

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 42 Mosquito-borne Viruses in Other Areas of Thailand.

Project No. 3A 025601 A 811 Military Medical Research Program  
S.E. Asia

Task 01: Military Medical Research Program  
S.E. Asia

Subtask 01: Military Medical Research Program  
SEASIA (Thailand)

Reporting Installation US Army-SEATO Medical Research Laboratory  
APO 146, San Francisco, California  
Division of Medical Research Laboratories  
Department of Medical Entomology

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Major John E. Scanlon, MSC

Assistant Investigators: Mr. Sumeth Chunchulcherm  
Mr. Kol Mongkolpanya

Reports Control Symbol: MEDDH-288

Security Classification: UNCLASSIFIED



strains of dengue virus had been isolated from A. aegypti collected in Southeastern Thailand during an epidemic. The distribution of A. aegypti in Thailand considerably exceeded the known distribution of hemorrhagic fever, and the species was very abundant in some areas which appeared to have no cases. Culex gelidus and C. tritaeniorhynchus, potential vectors of encephalitis virus, were found in many parts of the country, often in fairly heavily forested areas. In areas with heavy vegetation other Stegomyia species, particularly Aedes albopictus tended to replace A. aegypti. A complete interpretation of the collection data will be published when the virological procedures have been completed.

BODY OF REPORT

SEATO Medic Study No. 42 Mosquito-borne Viruses in Other Areas  
of Thailand

Project No. 3A 025601 A 811 Military Medical Research Program  
S.E. Asia

Task 01: Military Medical Research Program  
S.E. Asia

Subtask 01: Military Medical Research Program  
SEASIA (Thailand)

Reporting Installation: US Army-SEATO Medical Research Laboratory  
APO 146, San Francisco, California  
Division of Medical Research Laboratories  
Department of Medical Entomology

Period Covered by Report: 1 April 1963 to 31 March 1964

Principal Investigator: Major John E. Scanlon, MSC

Assistant Investigators: Mr. Sumeth Chunchulcherm  
Mr. Kol Mongkolpanya

Report Control Symbol: MEDDH-280

Security Classification: UNCLASSIFIED

FINAL REPORT

Objective: To obtain frozen female mosquitoes from a variety to ecological situations in different parts of the country for attempted virus isolation. In addition, observations are made on the distribution of several important mosquito species in Thailand. Species obtained in insufficient numbers for virus isolation, or of unusual interest, are used for taxonomic studies (Study No. 47).

Description: Small teams of collectors worked in various parts of Thailand under a variety of circumstances. In some cases this was in response to a specific epidemic, in others it was part of a planned collection program for virus study, and sometimes as a by-product of other studies in progress in the area. In each case, mosquitoes were collected by several methods, always including human biting and house collecting. At times light trap and animal biting collections were also made. Female mosquitoes were retained alive in the collection bags for one day, then killed, identified and frozen. The teams carried dry ice, and the frozen mosquitoes were sealed in ampoules and stored in a dry ice chest until they were given to the Virus Department for isolation. All males and samples of females were returned to the Entomology Department for taxonomic study. Because of an initial interest in the hemorrhagic fever epidemic of 1962, much of the collection effort was devoted to the collection of Aedes aegypti. In a parallel study, members of the Virus Department bled inhabitants of some of the areas (especially children) for serological evidence of virus infection. For the results of the serological examinations see Study No. 5.

Progress: Results of the virus isolation program are reported by the Virus Department (Study No. 7). These will be referred to here only to clarify the progress of the collection program. The areas in which mosquito-virus collections were made from 1962 to 1964 are shown in Figure 1. Most of the collection sites were located within one day's drive of Bangkok. Exceptions to this were: Chiangmai, where a number of collections were made by a team stationed in that area; and Klong Chandi, Surathani Province, where collections were made during an outbreak of hemorrhagic fever. The results of mosquito collections in each of these areas will be discussed separately.

#### Chiangmai

Approximately ten thousand female mosquitoes were collected in animal and human biting collections on the outskirts of Chiangmai in 1962. These were chiefly the common species of Culex. A smaller number of Stegomyia and Finlaya species were collected at the same time (1962). None of the lots collected yielded virus. Aedes aegypti were collected in the main part of the city, and breeding of the species was detected a number of times in urban and suburban sites. However, the numbers were small compared to Bangkok and other cities examined. Many hundreds of hours of biting and larval collection failed to detect this species in the forest covered hills ringing the city.

