, Ae. pampangensis, Mi. hybrida, Ficalbia ludlowae, Fi. luzonensis, Aedeomyia catasticta,* and *Armigeres subalbatus.*

** Species was not collected using that technique.

*** Species was collected but at <0.005%.

RESULTS AND DISCUSSION

Forty-four species of mosquitos in 8 genera were collected during 49 trips to this field site (Table 1). *Culex vishnui* was the most abundant species collected on man and in the 3 trapping techniques. When comparing the first 4 techniques, *Anopheles peditaeniatus* (Leicester), *Cx. pseudovishnui*, and *Cx. quinquefasciatus* comprised a much greater percentage of the total from man collections, while *Cx. fuscocephala* comprised a much smaller percentage. Comparing the carabao- and pig-baited traps shows that while some minor variations exist, they show the most similarity among these first 4 techniques. For *Cx. vishnui* and *Cx. tritaeniorynchus*, the light trap was the most effective collecting technique. Indoor resting showed very different results. The number of mosquitos was influenced not only by species abundance, but to a greater degree by their behavior — whether they enter houses and rest on the walls. *Anopheles vagus*, *An. subpictus*, and *An. indefinitus* (Ludlow) comprised a much greater percentage of the total from indoor resting collections than the other techniques. Although *Cx. vishnui* was the most abundant species, it comprised only about 8% of the mosquitos resting indoors. Larval surveys in the rice fields surrounding the village showed that *Cx. fuscocephala*, *Cx. vishnui*, and *Cx. quinquefasciatus* were the most common species.

Among the 13 most common species collected biting man, some showed a definite exophagic behavior while others were endophagic (Table 2). All of the *Anopheles* species listed were exophagic to some degree, while all of the *Culex* were endophagic. The indoor-outdoor ratio did not change significantly for any of the species between the rainy and dry seasons.

Examining the ratio of mosquitos collected from man to those collected from carabao for each species indicated their anthropophilic or zoophilic host selection patterns (Reid, 1961). *Culex quinquefasciatus* had the strongest anthropophilic preference, while *An. vagus* was the most zoophilic (Table 3). In a similar comparative study at a JE endemic area in India, Reuben (1971b) also found *Cx. pipiens fatigans* (= *Cx. quinquefasciatus*) to be the only strongly anthropophilic species. She determined the order of increasing zoophility to be *Cx. bitaeniorhynchus*, *Cx. pseudovishnui*, *Cx. vishnui*, *Cx. tritaeniorhynchus*, *Cx. fuscocephala*, *An. vagus*, and *An. subpictus*, which is generally supported by the present results.

During the 2 years of surveying mosquitos, 4 distinct periods of mosquito abundance were observed (Fig 1). These periods occurred either several months following the onset of the rainy season, or several months after the start of the irrigation period. Although some differences were evident when comparing the 4 techniques, the

Table 2

Number of mosquitos collected biting man both indoors and outdoors and their ratio.

Species	Indoor	Outdoor	Ratio (indoor : outdoor)
Anopheles annularis	48	188	1 : 3.9
An. peditaeniatus	1,452	2,150	1 : 1.5
An. subpictus	88	130	1 : 1.5
An. vagus	12	15	1 : 1.2
Culex annulirostris	45	36	1.3 : 1
Cx. bitaeniorhynchus	178	141	1.3 : 1
Mansonia uniformis	380	280	1.4 : 1
Cx. quinquefasciatus	1,368	858	1.6 : 1
Cx. fuscocephala	509	292	1.7 : 1
Cx. gelidus	44	22	2.0 : 1
Cx. vishnui	3,876	1,790	2.2 : 1
Cx. pseudovishnui	1,541	686	2.2 : 1
Cx. tritaeniorhynchus	266	108	2.5 : 1

Table 3

Number of mosquitos collected biting man and carabao and their ratio.

