

ANNUAL PROGRESS REPORT

SEATO Medic Study No. 7	Ecology of Arboviruses in Thailand. Virus Isolation from Wild Caught Mosquitoes.
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 Virology
Period Covered by Report:	1 April 1963 to 31 March 1964
Principal Investigator:	Major Scott B. Halstead, MC
Associate Investigator:	Dr. Precha Singharaj
Reports Control Symbol:	MEDDH-288
Security Classification:	UNCLASSIFIED

ABSTRACT

SEATO Medic Study No. 7 Ecology of Arboviruses in Thailand. Virus Isolation from Wild Caught Mosquitoes.

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The objective is to determine the spectrum and frequency of arthropod borne virus transmission in Thailand and to relate virus to arthropod vector. This study is concerned with the isolation and identification of viruses recovered from arthropods. Mosquitoes are collected by the Entomology Department, SEATO Medical Research Laboratory and at the Red Cross Horse Farm, Bangphra, By Dr. Skon Rohitayodhin. Linear isolation attempts from anthropophilic mosquitoes in urban areas of Thailand over a 2 and one half year period have established Aedes aegypti as the chief vector of disease-producing virus. Over the same time period collections made in animal baited and light traps at the rural collecting station at Bangphra have yielded Japanese encephalitis, chikungunya and Cg Bt 10 (a group B virus) from Culex gelidus

and Culex tritaeniorhynchus. Dengue types 1, 2, 3 and 4 were recovered from Aedes aegypti in Bangkok in 1962. Seven unidentified agents have been recovered from Culex quinquefasciatus.

BODY OF REPORT

SEATO Medic Study No. 7

Ecology of Arboviruses in Thailand. Virus Isolation from Wild Caught Mosquitoes

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S.E. Asia

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Objectives: To determine the spectrum and frequency of arthropod borne virus transmission in Thailand and to relate virus to arthropod vector. Mosquitoes to be collected from areas representing different ecologic habitats.

Description: Mosquitoes collected by the Entomology Department of the SEATO Medical Research Laboratory are tested for virus and viruses recovered at the Red Cross Horse Farm are identified. Mosquitoes of the same species captured in the same area on same day or not more than 7 days apart are pooled together. Each pool contained about 50-100 mosquitoes. Mosquito pools stored whole at -70°C . until virus isolation attempt. Pooled mosquitoes are ground in a mortar. For each mosquito 0.04 ml of phosphate buffered saline

Mosquitoes collected in 1963 in Bang Pa-In and Pakchong (SMRL 42) are still under study and no results can be reported.

Dr. Skon Rohitayodhin of the Red Cross Horse Farm, Bangphra, recovered 1 strain of Japanese encephalitis virus from Culex tritaeniorrhynchus captured in November 1963.

Summary and Conclusions: Linear studies of anthropophilic mosquitoes in urban areas over a 2½ year period has established Aedes aegypti as the chief vector of disease-producing viruses. Over the same period of time collections made in light and animal baited traps in a rural area of Southeast Thailand has yielded Japanese encephalitis, chikungunya and CG BT 10 (a group B virus) from Culex gelidus and Culex tritaeniorrhynchus. Dengue type 1, 2, 3 and 4 were recovered from Aedes aegypti in Bangkok in 1962. Dengue 3 was recovered proportionately more frequently from mosquitoes than from human hemorrhagic fever cases.

Table 37. Virus isolation from mosquitoes captured in Bangkok, 1962.

	Total tested		Isolation			
	No. tested	Pools	Positive pools	Dengue	Chik	Unk.
<u>C. quinquefasciatus</u>	79,453	753	8	-	1	7
<u>Ae. aegypti</u>	8,631	150	28	20	7	1
<u>M. uniformis</u>	1,693	23	-	-	-	-
<u>C. tritaeniorrhynchus</u>	1,938	18	-	-	-	-
<u>C. gelidus</u>	1,036	16	-	-	-	-
<u>Arm. subalbatus</u>	128	8	-	-	-	-
<u>An. vagus</u>	19	1	-	-	-	-
<u>M. annuliferra</u>	8	1	-	-	-	-
<u>An. sublictus malayansis</u>	5	1	-	-	-	-
Total	92,911	971	36	20	8	8

Table 40. Tentative dengue virus type designations for viruses recovered from mosquitoes, Bangkok, 1962.

Dengue 1 TH-Sman Complex	Dengue 2 TH-36 Complex	Dengue 3	Dengue 4
5	6	7	2

ABSTRACT

SEATO Medic Study No. 8 based on Ecology of Arboviruses in Thailand, Arbovirus Infection Rates in Residents 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)

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Division of Medical Research Laboratories

Department of Virology

Period Covered by Report:

1 April 1963 to 31 March 1964

Principal Investigator:

Major Scott B. Halstead, MC

Associate Investigator:

Dr. Suchinda Udomsakdi

Assistant Investigator:

Miss Prabhasri Umpaivit

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The objective of this study is to determine what arthropod borne viruses infect man, when and where. Specific infection rates are correlated with mosquito collection, climatologic and ecologic data and also correlated with specific disease attack rates. This study is planned to observe the rate and temporal distribution of specific arthropod borne viral infections in humans. During 1962 sera were collected at intervals from residents of 19 study areas in Bangkok. From 5 of these areas arthropods were also collected. In 1963, serum specimens were obtained every 3 months from school children living in Bangkok, Bang Pa-In (Central Plain), Pak Chong (western fringe of the Korat Plateau) and Bangphra-Sriracha (Southeast Thailand). Mosquito

collections were made throughout the year at each of these sites. Preliminary serological study of the 1962 Bangkok material has shown that the city is inhomogeneous with respect to dissemination of dengue and chikungunya viruses. Upper class living areas, characterized by sparse housing and screened houses were found to have very low virus transmission. Serologic study of 1963 specimens is awaiting isolation and characterization of viruses from collected arthropods.

