

BODY OF REPORT

SEATO Medic Study No. 40                      Arthropod-borne Viruses in the Bangkok Area.

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 San Francisco 96346

Division of Medical Research Laboratories

Department of Medical Entomology

Period Covered by Report:                     1 April 1964 to 31 March 1965

Principal Investigators:                        Major John E. Scanlon, MSC (1)  
Douglas J. Gould, Ph.D (2)

Associate Investigators:                        1st Lt James M. Neely, MSC (3)  
1st Lt Dick L. Deonier, MSC (4)

Reports Control Symbol:                        MEDDH-288

Security Classification:                        UNCLASSIFIED

Objective: To determine the presence of Japanese encephalitis virus, dengue virus, chikungunya virus, and other viruses in mosquitoes on the artificial island of Pak Kret, Nonthaburi Province. Pak Kret is on the Maenam Chao Phrya, about 25 kilometers north-northwest of Bangkok. Mosquito collection data were gathered also for any possible indications as to population dynamics of the species.

Description: The land area called Pak Kret is an island in the sense that an oxbow and chute of the river encompass it. The frequent nightly wind currents over the water may reinforce the small barrier effect of the river stretches as regards mosquito flights to and from Pak Kret. Pak Kret has the obvious advantage

- (1) Departed for CONUS on PCS June 1964
- (2) Arrived from CONUS on PCS July 1964
- (3) Departed for CONUS on PCS October 1964
- (4) Arrived from CONUS on PCS January 1965

Table 1

## SPECIES OF MOSQUITOES COLLECTED ON PAK KRET, APRIL 1964-MARCH 1965

<u>Anopheles</u>	23. <u>uniformis</u> (Theobald), 1901	<u>Culex</u>
( <u>Anopheles</u> )	<u>Uranotaenia</u>	( <u>tutzia</u> )
1. <u>argyropus</u> (Swellengrebel), 1914	24. <u>edwardsi</u> Barraud, 1926	40. <u>fuscus</u> Wiedemann, 1820
2. <u>barbistris</u> Van der Wulp, 1884	25. <u>lateralis</u> Ludlow, 1905	41. <u>halifaxi</u> Theobald, 1903
3. <u>campestris</u> Reid, 1962	26. <u>orientalis</u> Barraud, 1926	( <u>Neoculex</u> )
4. <u>lesteri</u> <u>paraliae</u> Sandosham, 1959	27. <u>recondita</u> Edwards, 1922	42. <u>brevipalpis</u> (Giles), 1902
5. <u>nigerrimus</u> Giles, 1900	<u>Aedes</u>	( <u>Mochthogenes</u> )
6. <u>peditaeniatatus</u> (Leicester), 1908	( <u>Stegomyia</u> )	43. <u>malayi</u> (Leicester), 1908
7. <u>sinensis</u> Wiedemann, 1828	28. <u>aegypti</u> (Linnaeus), 1762	( <u>Lophoceraomyia</u> )
( <u>Cellia</u> )	29. <u>albopictus</u> (Skuse), 1894	44. <u>rubithoracis</u> (Leicester), 1908
8. <u>aconitus</u> Donitz, 1902	30. <u>desmotes</u> (Giles), 1904	45. <u>species B</u>
9. <u>annularis</u> Van der Wulp, 1884	31. <u>w-albus</u> (Theobald), 1905	46. <u>infantulus</u> Edwards, 1926
10. <u>kochi</u> Ludlow, 1902	( <u>Diceromyia</u> )	( <u>Culicimya</u> )
11. <u>philippinensis</u> Ludlow, 1902	32. <u>iyengari</u> Edwards, 1923	47. <u>nigropunctatus</u> Edwards, 1926
12. <u>subpictus</u> Grassi, 1899	( <u>Aedimorphus</u> )	( <u>Culex</u> )
13. <u>tessellatus</u> Theobald, 1901	33. <u>mediolineatus</u> (Theobald), 1901	48. <u>annulus</u> Theobald, 1901
14. <u>subpictus</u> <u>malayensis</u> Hacker, 1921	34. <u>taeniorhynchoides</u> (Christophers), 1911	49. <u>bitaeniorhynchus</u> Giles, 1901
15. <u>vagus</u> Donitz, 1902	35. <u>vexans</u> (Meigen), 1830	50. <u>fuscitarsis</u> Barraud, 1924
<u>Ficalbia</u>	( <u>Neomelaniconion</u> )	51. <u>uscocephalus</u> Theobald, 1907
( <u>Ficalbia</u> )	36. <u>lineatopennis</u> (Ludlow), 1905	52. <u>gelidus</u> Theobald, 1901
16. <u>minima</u> (Theobald), 1901	( <u>Aedes</u> )	53. <u>quinguefasciatus</u> Say, 1823
( <u>Mimomyia</u> )	37. <u>dux</u> Dyar and Shannon, 1925	54. <u>pseudovishnui</u> Colless, 1957
17. <u>hybrida</u> (Leicester), 1908	<u>Armigeres</u>	55. <u>sinensis</u> Theobald, 1903
18. <u>chamberlaini</u> (Ludlow), 1904	( <u>Armigeres</u> )	56. <u>sitiens</u> Wiedemann, 1828
( <u>Etoleptomyia</u> )	38. <u>subalbatus</u> (Coquillett), 1898	57. <u>tritaeniorhynchus</u> <u>summorosus</u> Dyar, 1920
19. <u>luzonensis</u> (Ludlow), 1905	( <u>Leicesteria</u> )	58. <u>whitmorei</u> (Giles), 1904
<u>Mansonia</u>	39. <u>annulitarsis</u> (Leicester), 1908	
( <u>Coquillettidia</u> )		
20. <u>crassipes</u> (Van der Wulp), 1881		
( <u>Mansonioides</u> )		
21. <u>annulifera</u> (Theobald), 1901		
22. <u>indiana</u> Edwards, 1930		

