ACTIVITY OF TEMBUSU AND UMBRE VIRUSES IN A MALAYSIAN COMMUNITY: MOSQUITO STUDIES[1]

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ABSTRACT. Either tembusu or Umbre virus or both were recovered from three species of the *Culex (Culex) vishnui* subgroup of mosquitoes: *Cx. vishnui, Cx. tritaeniorhynchus* and *Cx. pseudovishnui*, which were collected in and near a chicken farm at the Dusun Tua study site in Malaysia. *Cx. vishnui*, the most abundant species collected, was more successfully attracted to light traps augmented by CO_2 than to any of the other five collection methods used. Ten out of 12 tembusu and four out of six Umbre virus strains were isolated from *Cx. vishnui*, the majority of which were recovered during June, the peak month for mosquito collections. Tembusu virus activity appeared in late April and continued throughout July, whereas Umbre was active in June and July. Tembusu and Umbre viruses appear to utilize the same hosts, *Cx. vishnui* subgroup mosquitoes and domestic fowl in their transmission cycles.

INTRODUCTION. Epidemiologic studies of tembusu and Umbre viruses were conducted in the Dusun Tua Youth Training Centre in Ulu Langat, Selangor State, Malaysia to determine the extent and significance of human infections. Tembusu, a group B arbovirus, was first isolated in Peninsular Malaysia in 1955 from *Culex (Culex) tritaeniorhynchus* (Institute for Medical Research, Annual Report 1957). In the past 14 years, isolations have been reported in peninsular Malaysia (University of California International Center for Medical Research and Training, [UC-ICMRT] Annual Progress Report 1972), Sarawak (Simpson et al. 1970) and Thailand (International Catalogue of Arboviruses, [ICA]1975a) mainly from *Cx. tritaeniorhynchus* and 2 other species of the *vishnui* subgroup: *Cx. vishnui* and *Cx. pseudovishnui*, and from *Cx. gelidus* of the *gelidus* subgroup. *Cx. vishnui* was identified as *Cx. annulus* in Malaysia until Sirivanakarn (1975) considered the latter as a synonym. Tembusu antibody has been found in man in Malaysia although disease associated with infection has yet to be reported. Simpson et al. (1970) reported high levels of neutralizing antibody in domestic fowl in Sarawak. All the mosquito species mentioned are known to be the principal or potential vectors of Japanese encephalitis virus which causes severe and often fatal disease in man.

The first isolation of Umbre, a Turlock group virus, was made in India in 1955 from *Cx. bitaeniorhynchus* (ICA 1975b). Since then, Umbre virus has been isolated from *Cx. vishnui* subgroup mosquitoes in southern India (Dandawate et al. 1969) and Malaysia (UC-ICMRT 1972). The discovery by the UC-ICMR Arbovirus Research Unit in Kuala Lumpur of a dual infection of tembusu and Umbre viruses in a sentinel chicken led to the conclusion that in Malaysia both viruses may utilize the same hosts, birds, and mosquitoes of the *vishnui* subgroup in their transmission cycles.

A one-year investigation was con-

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ducted to study antibody prevalence, infection rates and febrile or inapparent infections in a population of vocational trainees residing at the Dusun Tua Centre. Also, transmission studies of these viruses were initiated simultaneously. A sentinel chicken flock was maintained to determine transmission indices by virus isolation and antibody changes, and wild birds were trapped monthly for antibody studies. Potential vector populations, mainly *Culex* (*Culex*) species, were collected for virus isolation attempts.

MATERIALS AND METHODS. The Dusun Tua Youth Training Centre, which is located 25 miles east of Kuala Lumpur, was chosen as the study site because tembusu and 2 strains of Umbre virus had been recovered from pools of *Cx. vishnui* and *vishnui* subgroup mosquitoes collected there during an investigation conducted in 1970 (UC-ICMRT 1971). The center is in an inland rural area that is traversed by the main road and by a tidal river, the Sungai Langat, along its western perimeter (Figure 1). Barracks, offices and training buildings occupy the main areas of the center with crops planted behind the barracks to the north and a chicken farm located to the south. *Lalang*—tall grass—and brush surround the fields and a rubber tree forest lies behind the farm, extending over half of the southeast perimeter of the center. Secondary forest exists in abandoned portions of the rubber forest. A half mile north of the center is a large Malay village.