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Arbovirus Infection Rates in Residents of
Thailand.

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Table 38. Virus isolation from mosquitoes captured in Rayong, June 1962.

	Total tested		Isolation		
	No. tested	Pools	Positive Pools	Dengue	Chik
<u>C. quinquefasciatus</u>	450	7	-	-	-
<u>Ae. aegypti</u>	1,220	13	11	11*	-
<u>C. sitiens</u>	65	2	-	-	-
<u>An. vagus</u>	53	1	-	-	-
<u>C. tritaeniorrhynchus</u>	3	1	-	-	-
<u>Arm. subalbatus</u>	1	1	-	-	-
Total	1,792	25	11	11*	-

* All identified as dengue 2-TH 36 complex.

Table 39. Virus isolation from mosquitoes captured in Bangkok, January-June 1963.

	Total tested		Isolation			
	No. tested	Pools	Positive Pools	Dengue	Chik	Unk.
<u>C. quinquefasciatus</u>	10,133	96	-	-	-	-
<u>Ae. aegypti</u>	1,720	36	3	3	-	-
<u>An. vagus</u>	105	4	-	-	-	-
<u>Arm. subalbatus</u>	116	2	-	-	-	-
<u>C. sitiens</u>	94	1	-	-	-	-
Total	12,168	139	3	3	-	-

Table 36. Virus isolation attempts from mosquitoes captured in Bangkok, August-December, 1961.

Species	Total tested		Isolation
	No. tested	Pools	
<u>Culex quinquefasciatus</u>	20,600	142	Neg.
<u>Aedes aegypti</u>	739	16	Neg.
<u>Mansonia uniformis</u>	1,283	12	Neg.
<u>Culex tritaeniorrhynchus</u>	1,189	11	Neg.
<u>Culex gelidus</u>	852	11	Neg.
<u>Armigeres subalbatus</u>	103	5	Neg.
Total	24,766	197	Neg.

containing 0.75% bovine albumin, 500 units of penicillin and kanamycin, 500 micrograms of streptomycin and 25 micrograms of mycostatin per ml is added. The mixture is centrifuged at 10,000 rpm for 30 minutes in a Servall refrigerated centrifuge. The supernatant fluid is inoculated into 1-day old suckling mice, using 0.01 ml and 0.02 ml for i.c. and i.p. inoculation respectively. Each mosquito-pool is blind passed 3 times. Infected mice are observed for 10 days at first and second passage and 21 days at the third passage.

Progress: Table 36 shows virus isolation attempts from mosquitoes in 1961. No virus was isolated from 24, 766 mosquitoes of 6 species. However, in 1962, dengue virus was successfully isolated from mosquitoes captured in Bangkok as shown in Table 37. From 92,911 mosquitoes of 9 species, 36 viruses were isolated. Of 28 viruses recovered from 8,631 Aedes aegypti, 20 were dengue, 7 were chikungunya and one virus was unidentified. Of 8 viruses recovered from 79,453 Culex quinquefasciatus, 1 was chikungunya and other viruses are unidentified. These findings confirm the hypothesis that Aedes aegypti serves as the urban vector of Thai hemorrhagic fever. Culex quinquefasciatus which yielded only one chikungunya virus, on the other hand, appears to serve as vector of as yet unidentified viral agents. Chikungunya isolation from this species may be the result of virus remaining from a recent blood meal from a viremic individual. In one experiment C. quinquefasciatus did not transmit chikungunya from viremic mice to susceptible suckling mice. Mosquitoes which yielded no virus in this study were M. uniformis, C. tritaeniorhynchus, C. gelidus, Arm. subalbatus, An. vagus, M. annulifera and An. sublitus malayansis.

Table 38 shows virus isolations from mosquitoes caught in Rayong, Southeast Thailand in June 1962. Eleven strains of dengue virus were isolated from 1,220 Aedes aegypti. The dengue pool recovery rate in Aedes aegypti was 85% as opposed to an overall rate for Bangkok of 18%. No virus was isolated C. quinquefasciatus or from other species captured in Rayong.

Table 39 shows virus isolation results in mosquitoes captured from January - June, 1963 in Bangkok. Only 3 dengue viruses were isolated from 36 pools containing 1,720 Aedes aegypti. No virus was obtained from other mosquito species.

Table 40 indicates the tentative dengue type designation for virus recovered from mosquitoes in Bangkok in 1962. Dengue typing was performed by producing mouse anti-dengue serum with 2 or 3 injections of low passage virus. Resulting sera have proven to be rather type specific when tested by CF using high mouse passage prototype dengue viruses. Neutralization tests using standard dengue hyperimmune sera are also being performed in tissue culture. Final type designation can not be established until all antisera are retested against prototype Bangkok dengue viruses.