

## STUDIES ON JAPANESE ENCEPHALITIS VECTOR MOSQUITOES IN SELANGOR, MALAYSIA

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**ABSTRACT.** Mosquito collections were carried out from May to June 1992 and from September to December 1992 in an area where a case of Japanese encephalitis was confirmed. A total of 40,072 mosquitoes belonging to 35 species and 8 genera were collected. The dominant species in that locality were *Culex vishnui*, *Culex tritaeniorhynchus*, *Culex pseudovishnui*, *Culex gelidus*, *Aedes butleri*, and *Mansonia uniformis*.

Japanese encephalitis (JE) virus is common over a wide part of Asia. The natural transmission cycle involves *Culex tritaeniorhynchus* Giles and other closely related *Culex* mosquitoes that breed primarily in rice fields and feed on man in the early evenings (Shope 1990). Human cases of Japanese encephalitis follow temporally the peak in *Cx. tritaeniorhynchus* mosquitoes. Japanese encephalitis is endemic and occurs sporadically throughout Malaysia all year around (Fang et al. 1980). Other studies in peninsular Malaysia were carried out primarily in the 1950s (Cruikshank 1951). However, the situation in Sarawak has been studied more recently by Simpson et al. (1970, 1974) and Macdonald et al. (1965, 1967). When one positive case of JE was confirmed from Kampong Pasir Panjang, Sabak Bernam District, Selangor, Malaysia, it was decided to carry out entomological surveys to determine the vectors and attempt to isolate the virus. This study describes the entomological surveys.

The study area is situated in Kampong Pasir Panjang, which is 80 km northwest of Kuala Lumpur. This is an open village ecotype; the land is flat and in some areas low lying. The houses are mostly spread out, each built on a large piece of land surrounded by coconut palms and other fruit trees. There are also large areas of rice fields directly opposite the houses, on the other side of the road, adjoining the village.

This study was carried out in 2 phases, the first during the very dry season of May-June and the second during the wet season of September-December 1992. The study was standardized as follows: CDC battery-operated light traps with CO<sub>2</sub> were used throughout the study. The traps were operated each night from 1800 to 0700 h. Light trap bags were changed at intervals of 2 h. Carbon dioxide was obtained from a piece of dry ice placed in a wooden box suspended adjacent to

the light trap. The light traps were hung on trees or poles outdoors.

Landing catches on human bait were conducted outdoors near houses by a team of 3 men commencing at 1900 h and terminating at 2100 h. The men using flashlights actively collected mosquitoes landing on them in 50 × 19-mm vials, plugging the mouth of the tube with cotton wool.

All mosquitoes caught in the tubes were identified alive, grouped according to species, recorded, and then killed by placing them on dry ice. The mosquitoes in the trap bags were also killed on dry ice and then sorted according to species, recorded, and pooled. Pools of mosquitoes, 50 per tube, were then placed in liquid nitrogen and returned to the laboratory for virus isolation. The results of virus isolation attempts will be reported elsewhere.

A total of 40,072 mosquitoes was caught during the study. *Culex* species constituted 75.2% of the total catch; this was followed by *Aedes* species (19.6%). *Aedes* were more attracted to human bait than to light traps. They composed 32.6% of the total catch in human bait collections.

*Culex tritaeniorhynchus* was the main species obtained in light traps, composing 28.7%; this was closely followed by *Culex vishnui* Theobald, which composed 27.4% (Table 1). However, in the human landing catch *Aedes butleri* Theobald was the predominant species, composing 30.8% of the catch (Table 2). This was followed by *Anopheles sinensis* Wied. (13.5%) and *Mansonia uniformis* (Theobald) (12.2%).

The total number of *Cx. tritaeniorhynchus* exceeded that of *Cx. vishnui* by only about 500. However from May to October, *Cx. vishnui* was found in larger numbers than *Cx. tritaeniorhynchus*. *Aedes butleri* was the predominant species in human bait collections, followed by *Mansonia* spp. This shows that both these species are attracted more to humans than to light traps. The peak collection time for all species was between 1900 and 2100 h. *Culex tritaeniorhynchus* and

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Table 1. Number of mosquitoes collected for the predominant species in Kampong Pasir Panjang from May to December 1992.