Species	Man	Carabao	Ratio (man : carabao)
Cules quinquefasciatus	1,338	712	1.9 : 1
Mansonia uniformis	659	2,638	1 : 4.0
Cx. pseudovishnui	2,228	10,919	1 : 4.9
Cx. bitaeniorhynchus	319	1,967	1 : 6.2
Anopheles peditaeniatus	3,602	37,354	1 : 10.4
Cx. tritaeniorhynchus	375	14,509	1 : 38.7
Cx. vishnui	5,666	225,357	1 : 39.8
An. annularis	238	11,256	1 : 47.3
Cx. gelidus	66	6,079	1 : 92.1
An. subpictus	219	25,621	1 : 117.0
Cx. fuscocephala	802	122,766	1 : 153.1
An. vagus	27	8,775	1 : 325.0

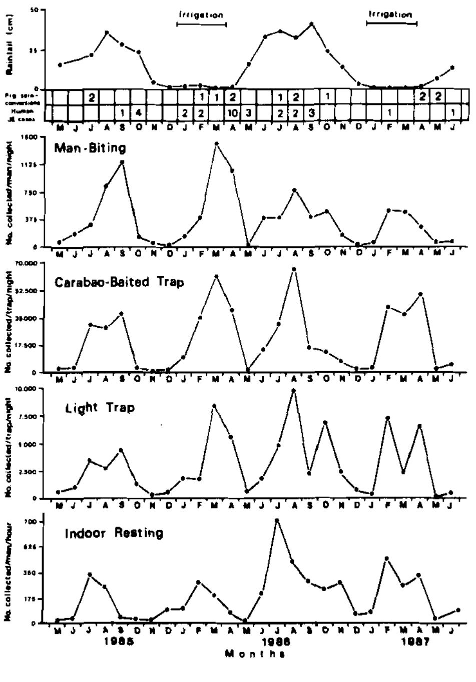

Fig 1—Comparison of 4 collecting techniques showing seasonal population changes. Top graph shows total rainfall for each month and period of irrigation. Number of pig sero-conversions and confirmed human JE cases each month are shown.

overall trends were the same. Both the rainy season and the irrigation period seem to produce comparable numbers of mosquitos.

During each of the rainy seasons and irrigation periods, there were about 3 months when mosquito abundance in the carabao- baited trap was elevated (Fig 1). This increase represents the accumulation of all species' population curves. By examining the population curves of individual species, more specific periods of abundance were recognized (Fig 2). Three distinct patterns were noted: 1) populations are high during the rainy season and very low during the irrigation period — *Cx. quinquefasciatus, An. vagus, Cx. bitaeniorhynchus,* and *Cx. tritaeniorhynchus;* 2) populations were equally strong during both periods — *Cx. fuscocephala, Cx. pseudovishnui,* and *Cx. vishnui;* 3) populations were highest during the irrigation periods — *An. subpictus, An. annularis,* and *An. peditaeniatus.* In the first group each of the first 3 species shows a slightly different period of dominance. *Cx. quinquefasciatus* reached its peak earliest in July. *Anopheles vagus* started to increase in July, attaining its peak in August and dropped to near zero in September. *Culex bitaeniorhynchus* showed a low population in July, peaking in August, still remaining high in September, and then dropping off in October. *Culex tritaeniorhynchus* had its peak population in August, while maintaining a substantial population during the irrigation period. In the second group, both peaks for *Cx. fusco-*

