STUDY REPORTS

1. Title: A Dengue Control Program in an Insular Setting

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OBJECTIVE

To determine whether an A. aegypti control program could effectively reduce transmission of dengue viruses to humans, particularly as reflected in a reduction in incidence of dengue hemorrhagic fever.

DESCRIPTION

Epidemics of hemorrhagic fever occurred on the island of Koh Samui in 1966 and 1967; the results of investigations into these epidemics were described in SMRL Annual Reports prepared 15 April 1967 and 15 April 1968.

Koh Samui has been described extensively in these previous reports. Briefly, it is the main island in a small archipelago in the gulf of Thailand, about 400 miles south of Bangkok, with a population of about 35,000, living chiefly on coastal plains. There are an estimated 500 dwellings on the island. Large jars are the usual means of water storage, with an estimated 25,000 in use; these jars commonly serve as breeding sites for A. aegypti.

In both 1966 and 1967, epidemic patterns of dengue hemorrhagic fever were similar. Cases began to occur in July, after the onset of the rainy season. Peak incidence was late in July, and cases continued to occur, with diminishing frequency until October or November. During the epidemics, most clinical dengue cases occurred in the age group under 15. Approximately 30—40% of the pediatric population had serologic evidence of infection sometime during the year. The ratio of clinical cases to subclinical or missed

infections was about 1:50. Aedes aegypti and Aedes albopictus were implicated in dengue transmission on the island. Dengue virus was recovered from both species on numerous occasions. A pilot project conducted in 1967 in a small village on the island and described in last years report indicated that control of Aedes aegypti was probably an achievable goal for the entire island.

This led to speculation whether in this setting, control of A. aegypti alone would be sufficient to interrupt, or even markedly reduce, dengue transmission. Therefore a study was designed to measure the effect of island—wide Aedes aegypti control on (1) mosquito population indices (2) dengue transmission, as indicated by monthly sero—conversion rates in a sample of the pediatric population and (3) incidence of clinically—recognized dengue and dengue hemorrhagic fever.

It was anticipated that as in the two preceding years, a clearly defined epidemic would occur in 1968. Based on this assumption, and upon the expected monthly attack rates, criteria were established for beginning the Aedes aegypti control program. By these criteria the control program would be instituted as soon as feasible after either: (1) the incidence of suspected cases of clinical dengue reached 5 in any calender week, or (2) the monthly inapparent infection attack rates reached 5% in any 4 week interval. The incidence of suspect dengue cases was estimated by screening patients at the two medical clinics in the village of Ang Thong (the only source of western medical care on the island).

To assess inapparent infection, a sample of school children was selected for monthly determination of dengue antibody. This survey sample came from 3 widely separated schools, and consisted chiefly of children aged 7—14. Sample size was determined on the assumption that the inapparent attack rate would rise from less than one percent per month during the dry season to from five to ten percent, during the epidemic period. It was felt that attempted control of Aedes aegypti at the height of the dengue transmission season would provide the most rigorous test of the control methods. In addition, the proposed method might have application as a public health measure, employed in a similar manner in the face of an epidemic.

During the week of 7 July, five suspect dengue cases were reported. Accordingly, it was decided to institute the A. aegypti control program beginning the week of 28 July. Preliminary serologic data subsequently available from the school servey indicated that attack rates in the period mid June—mid July were approaching 5%.

The control program, carried out between 29 July and 14 August, consisted primarily of the application of the larvicidal organophosphate Abate to water jars throughout the island and the use of Malathion, dispersed in a fog throughout the villages. The rationale, methodology and techniques employed to estimate effects on mosquito populations are described in the SEATO Medical Research Study on Mosquitoes section of this report.

Methods employed for monitoring of clinical cases of dengue infection, specimen collection, and virologic study were identical to techniques described in last year's report. All patients with clinical disease compatible with dengue seen at the medical clinics of Ang Thong were considered for inclusion into the study. As before, clinical records of each case were the basis for inclusion into clinical categories. Dengue shock syndrome included cases with fever, and evidence of shock (fall in Blood pressure or narrow pulse pressure) regardless of presence or absence of hemorrhagic signs or symptoms. Hemorrhagic fever syndrome was diagnosed in the presence of fever, petechiae and evidence of gross bleeding, i.e., purpura, epistaxis, hematemesis. etc. Patients with fever alone or with fever and scattered petechiae or positive tourniquet test were grouped as dengue fever; a departure from the practice of previous investigations.

