

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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Division of Medical Research Laboratories

Department of Virology

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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: Since Thai hemorrhagic fever was first recognized in 1958 arboviruses have been reported from several mosquito species. Hammon, Rudnick and Sather ¹ reported recovery of dengue type 1 and 2 viruses from Aedes aegypti and chikungunya virus and EEE virus from Culex pipiens quinquefasciatus captured in 1958 and 1960 ². To extend these early observations collaborative studies

1. Science; 131:1102-1103, 1960

2. SEATO Medical Research Monograph No. 2: 24-26, 1962.

were undertaken by the Virus and Entomology Departments.

Mosquito collections. Initially, mosquitoes were collected in a variety of sites in Bangkok and surrounding rural areas. In order to determine the species present and their relative abundance, collections were made by standard methods including: houses and animal shelter resting collections, New Jersey mosquito light traps, human and animal biting collections. All living mosquitoes were brought to the laboratory for identification. Females taken alive in satisfactory condition were held for 24 hours, frozen at -65°C and turned over to the Virus Department for isolation attempts. From February 1962 to January 1963, collections were concentrated in five areas of the city with approximately equal human population but widely diverging character. From April 1963 to March 1964, the mosquito collections were repeated in two areas surveyed in 1962-1963. In addition, mosquitoes were captured in horse baited traps or light traps by Dr. Skon Rohitayodhin at the Bangkok Phra Horse Farm. Primary virus isolation was done on some lots of mosquitoes at Bang Phra. From March 1964 to April 1965 mosquito collections were made at Pak Kret, 10 miles north of Bangkok municipality. These studies are summarized under SEATO Medic No. 13.

Preparation of mosquito suspension and mouse inoculations. Mosquitoes were pooled in lots of approximately 50 each for Aedes aegypti and 100 each for Culex quinquefasciatus. Mosquitoes were ground in a mortar. For each mosquito, 0.04 ml. of phosphate buffered saline containing 0.75% bovine albumin, 500 units/ml. each of penicillin and kanamycin, 500/ml. micrograms of streptomycin and 25/ml. micrograms of mycostatin were added. The mixture was centrifuged at 10,000 rpm for 30 minutes in Servall Model RC-2, Rotor SS-34. Supernatant fluid was inoculated in mice. Each specimen was blind passed three times.

Control measures to prevent or monitor laboratory cross-contamination. Because of the danger inherent in routine blind passage or recovery of extraneous agents either by cross-contamination in the laboratory or from enzootic infection in the mouse colony, every 5th pair of litters inoculated in April-July 1962 was inoculated with mosquito diluent only. Following this period, two litters were inoculated as controls on each day that mosquito suspensions were inoculated. Brain suspensions from control litters were passaged 2 times. Further measures to prevent laboratory contamination were the preparation and dispensing of all diluents in small bottles. Only one bottle was used per suspension. Pipettes and syringes were individually wrapped and sterilized. Mice were inoculated on paper towels which were then discarded and technicians washed their hands after handling each specimen. No prototype viruses were allowed in the isolation laboratory.

Progress: No virus was recovered in this laboratory from mosquitoes captured in 1961. (One dengue virus was recovered by Dr. Ambhan Dasanayavaja from several Aedes aegypti pools collected by SMRL Entomology Department. The results of virus isolation from mosquitoes captured 1962-1964 are shown in Table 1. Of 7 viruses recovered from Culex quinquefasciatus, one was chikungunya (recovered