### Klong Chandi, Surathani Province

A small number of collections, chiefly resting collections in houses, were made in this town along the main railroad in Southern Thailand in 1962, in response to a request for assistance in investigation of an outbreak of hemorrhagic fever. No viruses were isolated from the small number of Aedes aegypti and C. quinquefasciatus preserved. Some of the specimens collected on this first trip were taken from homes where hemorrhagic fever cases had resided. Collections were made difficult by fogging operations which were being conducted by the Ministry of Health in order to bring the epidemic under control. Additional collections were made in the area in September, both in the settled area along the railroad where most of the cases had occurred, and in the jungles to the east of the railroad. While Aedes aegypti was quite common along the railroad, it was not found at all in the jungle or in isolated homesteads. In these localities Aedes albopictus was the dominant day-biting species, particularly in bamboo groves. The phenomenon of replacement of A. aegypti by A. albopictus in areas away from human concentrations has been noted elsewhere in SE Asia. A list of all of the species preserved for virus isolation in the second visit to the area is presented in table 1. No viruses were isolated from these mosquitoes. All of the specimens were obtained by human, pig and cattle biting collections.

### Rayong and Ban Phe, Rayong Province

Rayong and the nearby village of Ban Phe are on the Southeastern coast of Thailand, facing southward across the Gulf of Thailand. The area is sandy with little vegetation, but scrub covered hills come close to the beach. The area experienced a considerable outbreak of hemorrhagic fever in 1962, and a number of resting and human-biting collections were made during and just after the epidemic. A list of the females preserved for virus isolation will be found in table 2. The collections were heavily weighted toward A. aegypti since it was assumed that this was the most important vector in the area. However, most of the collections were made by inexperienced collectors, and the very large percentage of Aedes aegypti taken indicates an unusually large population in the villages, since this mosquito is generally quite difficult to capture. Eleven strains of dengue virus were obtained from the 13 pools of A. aegypti inoculated, an unusually high percentage (See Virus Department, Study 7). A contributing factor to the unusual A. aegypti population was the very large number of earthenware jugs in the villages. These were used for water storage, and for the production of "nam pla", a fermented fish sauce. No A. aegypti were found in the jars containing fermenting fish, but a number of these had been discarded carelessly

Table 1. Mosquitoes preserved for virus isolation, Surathani Province

<u>Species</u>	<u>Female preserved</u>
<u>Aedes albopictus</u>	188
<u>Aedes desmotes</u>	8
<u>Aedes lineatopennis</u>	9
<u>Aedes niveus*</u>	24
<u>Aedes taeniorhynchoides</u>	2
<u>Aedes vexans</u>	3
<u>Anopheles barbirostris</u>	127
<u>Anopheles kochi</u>	140
<u>Anopheles maculatus</u>	5
<u>Anopheles philippinensis</u>	17
<u>Anopheles stephensi</u>	84
<u>Anopheles vagus</u>	62
<u>Armigeres cingulatus</u>	2
<u>Armigeres magnus</u>	22
<u>Culex annulus</u>	58
<u>Culex fuscocephalus</u>	47
<u>Culex gelidus</u>	436
<u>Culex pseudovishnui</u>	280
<u>Culex sinensis</u>	7
<u>Culex tritaeniorhynchus</u>	4
<u>Mansonia uniformis</u>	15
Total	1,540

\* species complex

Table 2. Mosquitoes collected for virus isolation, Rayong, Thailand.

Species	June 1962		November 1963	
	Females	%	Females	%
<u>Aedes aegypti</u>	1,220	63.1	812	33.5
<u>Aedes mediolineatus</u>	-	-	10	0.4
<u>Aedes vexans</u>	-	-	41	1.7
<u>Anopheles vagus</u>	53	3.0	-	-
<u>Armigeres subalbatus</u>	1	-	-	-
<u>Culex fuscocephalus</u>	-	-	12	0.5
<u>Culex gelidus</u>	-	-	63	2.6
<u>Culex quinquefasciatus</u>	450	25.1	1,429	58.9
<u>Culex sitiens</u>	65	3.6	-	-
<u>Culex tritaeniorhynchus</u>	3	-	45	1.9
<u>Mansonia uniformis</u>	-	-	13	0.5
Total	1,792		2,425	

and had become filled with rain water. The Rayong area was revisited in November 1963, and a list of the species collected at that time will be found in table 2. These mosquitoes have not been tested as yet. The A. aegypti population was again high, although more C. quinquefasciatus were collected at that time.