Table 2

SPECIES AND NUMBERS OF FEMALE MOSQUITOES FROM PAK KRET  
PRESERVED FOR VIRUS ISOLATION

Species	9 April 1964- 17 October 1964	1-27 March 1965	Total	%
<u>Aedes aegypti</u>	2,570	267	2,837	33.58
<u>Aedes albopictus</u>	2	0	2	0.02
<u>Anopheles vagus</u>	91	0	91	1.07
<u>Armigeres subalbatus</u>	1	0	1	0.01
<u>Culex annulus</u>	0	2	2	0.02
<u>Culex fuscitarsis</u>	0	16	16	0.18
<u>Culex fuscocephalus</u>	78	86	164	1.94
<u>Culex gelidus</u>	1,914	688	2,602	30.80
<u>Culex quinquefasciatus</u>	784	41	825	9.76
<u>Culex nigropunctatus</u>	0	40	40	0.47
<u>Culex tritaeniorhynchus</u>	1,426	346	1,772	20.97
<u>Culex sitiens</u>	6	0	6	0.07
<u>Mansonia annulifera</u>	3	0	3	0.03
<u>Mansonia indiana</u>	3	0	3	0.03
<u>Mansonia uniformis</u>	84	0	84	0.99
TOTAL:	6,962	1,486	8,448	100.00

as a study area of having real boundaries. In so far as year-round data collecting is concerned, this small advantage has been annulled at least once by inaccessibility during a flood.

From 1 April to 17 October 1964 mosquitoes were collected on a five-day per week schedule with the following methods: two light traps (New Jersey), one cow-baited magoon trap, one chicken-baited magoon trap, man-biting (day and night), and buffalo-biting (day and night). Mosquitoes, viz., Aedes aegypti, Culex quinquefasciatus, C. tritaeniorhynchus, C. fuscocephalus, and C. gelidus were collected and kept alive long enough for blood-meal digestion, then anaesthetized, identified, and frozen for virus isolation. These daily routine operations were halted suddenly in October, when extensive flooding made the study area inaccessible.

On 1 March 1965, the following weekly program was initiated: 12-hour operation of seven Chamberlain (CDC) and two New Jersey light traps each of three nights, 12-hour operation of one cow-baited magoon trap each of two nights, man-biting and buffalo-biting collections four nights, and resting-site collections during each of four days. These activities are carried on at map-recorded sites so that a potential for routine acquisition of ecologic data exists.

Progress: From 1 April to 17 October 1964 and from 1 March to 31 March 1965, 89,466 females of 57 species of Culicidae (see Table 1) were collected and identified from Pak Kret. The seven most abundant species were Aedes aegypti (4,570

Table 3

MOSQUITOES COLLECTED FROM MAN AND BUFFALO AFTER TWILIGHT  
ON PAK KRET, APRIL 1964 - MARCH 1965.