Acute and convalescent sera were obtained as before, and examined for hemagglutination inhibition (HI) and complement—fixing (CF) antibodies. Attempts were also made to recover dengue virus from acute phase sera. Serologic response was classified as not dengue, dengue (primary type response), dengue (secondary response), dengue (not exactly classifiable), and probable dengue, following previously decribed criteria (SMRL Annual Report 1968).

Mosquito collections for virus recovery attempts were made in and around the homes of all suspected dengue cases. A team of 4 collectors made biting collections which were ordinarily completed within 48 hours after the case was seen. Collections were routinely carried out from 8—10 AM and 4—6 PM since experience had indicated that both Aedes aegypti and Aedes albopictus were crepuscular biters. At each visit, 2 men collected indoors and 2 outdoors. Collections were repeated at most sites from 2 to 4 months after the initial collection. All mosquitoes collected were identified and shipped on dry ice to Bangkok, in pools of about 10 mosquitoes each.

A sample of school children was selected for monthly determination of dengue antibody. Demographic data on which to base a stratified random sampling procedure was not available. Age—specific antibody prevalence was thought to be fairly uniform from place to place on the island, based on fragmentary data gathered in prior years. Similarly, attack rates, as judged by serologic data, were judged to be fairly uniform in the narrow age range (7—14 years) from whom repeated sampling was feasible. It was anticipated that these attack rates on a monthly basis would be low, on the order of 5—10% per month. This indicated the need for a large sample in order to reliably detect small differences.

These considerations, plus the likelihood of difficulty in gaining access to many areas of the island during the rainy season (dengue season) seemed to dictate a "sentinel flock" type of approach. Accordingly the student population age 7—14, attending 4 island schools, was designated as the survey sample. The first school, in Bang Po, was located on the north coast of the island, in a rural area. The second was in the main village, Ang Thong, and the third, at Wat Saket, south of Li Pa Noi, was also chiefly rural (Figure 1).