Note: The collections which follow were all made by the same team of experienced collectors, who alternated assignment between Bangkok and the areas discussed. The collections are therefore roughly comparable, although different amounts of time were devoted to the various collection sites during the year. The species composition through the year has been computed for the more frequently visited sites. Human and animal biting collections were the most frequently employed techniques in each of the areas.

#### Klong Rangsit, Phatumthani Province

This is a community approximately ten miles north of the Bangkok municipal limits. It was selected for study in April 1963, in the belief that the linear nature of the community (along a major klong or canal) would readily permit a detailed study of the mosquito population throughout the year. It quickly became apparent that the area was unsuitable, since high evening winds along the canal severely limited the mosquito catch. Aedes aegypti and Culex quinquefasciatus were the most abundant species present, and no viruses were isolated from them.

#### Bang-Pa-In, Ayudhya Province

A complex of villages along the Menam Chao Phrya, the principal river of the central rice plain of Thailand north of Bangkok. The area is extremely flat, and very little above sea level. Almost all of the plain is devoted to rice culture, with trees found only along the river and canals, and in the villages. Collections were begun in May, 1963 and continued for at least part of each month until November 1963. Over 14,000 females were preserved for virus isolation and turned over to the Virus Department. No viruses had been isolated at the time of this report, but the series was incomplete. In table 3 it will be noted that the number of A. aegypti for the entire year was slightly more than the number of C. quinquefasciatus, whereas in Bangkok (Study 40) C. quinquefasciatus exceeded A. aegypti over ten to one. There is far less breeding of C. quinquefasciatus in this more rural area. On the other hand, the relative proportion of Anopheles species was quite high. Most of the Anopheles were collected in animal (cow) biting collections, and most were rice paddy breeding species.

Table 3. Female mosquitoes preserved for virus isolation, Bang-Pa-In Ayudhya, Thailand. April 1963 to March 1964.

Species	Females	Percent of Total
<u>Aedes aegypti</u>	1,892	13.3
<u>Aedes albopictus</u>	28	-
<u>Anopheles annularis</u>	205	1.4
<u>Anopheles barbirostris</u>	184	1.3
<u>Aedes mediolineatus</u>	4	-
<u>Anopheles argyropus</u>	222	1.5
<u>Anopheles nigerrimus</u>	133	0.9
<u>Anopheles peditaeniatus</u>	391	2.7
<u>Anopheles sinensis</u>	192	1.3
<u>Anopheles subnictus</u>	1,321	9.3
<u>Anopheles vagus</u>	4,763	33.4
<u>Armigeres subalbatus</u>	7	-
<u>Culex bitaeniorhynchus</u>	12	-
<u>Culex fuscocephalus</u>	505	3.5
<u>Culex gelidus</u>	696	4.9
<u>Culex quinquefasciatus</u>	1,755	12.3
<u>Culex sinensis</u>	235	1.6
<u>Culex tritaeniorhynchus</u>	1,375	9.6
<u>Mansonia annulifera</u>	124	0.9
<u>Mansonia uniformis</u>	234	1.6
Total	14,278	

### Pakchong, Nakornratchasima (Korat) Province

This village is some 100 kilometers northeast of Bang-Pa-In, but quite different in general character. It is located near the edge of the Korat Plateau, which rises abruptly from the Menam Chao Phrya plain. During the most of the year the area is considerably drier than the plain, with few waterways. Tree covered hills come quite close to the town, which is largely concentrated along the only major highway. Collections were made at frequent intervals from April 1963 to March 1964. The specimens preserved for virus isolation are listed in table 4. It is particularly interesting to note that Pakchong is located in an area notorious for malaria transmission. The town itself is apparently not in the transmission zone (see study No. 51), but it has been sprayed with DDT for a number of years. Despite this, large numbers of *A. aegypti* were found. The population proved to be resistant to DDT (Study No. 50). The percentage of *Anopheles* species collected was much lower than at Bang-Pa-In, and neither of the known malaria vectors in the area was collected during the study.

The following areas were visited on a much less extensive schedule than Pakchong or Bang-Pa-In, most of them being visited but once during the year. These collections were intended chiefly to give a rapid impression of the mosquito population, including the incidence of *Aedes aegypti*, and to provide additional material for taxonomic and mosquito distribution studies (Study No. 47). The collection records for all of the remaining localities have been grouped together in table 5. It will be noted that the total number of most species is quite small, compared to the localities discussed above. Processing of the mosquitoes from these collections for virus isolation was not completed at the time of this report.