The population of young adult males at the center were tested for antibodies, and a large flock of birds from the chicken farm on the premises was used as sentinels and as bait for mosquito collections. The chicken farm is adjacent to small fish ponds and the rubber forest. The pen for the sentinel chickens is located near the chicken houses. Wild birds were mist-netted near the farm and in the nearby secondary forest.

Adult mosquito collections were made twice weekly from August 28, 1973 to August 29, 1974 by 6 different methods. These included modified CDC light traps, Magoon traps with sentinel chickens as bait, malaise traps, a sentinel chicken pen trap modified after Rainey et al. (1962), and daytime sweep collections with hand-operated vacuum aspirators of mosquitoes resting on vegetation or in chicken houses. Tree hole collections were also made. The light, Magoon, and Malaise traps were augmented with blocks of dry ice in most of the 1974 collections. Mosquitoes were identified with the aid of a stereoscopic microscope and pooled by species. Freshly engorged specimens were held for 24 hr so that blood meals would be digested before pooling, or the specimens were dissected and the abdomens discarded. Some of the abdomens were held for subsequent blood meal determination by precipitin test. Pools were stored at −70°C until they were processed for detection of virus.

Species of the *vishnui* subgroup and other suspect vector species were pooled into groups of 50 specimens each; the rest, including *Cx. quinquefasciatus* Say (=*fatigans*) were pooled into groups of 100 or more. A diluent of 3 ml phosphate-buffered saline with 25% normal rabbit serum and antibiotics (1000 U/ml penicillin and 1600 μg/ml streptomycin) was added to each mosquito pool in a chilled mortar and ground. Triturated pools were centrifuged for 20 min. at 2500 RPM at 4°C. Each of a litter of eight 1 to 2-day-old suckling mice was inoculated intracerebrally with 0.01 ml and intraperitoneally with 0.03 ml of the supernatant. During the observation period of 14 days, brains of sick or dying mice were harvested and stored at −70°C.