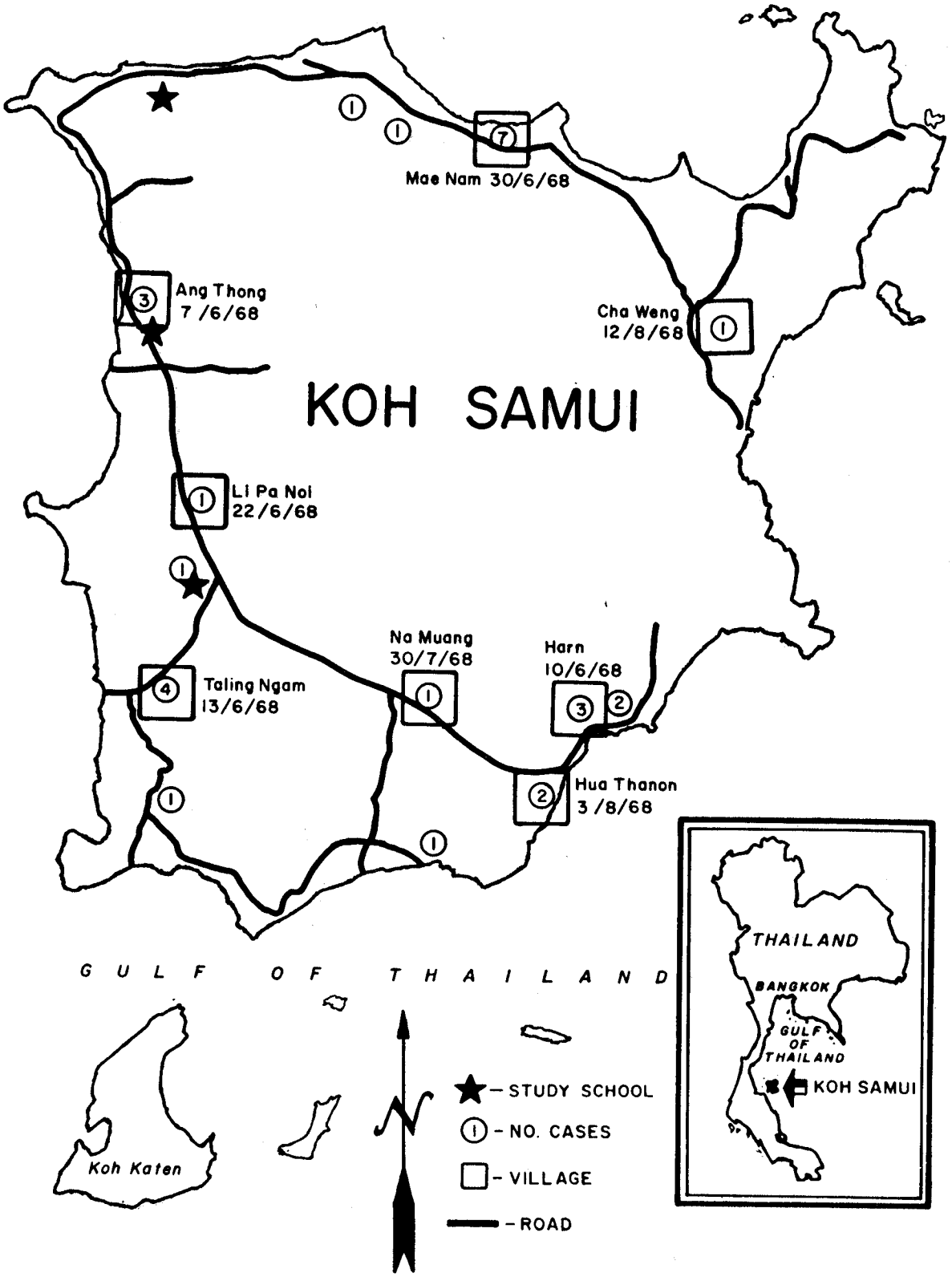

Fig. 1 Map of Koh Samui, with distribution of cases, location of study schools. Dates refer to dates of earliest onset in each village.

Serum collections were made at 4—week intervals, from students at each of the 3 schools, beginning on 21 May 1968. Parental consent was obtained at the outset; parental permission could be, and was, withdrawn at any time during the study. Because of increasing parental resistance (and the absence of the anticipated dengue epidemic) the survey was terminated in October 1968.

All blood was collected in sterile vacutainers. After clot formation and retraction, serum was removed and shipped on dry ice to Bangkok. HI antibody to dengue 1—4, chikungunya and Japanese encephalitis was determined, using paired sera from each individual for each monthly interval. A four—fold or greater rise in HI antibody was considered evidence of infection during the preceding period.

In this way, sera from a child bled in May, June and July were first paired as May—June. Then, after the July collection, the June—July pairs were done in parallel.

PROGRESS

Mosquito indices The effects of the control program on population indices of A. aegypti and A. albopictus are described in detail in the Study on Mosquitoes section of this report. Briefly, striking reduction was noted in the A. aegypti adults in all surveillance sites; however a return to pre—treatment levels occurred by 16 weeks. Appreciable numbers of A. albopictus adults continued to be collected outdoors in the same villages during this 16 week period. More prolonged reductions were noted in the rates of infestation of household water containers with Stegomyia larvae, which persisted in most areas through the end of the reporting period.

Clinical Case Studies

Four cases of hemorrhagic fever were reported in early May prior to the beginning of the study. No attempt was made to establish an etiologic diagnosis in these cases, but mosquito collections were made in the homes of all four. Dengue virus was recovered from mosquito pools of 2 of the 4 households. These cases are not included among the cases described below.

The first case under our surveillance was seen 7 June. Between that date and 26 August, a total of 27 dengue and probable dengue cases were seen at the treatment facilities on the island. FIGURE 2 presents the distribution of these 27 cases by week of onset. Case incidence appeared to increase through July and to fall off in August. No further cases were seen, even through observation continued until mid-December.