Month	<i>Culex tritaeniorhynchus</i>	<i>Culex gelidus</i>	<i>Culex pseudovishnui</i>	<i>Culex vishnui</i>	<i>Aedes butleri</i>	<i>Mansonia</i> spp.
May	517 (28.7) <sup>1</sup>	31 (1.7)	371 (20.6)	1,001 (55.6)	471 (26.2)	173 (9.6)
June	51 (2.7)	73 (3.8)	46 (2.4)	97 (5.1)	10 (0.5)	19 (1.0)
Sept.	2,415 (219.5)	792 (72.0)	1,839 (167.2)	3,300 (300.0)	42 (3.8)	108 (9.8)
Oct.	2,067 (206.7)	321 (32.1)	856 (85.6)	2,758 (275.8)	1,551 (155.1)	118 (11.8)
Nov.	4,635 (662.1)	382 (54.6)	669 (99.9)	2,940 (420.0)	2,926 (418.0)	350 (50.0)
Dec.	1,116 (558.0)	37 (18.5)	58 (29.0)	203 (101.5)	1,884 (942.0)	7 (3.5)

<sup>1</sup> Average number/trap/night.

*Cx. vishnui* showed a gradual decline from 2100 to 0100 h, whereas *Cx. butleri* declined sharply after 2100 h. These main species were caught throughout the night but in lower numbers from 0300 to 0700 h.

There was a positive correlation between rainfall and the 3 predominant species (*Cx. tritaeniorhynchus*, *Cx. vishnui*, and *Culex pseudovishnui* Colless). These species are dependent on rainfall. During the dry months of May and June *Cx. vishnui* was found in greater numbers than *Cx. tritaeniorhynchus* but during the wet months *Cx. tritaeniorhynchus* was the dominant species.

The dominant species in this rice field ecosystem was *Cx. tritaeniorhynchus*. In Sarawak *Cx. tritaeniorhynchus* was also the predominant species in this ecosystem (Macdonald et al 1965, 1967; Hill 1970) and the greatest number of virus isolates was obtained from *Cx. tritaeniorhynchus* (Simpson et al. 1970).

In Sarawak, JE virus has also been isolated from *Culex gelidus* Giles, *Ma. uniformis*, *Mansonia* spp., and *Anopheles* spp. (Simpson et al. 1970). In India, JE virus has been isolated from *Culex bitaeniorhynchus* Giles, *Culex pseudovishnui* Giles, *Cx. vishnui*, *Anopheles barbirostris* Van der Wulp, *Anopheles subpictus* Grassi, *Mansonia annulifera* Theobald, *Cx. gelidus*, *Culex fuscocephala* Theobald, and *Culex quinquefasciatus* Say (Rodrigues et al. 1980, Chakravarti et al.

1981, George et al. 1987, Mourya et al. 1989). Most of these species were present in our study area.

Macdonald et al. (1967) showed that *Cx. tritaeniorhynchus* bites pigs more readily than humans. In our study *Cx. tritaeniorhynchus* was caught in small numbers compared to other species in the human landing catches. In our study, landing catches were carried out for only 2 h. All night catches should be conducted using pig-baited traps to determine the peak biting time and the man : pig biting ratio. Maximum collections of *Cx. tritaeniorhynchus* in light trap collections occurred in the early hours of the night when people are most active and no protective measures are taken.

Seasonal distribution of the mosquito species collected from the traps varied. Certain species such as *Cx. gelidus*, *Cx. vishnui*, and *Cx. pseudovishnui* showed population peaks in September, but the predominant species, *Cx. tritaeniorhynchus*, showed a peak population in November. Because the incidence of JE virus in this country is low and fairly evenly distributed throughout the year (Fang et al. 1980), further extensive collections should be carried out in order to correlate vector peaks and disease transmission.

*Culex tritaeniorhynchus* constituted 28.7% of the light trap collections but the actual number

Table 2. Number of mosquitoes collected landing on human bait for predominant species in Kampong Pasir Panjang from May to December 1992.