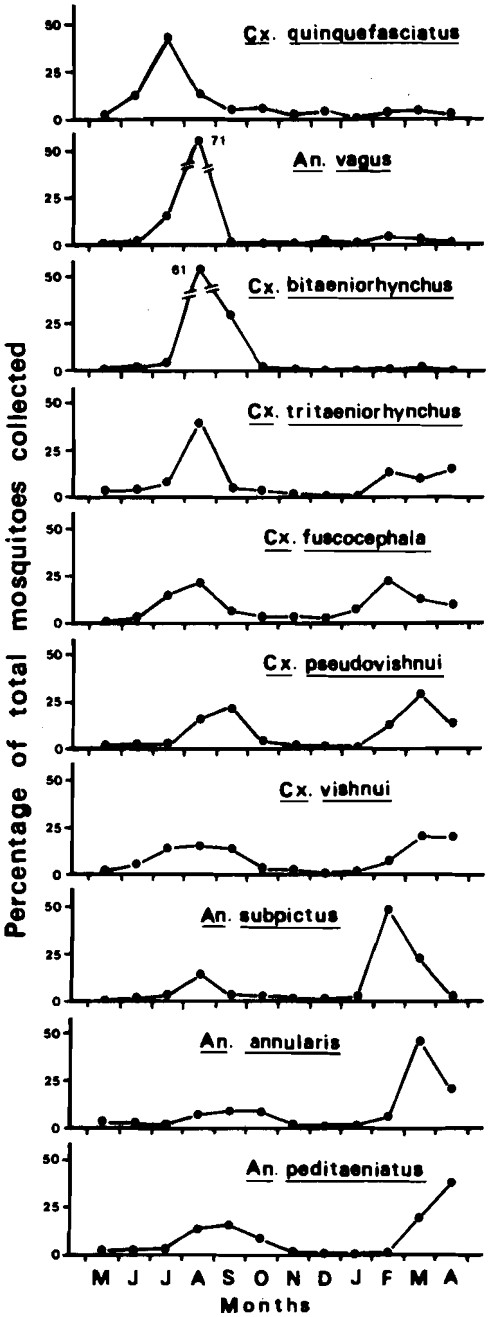

Fig 2—Comparison of 10 species showing seasonal population changes from carabao-baited traps.

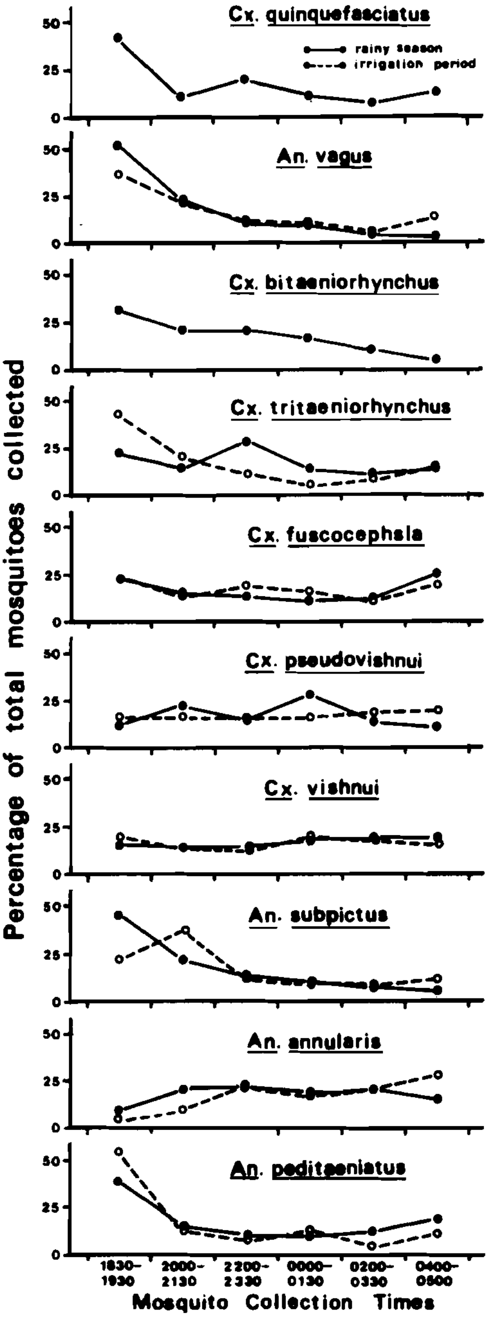

Fig 3—Comparison of 10 species showing nocturnal biting activity both during the rainy season and irrigation period. *Culex quinquefasciatus* and *Cx. bitaeniorhynchus* only show rainy season activity.

cephala shifted 1 month earlier in comparison to *Cx. pseudovishnui*. *Culex vishnui* did not have any peak months of activity and differed from the previous 2 species by maintaining a high population into April. The last group again showed shifting peak populations with *An. subpictus* peaking in February, *An. annularis* in March, and *An. peditaeniatus* in April. As the fields are flooded and the rice matures, the larval habitat continuously changes. Shifting abundance patterns probably represent the differing needs or limitations of larval habitats for each species. For example, peak larval populations of *Cx. quinquefasciatus* were found in early July, while those of *An. vagus* were in late July. At each stage of the rice crop, conditions are probably ideal for a few species and less ideal for many others. A similar succession of species in the *Cx. vishnui* group has been observed in rice fields in India (Reuben, 1971c).