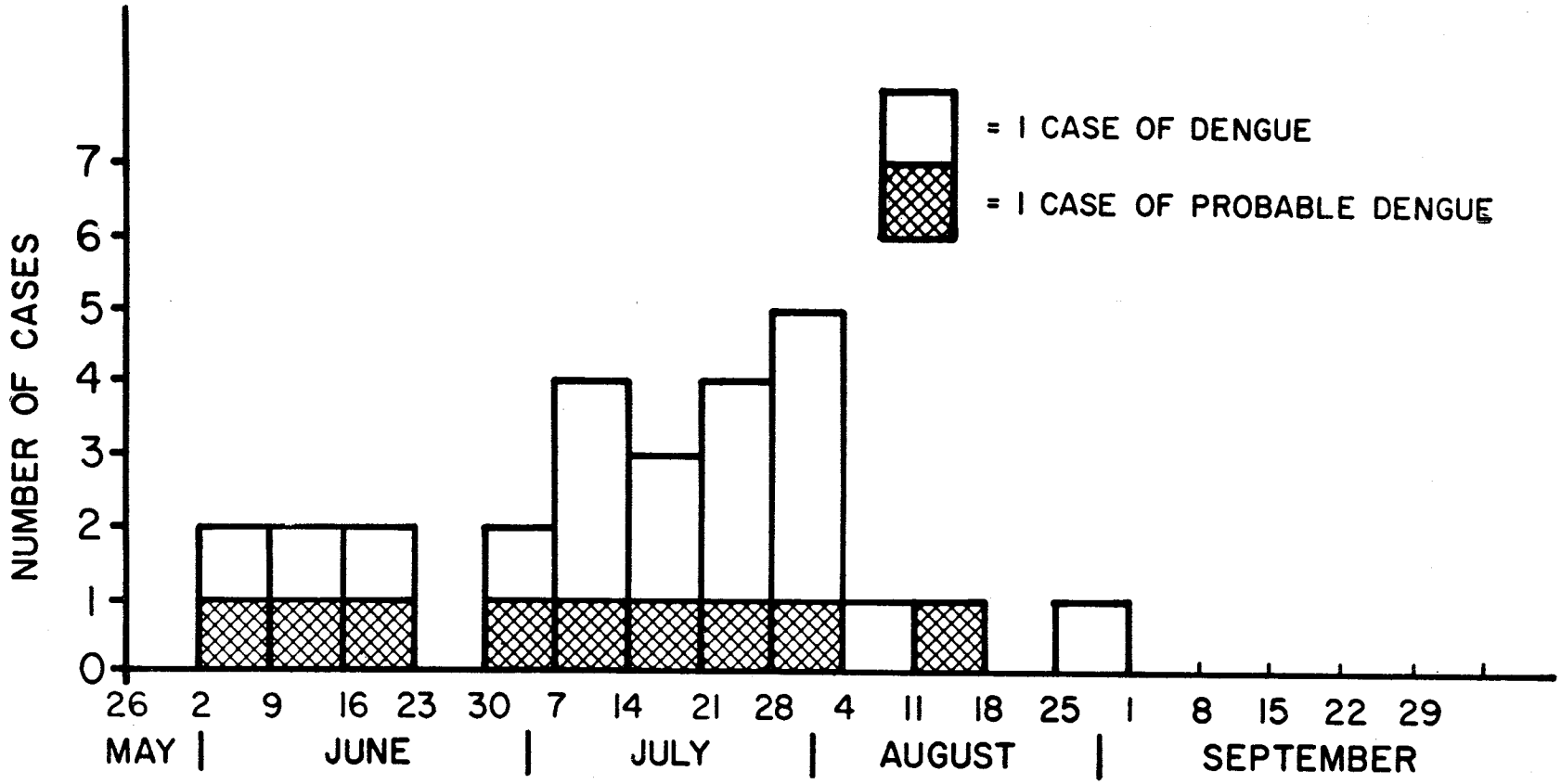

Figure 2. Distribution of 22 cases of dengue and probable dengue, by week of onset

Of the 27 cases, two were diagnosed as shock syndrome and six as hemorrhagic fever. Table 1 presents the distribution of these 27 cases by serologic response and clinical syndrome. Dengue virus type 4 was recovered from the acute phase sera of two patients, both with dengue fever syndrome. No primary-type responses were seen in this group of patients.