Species	Biting Man	Biting Buffalo
<i>Aedes aegypti</i>	348	0
<i>Aedes albopictus</i>	60	2
<i>Aedes desmotes</i>	1	0
<i>Aedes dux</i>	1	0
<i>Aedes w-albus</i>	1	0
<i>Aedes lineatopennis</i>	1	3
<i>Aedes mediolineatus</i>	11	17
<i>Aedes taeniorhynchoides</i>	3	2
<i>Aedes vexans</i>	1	6
<i>Armigeres subalbatus</i>	700	16
<i>Armigeres annuliferis</i>	5	14
<i>Anopheles annularis</i>	39	7
<i>Anopheles acoutus</i>	9	6
<i>Anopheles argyropus</i>	21	73
<i>Anopheles campestris</i>	20	29
<i>Anopheles barbostris</i>	11	7
<i>Anopheles lesteri baraliae</i>	19	100
<i>Anopheles nigerrimus</i>	8	28
<i>Anopheles pedanaeatus</i>	6	3
<i>Anopheles philippinensis</i>	10	4
<i>Anopheles subpictus malayensis</i>	6	21
<i>Anopheles subpictus subpictus</i>	3	11
<i>Anopheles tessellatus</i>	3	714
<i>Anopheles sinensis</i>	3	4
<i>Anopheles vagus</i>	215	2,914
<i>Culex annulus</i>	29	0
<i>Culex bitaeniorhynchus</i>	49	10
<i>Culex brevipalpis</i>	1	0
<i>Culex fuscocephalus</i>	88	362
<i>Culex gelidus</i>	583	3,148
<i>Culex pseudovishnui</i>	4	0
<i>Culex quinquefasciatus</i>	1,333	7
<i>Culex sinensis</i>	41	10
<i>Culex sitiens</i>	117	1
<i>Culex species B</i>	8	0
<i>Culex iritaeniorhynchus</i>	533	4,550
<i>Culex whitmorei</i>	20	1
<i>Culex rubithorecis</i>	1	0
<i>Mansonia annulifera</i>	347	564
<i>Mansonia crassipes</i>	1	0
<i>Mansonia indiana</i>	1,145	160
<i>Mansonia uniformis</i>	1,382	1,403
<b>TOTAL:</b>	<b>7,187</b>	<b>14,197</b>

Table 4

COMPARISON OF MOSQUITO-COLLECTION METHODS USED ON PAK KRET

Collection Method	Man-biting (indoors)	Man-biting (Outdoors)	Buffalo biting	Resting site	Cow-bait trap	Light trap	Total
<u><i>Aedes aegypti</i></u>							
Total No. females	237	16	0	4,038	7	177	4,475
Total No. collections	517	203	132	306	21	189	1,368
Per cent for method	5.29	0.35	0.00	90.23	0.15	3.95	100.00
<u><i>Culex tritaeniorhynchus</i></u>							
Total No. females	358	175	3,550	56	5,775	23,376	33,290
Total No. collections	517	203	132	306	21	189	1,368
Per cent for method	1.00	0.52	10.66	0.16	17.34	70.21	100.00
<u><i>Culex gelidus</i></u>							
Total No. females	421	157	2,423	25	2,458	19,521	25,013
Total No. collections	517	203	132	306	21	189	1,368
Per cent for method	1.68	0.67	9.68	0.00	9.68	78.04	100.00
<u><i>Culex quinquefasciatus</i></u>							
Total No. females	1,097	236	7	879	0	199	2,418
Total No. collections	517	203	132	306	21	189	1,368
Per cent for method	45.36	9.76	0.28	36.35	0.00	8.22	100.00
<u><i>Culex fuscocephalus</i></u>							
Total No. females	65	23	364	87	27	1,780	2,346
Total No. collections	517	203	132	306	21	189	1,368
Per cent for method	2.77	0.98	15.51	3.70	1.15	75.87	100.00

females), Culex tritaeniorhynchus (33,692), C. gelidus (25,913), C. quinquefasciatus (2,418), C. fuscocephalus (2,346), Anopheles vagus (5,170), and Mansonia uniformis (4,344). Comparative collection-method data were collated into Table 4.

During this period 8,448 females of 15 species were preserved for virus isolation attempts (Table 2). These were segregated into 600 pools, placed in sealed tubes and frozen at  $-70^{\circ}\text{C}$ . The pools were sent to the Virus Department where they were tested for presence of virus by inoculation suckling mice or tubes containing cultures of hamster kidney cells. Nine strains of chikungunya virus were isolated from pools of A. aegypti collected at Pak Kret between 16 June and 26 August 1964.

Some very tentative comparative data on humans and buffaloes as mosquito hosts were acquired during this study. They have been summarized in Table 3.

Summary: The collection of mosquitoes on Pak Kret for the virus survey resulted in the isolation of nine strains of chikungunya virus from pools of A. aegypti. A total of 8,448 females of 15 species were preserved into 600 pools for virus isolation attempts. Daily collecting efforts during seven months yielded 89,466 females of 57 species of mosquitoes on Pak Kret.