The biting activity of mosquitos can also be generally grouped into 2 patterns: 1) those which feed primarily from 1800-0000 — *Cx. quinquefasciatus, An. vagus, Cx. bitaeniorhynchus, Cx. tritaeniorhynchus, An. subpictus*, and *An. peditaeniatus;* and 2) those that feed throughout the night — *Cx. fuscocephala, Cx. pseudovishnui, Cx. vishnui*, and *An. annularis* (Fig 3). Those that feed primarily from 1800-0000 usually are most active during the first hour after sunset. Some differences in biting activity were noted between the rainy season and the irrigation period, but the patterns were generally similar.

Of the different techniques used to monitor seasonal abundance of mosquitos, the animal-baited trap was the most advantageous. Large numbers of mosquitos can be collected in a single trap operated by 1 or 2 men. Because people are less attractive to mosquitos, many men are needed to obtain a sufficient quantity of mosquitos, and frequent supervision is necessary. Although light traps were efficient in mosquito collecting, they require dry ice which can be difficult or impossible to obtain in most areas.

Japanese encephalitis in the Philippines has been characterized as having no apparent seasonality (Okuno, 1978). Although this may be true when looking at the country as a whole, most localities probably do have seasonal patterns. Weather patterns within and between islands vary as do the number of crops grown each year. JE viral transmission, measured by both pig seroconversion at the study site and human JE cases within the province, generally show 2 periods of activity each year corresponding to the rainy season and irrigation period (Fig 1).

The relationship between rainfall, rice crops, and mosquito populations has been noted in several other Southeast Asian countries. The abundance of *Culex vishnui* in India closely followed the rainfall pattern, with highest populations during the monsoon season of October through January (Reuben, 1971a). In Taiwan two peak populations of *Cx. annulus* (= *Cx. vishnui*) were found each year corresponding to the spring and late summer planting of rice (Detels *et al*, 1976). *Culex tritaeniorhynchus* in the Philippines was shown to have 2 or 3 peak populations each year depending on whether 2 or 3 rice crops were grown each year (Reisen *et al*, 1976). In Indonesia, populations of *Cx. tritaeniorhynchus* and *Cx. gelidus* were found to be directly correlated with total monthly rainfall (Olson *et al*, 1983).

Just as rainfall, rice crops, and mosquito population are related, virus activity is also related to these 3 factors. In Thailand rainfall was shown to precede epidemics of JE by about 2 months (Thongcharoen, 1985). In India, peak populations of the primary JE vectors *Cx. vishnui* and *Cx. tritaeniorhynchus* were found to precede epidemics by 4 to 5 weeks (Mishra *et al*, 1984). The usefulness of sentinel pigs in monitoring virus activity and forewarning potential transmission to man has been demonstrated. Initial isolations from sentinel pigs in Taiwan were found to precede cases of JE in man by 3 to 6 weeks (Detels *et al*, 1976). Also in Bangkok, Thailand, human cases occurred 1 month following JE seroconversion in pigs (Gingrich *et al*, 1987). This interrelated process begins with the flooding of rice fields or other similar habitats by rain or irrigation. Mosquito populations increase rapidly, then mosquitos infect pigs which act as amplifying hosts, and finally mosquitos feed on infected pigs, thus transmitting the disease to man.

The vector(s) of JE virus at this field site is open to conjecture. There were at least 15 species collected that have been identified in other sites in Southeast Asia to contain the JE virus. The most likely candidates are *Cx. vishnui* and *Cx. fuscocephala* because of their very high populations.

Culex vishnui is a vector in Taiwan, Japan, and India, while *Cx. fuscocephala* is a vector in Taiwan and Thailand. Other mosquitos, especially *Cx. tritaeniorhynchus* and *Cx. pseudovishnui*, may be important in spite of lower populations.

ACKNOWLEDGEMENTS

The authors would like to thank the people of Cardenas who graciously allowed us to stay in their village and helped us with our collections. We would also like to thank the technicians of the Entomology and Virology Divisions, US Naval Medical Research Unit No. 2, whose dedication and hard work made this research possible.