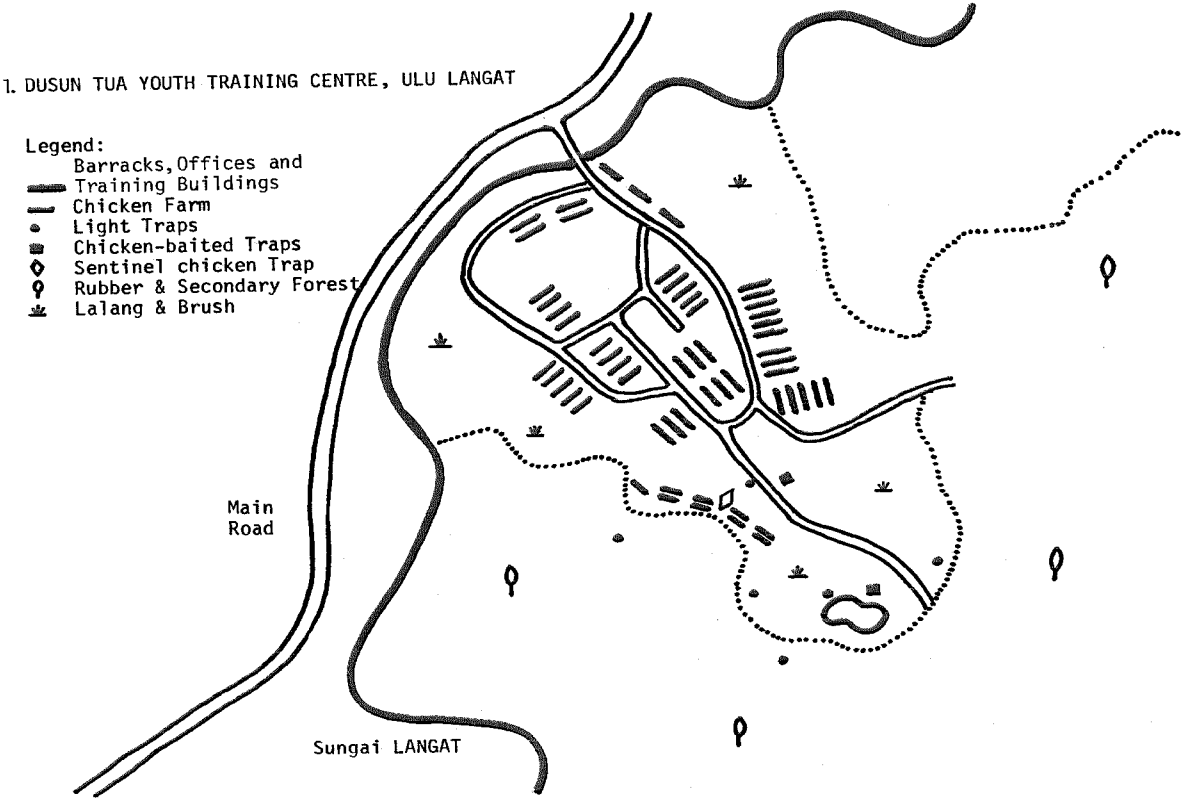

Fig. 1. Dusun Tua Youth Training Centre, ULU Langat.

All subsequent passages were made by inoculating fresh litters of suckling mice with 20% suspensions of triturated mouse brain in 0.75% bovalbumin phosphate saline with penicillin and streptomycin.

Final identification of virus isolates was made by plaque reduction neutralization tests on Vero cells. Specific hyperimmune mouse sera prepared against prototype strains of tembusu and Umbre viruses, AMM–1775 and Ig–1424, respectively, were used against all isolates.

RESULTS. Of the total of 23,853 mosquitoes representing 48 species that were collected, nine species of the *Cx. (Culex)* subgenus made up 91.7% (21,863) of the collection (Table 1). Mosquitoes from the *vishnui* subgroup comprised 53.6% (11,723) of the *Cx. (Culex)* mosquitoes collected, while *Cx. quinquefasciatus* was second most common, 34.3% (7,503); *Cx. pseudosinensis* made up 6.5% (1,413), *Cx. gelidus* 3.7% (801) and the remaining species 1.9% (423). Of the *vishnui* subgroup, *Cx. vishnui* was collected in the greatest numbers, 9,711 or 82.8%. A total of 482 *Cx. tritaeniorhynchus* or 4.1% and 300 *Cx. pseudovishnui* or 2.6% were collected. Classified as probable *Cx. vishnui* were 1,230 or 10.5%.

Of the resting *Culex* (*Culex*) mosquitoes taken by sweeping chicken houses in the daytime, 86.8% were *Cx. quinquefasciatus*. In contrast, sweeping vegetation outside the chicken houses in the evening yielded 78.0% *Cx. vishnui*. There were indications that certain species were attracted to or preferred certain types of traps: 55.0% of the *Cx. vishnui* were collected in the CO_2 light traps as were 62.2% of the *Cx. tritaeniorhynchus*. *Cx. gelidus* were attracted to both the light and Malaise traps, 31.5% and 27.6%, respectively, while *Cx. pseudovishnui* were found in the light and chicken-baited Magoon traps in similar numbers.

Twelve strains of tembusu and 6 strains of Umbre virus were isolated in suckling mice from 406 pools of mosquitoes, mainly *Cx. (Culex)* species. Ten of the 12 strains of tembusu and 4 of the 6 strains of Umbre were recovered from *Cx. vishnui*. The other two tembusu strains were recovered from *Cx. tritaeniorhynchus* and Umbre strains from both *Cx. pseudovishnui* and *Cx. pseudosinensis* (Table 2). A single pool of *Cx. vishnui* yielded strains of both viruses. All of these species are members of the *vishnui* subgroup except *Cx. pseudosinensis*. The identification of the mosquitoes in the *Cx. pseudosinensis* pool, however, is open to question since some *Cx. pseudovishnui* may have been included in that particular pool by error inadvertently.