TABLE 1

Distribution of 27 dengue and probable dengue cases by clinical syndrome and serologic response.

Clinical Syndrome	SEROLOGIC RESPONSE				
	Dengue Primary	Dengue Secondary	Dengue N.E.C.	Probable Dengue	Total
Shock	0	2	0	0	2
Hemorrhagic fever	0	3	0	3	6
Dengue fever	0	13*	1*	5	19
Total	0	18	1	8	27

* Virus isolation from 1 patient in this category.

Age distribution of the 27 cases is presented in FIGURE 3. The youngest was age 3, the eldest 24. This latter was a male with fever, widespread petechiae and purpura, tender hepatomegaly, gingival bleeding and hematemesis; typical clinical hemorrhagic fever. The median of the distribution was 6.8 years. There were 10 males and 17 females. As may be seen from FIGURE 1, cases were widely scattered on the island, with no evidence of clustering. Dates of onset showed no evidence of a pattern of spread.

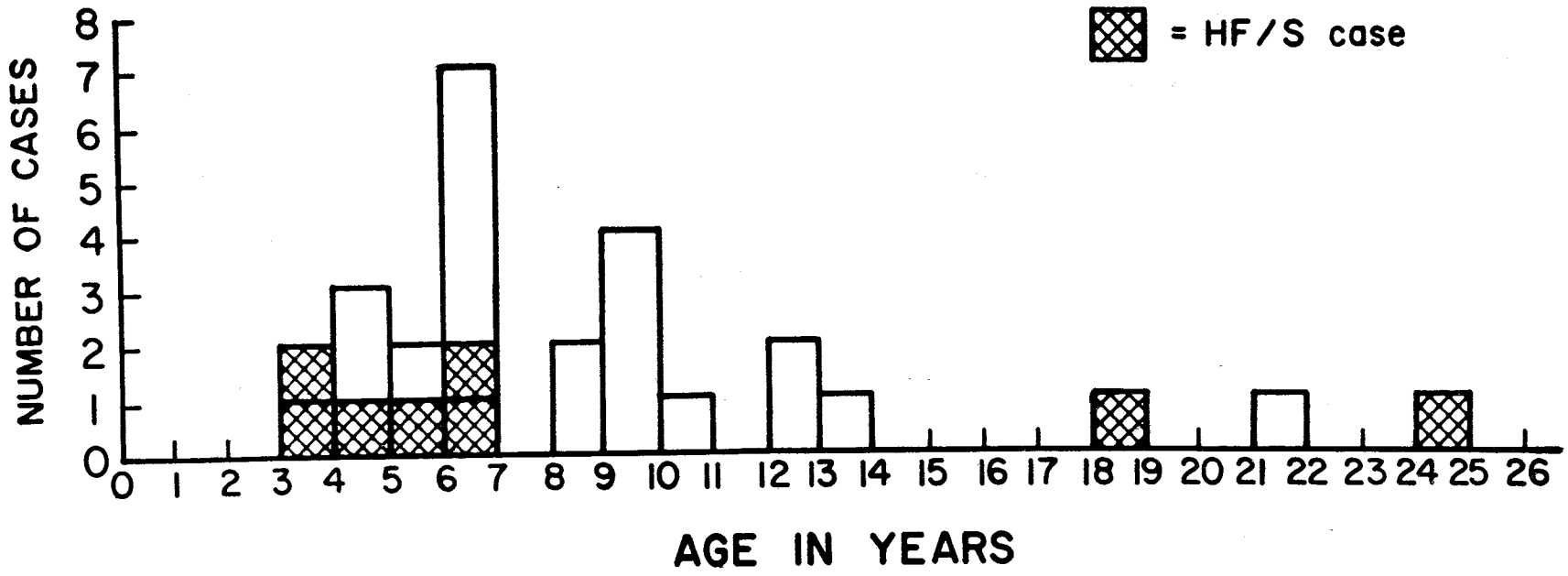

Figure 3. Distribution of 27 cases of dengue and probable dengue by age.