This study was supported through funds provided by the Naval Medical Research and Development Command, Navy Department, for Work Unit 3M161102BS10.AF429.

REFERENCES

Apiwathnasorn C. A list of mosquito species in Southeast Asia. SEAMEO-TROPMED, Mahidol University, Bangkok, Thailand. 1986; 74 pp.

Burke DS, Lorsomrudee W, Leake CJ, *et al.* Fatal outcome in Japanese encephalitis. *Am J Trop Med Hyg* 1985; 34 : 1203-10.

Clark DH, Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. *Am J Trop Med Hyg* 1958; 7 : 561-73.

Denning DW, Kaneko Y. Should travelers to Asia be vaccinated against Japanese encephalitis? *Lancet* 1987; 1 : 853-4.

Detels R, Cross JH, Huang WC, Lien JC, Chen S. Japanese encephalitis virus in northern Taiwan, 1969-1973. *Am J Trop Med Hyg* 1976; 25 : 477-85.

Gingrich JB, Nisalak A, Latendresse JR, *et al.* A longitudinal study of Japanese encephalitis in suburban Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 1987; 18 : 558-66.

Hayes CG, O'Rourke TF, San Luis AM, *et al.* Epidemiology of Japanese encephalitis in the Philippines. *Philippine J Microbiol Infect Dis* 1986; 15 : 35.

Ksiazek TG, Trosper JH, Cross JH, Basaca-Sevilla V. Additional isolations of Japanese encephalitis virus from the Philippines. *Southeast Asian J Trop Public Health* 1980; 11 : 507-9.

Mishra AC, Jacob PG, Ramanujam S, Bhat HR, Pavri KM. Mosquito vectors of Japanese encephalitis epidemic (1983) in Mandya district (India). *Indian J Trop Med Res* 1984; 80 : 377-89.

Okuno T. An epidemiological review of Japanese encephalitis. *WHO Stat Rep* 1978; 31 : 120-31.

Olson JG, Atmosoedjono S, Lee VH, Ksiazek TG. Correlation between population indices of *Culex tritaeniorhynchus* and *Cx. gelidus* (Diptera: Culicidae) and rainfall in Kapuk, Indonesia. *J Med Entomol* 1983; 20 : 108-9.

Olson JG, Ksiazek TG, Lee VH, Tan R, Shope RE. Isolation of Japanese encephalitis virus from *Anopheles annularis* and *Anopheles vagus* in Lombok, Indonesia. *Trans R Soc Trop Med Hyg* 1985; 79 : 845-7.

Reid JA. The attraction of mosquitoes by human or animal baits in relation to the transmission of disease. *Bull Ent Res* 1961; 52 : 43-62.

Reisen WK, Aslamkhan M, Basio RG. The effects of climatic patterns and agricultural practices on the population dynamics of *Culex tritaeniorhynchus* in Asia. *Southeast Asian J Trop Med Public Health* 1976; 7 : 61-71.

Reuben R. Studies on the mosquitoes of North Arcot District, Madras State, India, Part 1. Seasonal densities. *J Med Entomol* 1971a; 8 : 119-26.

Reuben R. Studies on the mosquitos of North Arcot District, Madras State, India. Part 2. Biting cycles and behavior on human and bovine bait at two villages. *J Med Entomol* 1971b; 8 : 127-34.

Reuben R. Studies on the mosquitoes of North Arcot District, Madras State, India. Part 5. Breeding places of the *Culex vishnui* group of species. *J Med Entomol* 1971c; 8 : 363-6.

Rosen L. The natural history of Japanese encephalitis virus. *Annu Rev Microbiol* 1986; 40 : 395-414.

Sever JL. Application of microtechnique to viral serological investigations. *J Immunol* 1962; 88 : 320-9.

Thongcharoen P. Japanese encephalitis in Thailand. *J Med Assoc Thai* 1985; 68 : 534-44.

Trosper JH, Ksiazek TG, Cross JH, Basaca-Sevilla V. Isolation of Japanese encephalitis virus from the Republic of the Philippines. *Trans R Soc Trop Med Hyg* 1980; 74 : 292-5.