Twelve of the virus strains were recovered from mosquitoes collected in light traps only, 4 from mosquitoes from pooled sources that included light trap collections and 1 from mosquitoes in a chicken-baited trap. Four strains of tembusu and 1 Umbre strain were recovered from mosquitoes collected from a single light trap near a shallow fish pond.

Tembusu strains were isolated from late April to August, with a peak of activity in June; Umbre isolates were made in June and July (Table 3). Infection rates of *Cx. vishnui* with tembusu virus were high from April to June: 1:432 for April, 1:556 for May, 1:566 for June and 1:2037 for July. Infection rates with Umbre were 1:1131 for June and 1:2037 for July. Overall *Cx. vishnui* infection rates with tembusu virus were 1:971 and with Umbre were 1:2428.

Sentinel chicken studies showed that almost two-thirds of the chickens exposed in a special sentinel pen were infected by 4 different viruses during the year. Infection rates were high for

Table 1. *Culex* (*Culex*) species of mosquitoes collected by various methods, Dusun Tua, Ulu Langat, August 1973–August 1974.

Species	Light trap with CO_2	Chicken-baited trap		Malaise trap with CO_2	Sentinel chicken trap	Sweeping collection		Other	All methods
		no CO_2	with CO_2			Vegetation	Coops and buildings		
Cx. vishnui	5,343	204	1,743	441	1,010	585	377	8	9,711
tritaeniorhynchus	300	15	27	60	29	24	26	1	482
pseudovishnui	99	66	86	11	18	8	5	7	300
"*vishnui*" group	200	264	..	..	479	10	266	11	1,230
bitaeniorhynchus	67	23	140	4	3	2	4	..	243
pseudosinensis	492	89	609	68	34	25	94	2	1,413
gelidus	252	11	67	221	39	68	140	3	801
mimulus	..	1	..	..	..	..	..	..	1
quinquefasciatus	12	727	117	13	517	25	6,032	60	7,503
fuscocephala	28	1	1	12	3	2	3	..	50
spp.	8	25	82	..	6	1	..	7	129
All species	6,801	1,426	2,872	830	2,138	750	6,947	99	21,863
No. of collections	148	171	34	41	84	44	173	240	935

Table 2. Distribution of virus strains by mosquito species, month and collection method, Dusun Tua, Ulu Langat.

Virus strain	Identification	Isolated from	Month (1974)	Collection method*			
				LT	BT	MT	SW
P74–1064	TMU	*Cx. vishnui*	Apr/May	X	X		
1065	TMU	*Cx. vishnui*	May	X	X		X
1060	TMU	*Cx. tritaeniorhynchus*	May	X			
1070	TMU	*Cx. vishnui*	May	X	X		X
1477	UMB	*Cx. vishnui*	Jun	X			
1283	TMU	*Cx. tritaeniorhynchus*	Jun	X			
1284	TMU	*Cx. vishnui*	Jun	X			
1299	TMU	*Cx. vishnui*	Jun	X			
1302	TMU	*Cx. vishnui*	Jun	X			
1285	UMB	*Cx. vishnui*	Jun	X			
937	TMU/UMB	*Cx. vishnui*	Jun	X			
950	TMU	*Cx. vishnui*	Jun	X			
955	TMU	*Cx. vishnui*	Jun	X			
1339	TMU	*Cx. vishnui*	Jul	X			
1318	UMB	*Cx. pseudosinensis* (?)	Jul	X			
1329	UMB	*Cx. vishnui*	Jul		X		
1333	UMB	*Cx. pseudovishnui*	Jul	X	X	X	X

* LT=light trap with CO_2; BT=chicken-baited trap with CO_2; MT=malaise trap with CO_2, and SW=sweeping vegetation and/or coops.

tembusu and Umbre and low for Sindbis and Japanese encephalitis. Ten virus strains recovered from sera drawn just prior to the serologic conversions included tembusu, Umbre and Sindbis.

Sera from wild birds captured around the chicken farm and in the nearby secondary forest showed neutralizing antibody levels to Umbre only. One pegu house sparrow, *Passer flaveolus* that was Umbre-negative in April was positive on recapture 2 months later in June. Besides the house sparrow, several other species of wild birds were found to have Umbre antibody.