Month	<i>Culex tritaeniorhynchus</i>	<i>Culex gelidus</i>	<i>Culex pseudovishnui</i>	<i>Culex vishnui</i>	<i>Aedes butleri</i>	<i>Mansonia</i> spp.
May	26 (1.6) <sup>1</sup>	19 (1.2)	35 (2.2)	43 (2.7)	297 (18.6)	116 (7.3)
June	19 (1.7)	43 (3.9)	27 (2.5)	22 (2.0)	9 (0.8)	33 (3.0)
Sept.	23 (2.6)	23 (2.6)	65 (7.2)	66 (7.3)	6 (0.7)	27 (3.0)
Oct.	7 (1.2)	3 (0.4)	7 (1.2)	11 (1.8)	295 (49.2)	14 (2.3)
Nov.	7 (1.2)	4 (1.3)	6 (2.0)	7 (2.3)	62 (20.7)	33 (11.0)
Dec.	36 (12.0)	18 (6.0)	4 (1.3)	6 (2.0)	87 (29.0)	11 (3.7)

<sup>1</sup> Average number/man/night.

of JE cases was not known. If these studies are continued together with case detection, it should be possible to determine what percentage of the overall mosquito captures *Cx. tritaeniorhynchus* has to reach before a severe epidemic could occur.

Light trap collections give an indication of what species of mosquitoes are abundant and the seasonal distribution of these species. However, light traps alone should not be considered the basis of a surveillance system for Japanese encephalitis virus. They should be supplemented by antibody surveys in animals.

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#### REFERENCES CITED

- Chakravarti, S. K., A. K. Chakraborty, K. K. Mukherjee, A. C. Mitra, A. K. Hati and M. S. Chakraborty. 1981. Isolation of Japanese encephalitis virus from *Mansonia annulifera* species of mosquitoes in Assam. *Bull. Calcutta Sch. Trop. Med.* 29: 3-4.
- Cruikshank, E. K. 1951. Acute encephalitis in Malaya. *Trans. R. Soc. Trop. Med. Hyg.* 45:113-118.
- Fang, R., Hsu and T. W. Lim. 1980. Investigation of a suspected outbreak of Japanese encephalitis in Pulau Langkawi. *Malays. J. Pathol.* 3:23-30.
- George, S., J. P. George and J. A. Rao. 1987. Isolation of Japanese encephalitis in West Nile viruses from mosquitoes collected in Kolar district of Karnataka State during 1977-79. *Indian J. Med. Res.* 85:235-238.
- Hill, M. N. 1970. Japanese encephalitis in Sarawak; studies on adult mosquito populations. *Trans. R. Soc. Trop. Med. Hyg.* 64:489-496.
- Macdonald, W. W., C. E. G. Smith and H. E. Webb. 1965. Arbovirus infections in Sarawak; observations on the mosquitoes. *J. Med. Entomol.* 4:335-347.
- Macdonald, W. W., C. E. G. Smith, P. S. Dawson, A. Ganapathipillai and S. Mahadevan. 1967. Arbovirus infections in Sarawak: further observations on mosquitoes. *J. Med. Entomol.* 4:146-157.
- Mourya, D. T., M. A. Ilkal, A. C. Mishra, P. G. Jacob, U. Pant, S. Ramanujam, M. S. Mavak, R. Bhat and V. Dhanda. 1989. Isolation of Japanese encephalitis virus from mosquitoes collected in Karnataka State, India from 1985-1987. *Trans. R. Soc. Trop. Med. Hyg.* 83:550-552.
- Rodrigues, F. M., P. Bright Singh, R. S. Danda Soman, S. N. Guttikar and H. N. Kaul. 1980. Isolation of Japanese encephalitis and West Nile from mosquitoes collected in Andhra Pradesh. *J. Parasitol.* 4:149-153.
- Shope, R. E. 1990. Epidemiology and control of dengue fever and Japanese encephalitis. *WPR/PHC/CDS* 1/90.79.
- Simpson, D. I. H., E. T. Bowen, H. J. Way, G. S. Platt, M. N. Hill, S. Kamath, T. W. Lim, P. J. E. Bendell and O. H. U. Heathcote. 1974. Arbovirus infections in Sarawak, October 1968-February 1970; Japanese encephalitis virus isolations from mosquitoes. *Ann. Trop. Med. Parasitol.* 68:393-404.
- Simpson, D. I. H., C. E. G. Smith, E. W. Bowen, G. S. Platt, H. Way, D. McMahon, W. F. Bright, N. M. Hill, S. Mahadevan and W. W. Macdonald. 1970. Arbovirus infections in Sarawak: virus isolations from mosquitoes. *Ann. Trop. Med. Parasitol.* 64:137-151.