Pratumthane lies just north of Bangkok on the flood plain of the Menam Chao Phrya. The collection site lay along the river, and closely resembled that at Bang-Pa-In. Collections were made in the area partially because of reports of malaria there in recent years for which the vector was unknown. The only unusual feature of the collection was the large proportion of *C. tritaeniorhynchus* found.

Rajburi, Petchburi and Prachuabkirikhan lie to the east and southwest of Bangkok, along the coastal highway. *Aedes aegypti* was found in all three areas. An unusually large number of *Mansonia* were collected at Petchburi.

Table 4. Female mosquitoes preserved for virus isolation, Pakchong, Nakornratchasima, Thailand. April 1963 to March 1964.

Species	Females	Percent of Total
<u>Aedes aegypti</u>	6,408	31.5
<u>Aedes lineatopennis</u>	6	-
<u>Aedes mediolineatus</u>	204	1.0
<u>Aedes taeniorhynchoides</u>	84	-
<u>Anopheles barbirostris</u>	6	-
<u>Anopheles kochi</u>	7	-
<u>Anopheles subpictus</u>	140	0.7
<u>Anopheles vagus</u>	676	3.3
<u>Armigeres subalbatus</u>	74	-
<u>Culex fuscocephalus</u>	331	1.6
<u>Culex quinquefasciatus</u>	11,838	58.1
<u>Culex sinensis</u>	20	-
<u>Culex tritaeniorhynchus</u>	566	2.8
Total	20,360	

Table 5. Female mosquitoes collected for virus isolation.  
Various areas of Thailand, April 1963 to March 1964

Females per locality

Species	Rajburi	Khao Mai Kaeo	Ko Chang	Prabu- tabat	Petch- buri	Khao Yai	Pathum- thane	Prachuab- kirikhan	Total
<u>Aedes aegypti</u>	59	-	84	72	65	3	6	176	465
<u>Aedes albopictus</u>	-	32	-	-	-	3	-	-	36
<u>Aedes butleri</u>	-	-	-	-	-	4	-	-	4
<u>Aedes taeniorhynchoides</u>	-	-	-	-	-	37	-	-	37
<u>Aedes vexans</u>	-	-	-	-	-	6	-	-	6
<u>Anopheles annularis</u>	-	-	-	-	-	-	23	-	23
<u>Anopheles nigerrimus</u>	19	-	-	-	-	-	-	-	19
<u>Anopheles peditaeniatus</u>	-	-	-	-	-	-	145	-	146
<u>Anopheles philippinensis</u>	98	-	-	-	-	-	-	-	98
<u>Anopheles sinensis</u>	49	-	-	-	-	-	-	-	49
<u>Anopheles subpictus</u>	-	-	-	-	-	-	-	103	103
<u>Anopheles vagus</u>	-	-	-	23	-	16	-	25	64
<u>Armigeres subalbatus</u>	-	-	-	-	-	154	-	-	154
<u>Culex fuscocephalus</u>	62	-	-	86	-	41	79	-	268
<u>Culex gelidus</u>	-	48	-	3	115	29	57	-	252
<u>Culex quinquefasciatus</u>	3	-	-	86	100	7	-	115	311
<u>Culex sinensis</u>	-	6	-	-	-	-	-	-	6
<u>Culex sitiens</u>	-	8	-	-	-	-	-	-	8
<u>Culex tritaeniorhynchus</u>	29	24	-	74	193	60	840	-	1,220
<u>Mansonia indiana</u>	-	-	-	-	19	-	-	-	19
<u>Mansonia crassipes</u>	-	5	-	-	-	-	-	-	5
<u>Mansonia uniformis</u>	-	-	-	-	125	-	8	-	133
							GRAND TOTAL -		3,426

Khao Mai Kaeo, Cholburi is the site of an intensive malaria study (Study No. 51). A few collections were made there in 1963 to provide additional background data on the culicine population of the area. Although not shown in the table a few A. aegypti were collected, too few for isolation attempts. The area is largely given over to tapioca culture, using land which was forest covered until recent years. A. albopictus (table 5), was much more abundant than A. aegypti, as noted elsewhere in sparsely settled areas.

Khao Yai, Nakornratchasima is almost due south of the major collection site at Pakchong. The region is covered by large stands of virgin forest interspersed with grass and savannah. A small number of A. aegypti were found in isolated forest huts along the highway, but not in the jungle proper. Armigeres subalbatus was the most common day biting species in the forest, and this species deserves additional attention as a arbo-virus vector suspect, since it occurs both in the deep forest and in highly urban areas.