Table 1

SUMMARY OF VIRUS ISOLATIONS FROM MOSQUITOES CAPTURED IN THAILAND, 1961-1964

Species	Year	Number								
		tested	D1	D2	D3	D4	Chik	JE	Uniden	Incomp
<u>A. aegypti</u>	1961	739	-	-	-	-	-	-	-	-
	1962	9,850	5	15	8	1	7	-	-	3
	1963	12,238	1	5●▲	-	1	-	-	-	2
	1964	6,314	-	-	-	-	9**	-	-	2★
<u>C. quinquefasciatus</u>	1961	20,600	-	-	-	-	-	-	-	-
	1962	79,903	-	-	-	-	1	-	3	-
	1963	56,015	-	-	1*	-	-	1	1	-
	1964	27,439	-	-	-	-	-	-	-	1
<u>C. tritaeniorhynchus</u>	1961	1,189	-	-	-	-	-	-	-	-
	1962	1,941	-	-	-	-	1↓	1↓	-	-
	1963	31,994	-	-	-	-	-	2A	-	-
	1964	5,318	-	-	-	-	-	4A	-	-
<u>C. gelidus</u>	1961	852	-	-	-	-	-	-	-	-
	1962	1,036	-	-	-	-	-	2↓	Cg Bt 10↓	-
	1963	24,911	-	-	-	-	-	1↓	-	-
	1964	3,268	-	-	-	-	-	-	-	-
<u>Mn. uniformis</u>	1961	1,283	-	-	-	-	-	-	-	-
	1962	1,693	-	-	-	-	-	-	-	-
	1963	133	-	-	-	-	-	-	-	-
	1964	493	-	-	-	-	-	-	-	-
<u>Arm. subalbatus</u>	1961	103	-	-	-	-	-	-	-	-
	1962	129	-	-	-	-	-	-	-	-
	1963	149	-	-	-	-	-	-	-	-
	1964	50	-	-	-	-	-	-	-	-
<u>An. vagus</u>	1962	71	-	-	-	-	-	-	-	-
	1963	3,959	-	-	-	-	-	-	-	-
	1964	200	-	-	-	-	-	-	-	-
<u>An. S. malayensis</u>	1962	5	-	-	-	-	-	-	-	-
	1963	680	-	-	-	-	-	-	-	-
	1964	42	-	-	-	-	-	-	-	-
<u>C. fascocephalus</u>	1963	7,336	-	-	-	-	-	-	-	-
	1964	492	-	-	-	-	-	-	-	-

* Reisolation - neg.

↓ Isolated at Bang Phra Horse Farm by Dr. Skon Rohitayothin.

A Mosquitoes captured at Bang Phra.

** Mosquitoes captured at Pak Kret, Nonthaburi Province.

● Mosquitoes captured at Pak Chong.

▲ Mosquitoes captured at Bang Pa-In.

★ Mosquitoes captured at Pisanuloke and Saraburi.

In addition the following species were tested without virus recovery (number mosquitoes tested indicates in parentheses): Mn. annulifera (324), C. sitiens (205), An. S. subpictus (823), An. peditaeniatus (539), Ae. medioliniatus (333), An. barbirostris (161), An. nigerinus (152), C. bitaeniorhynchus (43), An. philippi-nensis (55), C. Vishnui gr. (15), An. sinensis (447), An. argyropus (222), An. annularis (177), An. campastris (73), Ae. rexans (44), Ae. lineatopensis (13), Mn. annularis (12).

in Bangkok in 1962), one was Japanese encephalitis virus (mosquitoes captured in Rayong, May 1963), one was dengue 3 (Bangkok 1963 reisolation attempt was negative) and three were short incubation period agents identical or closely related to each other but not to group A, B or Bunyamwera viruses. One virus, incompletely studied, appears to be a group B agent. Results of reisolation of dengue viruses from 1962 mosquito pools in mice and tissue are compared in Table 2. Of 29 positive suspensions tested, 23 viruses were reisolated or about 80%. Chikungunya viruses were recovered from mosquitoes in 1962 and 1964. Of 17 strains recovered only 1 strain was recovered from C. quinquefasciatus (183,957 mosquitoes were tested in approximately 950 pools). One chikungunya strain was recovered from C. tritaeniorhynchus captured at Bang Phra in 1962. The very low rate of recovery of chikungunya virus from Culex quinquefasciatus is consistent with laboratory studies of this species in which virus survival and transmission could not be demonstrated. Aedes aegypti, on the other hand, have been shown experimentally to propagate and transmit both dengue and chikungunya viruses. While Culex quinquefasciatus may not be able to serve as a biologic vector of chikungunya, occasional recovery of this virus from this species suggests the possibility that the virus may survive long enough to be transferred to humans mechanically. This hypothesis deserves further inquiry.

Several strains of Japanese encephalitis virus have been recovered during these studies. Six strains from mosquitoes were collected at Bang Phra Horse Farm between 1962-4. One group B agent closely related but not identical to JE, CgBt 10 was recovered from C. gelidus in 1962.

Summary and Conclusions: Linear isolation attempts from anthropophilic mosquitoes in urban areas of Thailand over a two and one half year period have established Aedes aegypti as the chief vector of disease-producing viruses. 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 CgBt 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 1962-3. Three unidentified agents have been recovered from Culex quinquefasciatus.

Table 2

DENGUE VIRUS REISOLATION ATTEMPTS FROM 29 MOSQUITO SUSPENSIONS IN MICE AND TISSUE CULTURE, BANGKOK AND RAYONG MOSQUITOES (1962).

Virus	No. reisol. attempts	No. isolated both in SM* & BS-C-1 cell	No. isolated in SM only	No. isolated in BS-C-1 cells only	Virus not recovered
Dengue 1	5	4	-	-	1
Dengue 2	16	6	3	4	3
Dengue 3	7	1	4	-	2
Dengue 4	1	-	1	-	0
Total	29	11	8	4	6

SM * - Suckling mice