Specific Umbre neutralizing antibody was demonstrated in the human population studied and several serologic conversions occurred, indicating current infection in Dusun Tua. This is the first evidence of Umbre antibody in man in Malaysia. While a low prevalence of Umbre antibody was reported by Banerjee et al. (1973) in south India, current infection in man has not previously been reported. Neutralizing antibodies to group B arboviruses, including tembusu, were

Table 3. Virus isolations from *Culex vishnui* subgroup mosquitoes, Dusun Tua, Ulu Langat, August 1973–August 1974.

Species	Month collected	Number tested	Mosquito field infection rate	
			Tembusu	Umbre
Cx. vishnui	Apr	432	1 (1:432)	
	May	1112	2 (1:556)	
	Jun	3393	6 (1:566)	3 (1:1131)
	Jul	2037	1 (1:2037)	1 (1:2037)
Cx. tritaeniorhynchus	May	146	1 (1:146)	
	Jun	108	1 (1:108)	
Cx. pseudovishnui	Jul	108		1 (1:108)

demonstrated in over 50% of the human sera tested and antibodies to group A viruses (chikungunya and Sindbis) were present in lower percentages. Japanese encephalitis and Sindbis virus infections were also detected in the trainees in the center.

DISCUSSION. Tembusu and Umbre viruses were recovered from 3 species of the *Cx. vishnui* subgroup: *Cx. vishnui, Cx. tritaeniorhynchus* and *Cx. pseudovishnui*. The most abundant species collected during the year's study was *Cx. vishnui* and the majority of the strains of tembusu and Umbre viruses were isolated from this species.

From September 1973 to April 1974, *Cx. vishnui* mosquitoes were collected in low numbers. The number of *Cx. vishnui* collected increased in May, reached a peak in June and then maintained high levels until the end of the study in August 1974. Changes and improvements in the collection methods in May 1974 may have contributed to the increased numbers. The number of viruses isolated from the mosquitoes coincided with the increase in mosquitoes collected; the majority of isolations of tembusu virus strains were made in June and of Umbre in June and July. Mosquito infection rates for *Cx. vishnui* were high just before and during the peak month. However, rates for *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* were also very high in view of the numbers of these species collected and tested.

The majority of *Cx. vishnui* and *Cx. tritaeniorhynchus* and one-third of the *Cx. pseudovishnui* were collected in light traps augmented with CO_2. Almost all of the virus strains came from mosquitoes collected by light traps or pooled sources that included a light trap collection.

The *Cx. vishnui* subgroup generally breed in ground water collections. According to Bram (1967), larvae of *Cx. vishnui* have been collected from puddles in dry stream beds, rice fields, ponds, ditches and potholes; *Cx. tritaeniorhynchus* from a wide variety of temporary and semipermanent ground water habitats and from streams and swamps, and *Cx. pseudovishnui* from similar habitats. The physical environment of the Dusun Tua center is favorable for the breeding of all these species.

Colless (1959) reported the feeding preferences for the *Cx. vishnui* subgroup. *Cx. tritaeniorhynchus* feed mainly on cattle and pigs. However, in the absence of both, they may feed on birds and man. It has also been reported that *Cx. pseudovishnui* feed primarily on cattle, birds and pigs. Christopher and Reuben (1971) showed that although *Cx. vishnui* are strongly attracted to and feed on cattle, they feed on birds to a greater extent than *Cx. tritaeniorhynchus*. All 3 species were shown in precipitin tests to feed on humans, *Cx. vishnui* in the highest proportion. Persistent infection of sentinel chickens by tembusu and Umbre viruses and low-level infection of trainees by Umbre support these preferences. However, since cattle and pigs were not kept at the Dusun Tua Training Centre, this feeding preference could not be confirmed.

Our studies confirm that the *Cx. vishnui* subgroup and domestic fowl play important roles in the transmission cycles of tembusu and Umbre viruses in Malaysia. To determine whether these viruses cause only incidental infections as suggested by Platt et al. (1975) or actual disease in man requires further study.

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