Ko Chang, Trad is an island off the coast of Trad Province, not far from the Cambodian border. Very few mosquito collections appear to have been made on the island in the past, and most of the specimens collected in the present study were saved for taxonomic study. Collections were made in the fishing and agricultural communities along the perimeter of the island, and along streams and trails leading inland. Inner portions of the island are still forest covered. Twenty-eight species were collected, and the island appears to warrant further study from the arbo-virus viewpoint, since in a relatively small area one finds elements of both urban and jungle mosquito faunas.

#### Comments on the Distribution of Aedes aegypti

One of the purposes of the study was to determine the extent to which Aedes aegypti occurred in various parts of the country, and to relate this to the distribution of hemorrhagic fever. Initially, hemorrhagic fever appeared to be a strictly urban disease, restricted to the Bangkok-Dhomburi metropolitan complex. In 1962, however, the disease appeared in other parts of Thailand, including some smaller towns along the main arteries of communication. Generally speaking however, it did not appear in the very smallest villages, nor in isolated hamlets or dwellings in the deep forest.

There is every indication that the distribution of hemorrhagic fever tends to parallel the distribution of Aedes aegypti, which is almost entirely an urban species. However, there are still some puzzling aspects to this distribution. In one sense it is a clear-cut phenomenon, since no hemorrhagic fever has appeared in an area which did not have Aedes aegypti present in some numbers. The converse was not true however, since a number of communities were found (notably Pakchong) with very high populations of

A. aegypti and little or no hemorrhagic fever. The results of serological studies on the Pakchong population by the Virus Department may help to clarify this matter when they become available. The relationship of A. aegypti to classical dengue infection was not explored during this study, and the pattern of distribution of this clinical manifestation may also prove to be of interest.

A. aegypti appears to have originated in Africa, and to have been spread into SE Asia and elsewhere by the agency of human commerce. The time of its entrance into Thailand cannot be calculated at this late date, although it was first reported from this country in 1901. It may have arrived much earlier in Indian or Arab sailing vessels. The inland water commerce of Thailand must have permitted its rapid spread, and the current commerce in water jugs and other containers capable of supporting its growth have permitted it to spread far inland. No A. aegypti were found in the completely feral state during these studies. As noted above, a few specimens were in very isolated forest homes and farm houses. In each case, however, these were on reasonably good roads, or close to them, and there was evidence in the homes of frequent contact with urban centers. In the forests, and in the suburban fringes of cities, A. aegypti is replaced by A. albopictus, another member of the subgenus Stegomyia. This species is also capable of transmitting dengue virus, and there is some evidence that it may be the primordial vector of the virus in SE Asia. It apparently cannot compete successfully with A. aegypti in the more developed parts of the cities, but there is no evidence that A. aegypti is replacing it in the jungle or more remote areas. A. albopictus, therefore, is much more likely than A. aegypti to be involved in the often postulated jungle cycle of dengue. Some small pockets of A. albopictus persist even in the more developed parts of Bangkok, wherever bamboo is available as a breeding site and vegetation is well developed. It deserves additional attention as a virus vector, although present evidence is clearly against its being an important vector of hemorrhagic fever.

Summary: A large number of mosquito collections were made in many parts of Thailand from 1962 to 1964, and the females from these collections were frozen for virus isolation attempts whenever possible. Particular attention was given to collections of A. aegypti, and the distribution of this species was found to exceed the distribution of hemorrhagic fever. No evidence was found that Aedes aegypti is becoming a completely feral species in Thailand, and in jungle areas it is replaced by other species, particularly A. albopictus. The bulk of the collections are still in frozen storage, awaiting virus isolation attempts. However, 11 dengue strains were recovered from 13 pools of A. aegypti collected in the coastal region of Southeastern Thailand during a hemorrhagic fever epidemic. As a rule Anopheles mosquitoes were more common in the broad

rice growing plains, Culex species in the open uplands, and Stegomyia and Armigeres species in the forested areas.

Conclusions: Aedes aegypti is widely distributed in urban centers and along the main routes of travel in Thailand. The distribution of A. aegypti is not coextensive with the known distribution of hemorrhagic fever and further collections will be necessary to delineate the extent of each. A considerable potential for transmission of the arthropod-borne viruses exists in all of the portions of the country examined. No definitive conclusions on the extent of infection in the mosquitoes will be available until isolation attempts by the Virus Department are completed, but dengue virus carrying A. aegypti occur in at least one area outside of Bangkok.