Serologic Survey of School children

The three schools had a census of approximately 650 children aged 7-14 throughout the study. At the beginning of the survey, specimens were obtained from 490 (76%) of the students registered. In June, 566 (83%) were bled, but thereafter parental consent was obtained with difficulty. Permission was granted to bleed 246 (36%) in July, 241 (36%) in August, 178 (27%) in September and only 106 (16%) in October. Of this final group of 106 only 43 had been bled on all six survey visits. Table 2 summarizes the total experience for those subjects for whom at least 1 serum pair was obtained. From many children only one or two monthly specimens were obtained. The population from whom 4 or more specimens had been obtained numbered 201 (30% of the mean school census). Because of the increasing resistance, and in light of the apparent lack of cases following the control program, the survey was discontinued after October. Serologic data obtained were examined considering consecutive pairs from any given individual, regardless of the interval between the first and second serum of the pair. These results are summarized in Table 3. Number of attacks refers to number at risk who showed a 4-fold or greater rise in the period. None of the 27 clinical cases described occurred in students of the 3 survey schools.

TABLE 2

Overall crude results of subclinical infection survey by school.

School	Students included	Student—months of observation	No. of dengue infections	Crude attack rate/student month
1	170	494	26	5.3
2	196	504	13	2.6
3	144	395	5	1.3
Total	510	1393	44	3.2

TABLE 3

Pair period	Interval	N = pairs at risk*	No. of Attacks	Attack rate/ 100/mo.
May—Jun	4 wk	438	9	2.1
Jun—Jul	"	228	7	3.1
Jul—Aug	"	156	3	1.9
Aug—Sep	"	102	4	3.9
Sep—Oct	"	67	1	1.5
May—Jul	8 wk	6	0	—
Jun—Aug	"	72	6	4.2
Jul—Sep	"	19	4	10.5
Aug—Oct	"	14	3	10.7
May—Aug	12 wk	2	0	—
Jun—Sep	"	8	1	4.2
Jul—Oct	"	8	2	8.3
May—Sep	16 wk	1	0	—
Jun—Oct	"	8	2	6.2
May—Oct	20 wk	—	—	—

* Corrected for conversion in any prior period.

TABLE 4

RECOVERY OF DENGUE VIRUSES FROM AEDES MOSQUITOES COLLECTED IN AND AROUND HOMES OF SUSPECT DENGUE CASES ON KOH SAMUI, 1968

Case	Diagnosis[1]	Illness Onset Date	Mosquito Collection Date	Mosquito Species					
				A. aegypti			A. albopictus		
				No. of Mosq.	No. of pools		No. of Masq.	No. of poals	
					Tested	Pos.		Tested	Pos.
A	nt[2]		May 29—30	7	1	1	237	24	1
			Nov 13	0	0	0	620	62	1
B	nt		May 31—Jun 1	3	1	0	10	1	0
			Nov 15	1	1	0	430	43	0
C	nt		Jun 1—2	7	1	0	140	14	0
			Nov 18	1	1	0	80	8	0
D	nt		May 30—31	9	1	1	87	9	1
			Nov 14	1	1	0	128	13	0
1	Dengue	Jun 7	Jun 10—11	9	1	1	180	18	0
			Jun 11—12	15	2	1	0	0	0
2	Dengue	Jun 10	Jun 12—13	12	2	1	41	5	1
			Oct 28	3	1	0	351	36	0
3	Dengue	Jun 8	Jun 13—14	36	4	2	4	1	0
			Nov 21	14	2	0	110	11	0
4	Probable Dengue	Jun 13	Jun 17—18	29	3	3	225	23	3
			Oct 24	2	1	0	496	50	0
5	Probable Dengue	Jun 17	Jun 22—23	4	1	0	205	21	2
			Jul 4—5	19	2	0	260	26	0
			Oct 23	1	1	0	678	68	0
6	Negative	Jun 19	Jun 24—25	37	4	0	4	1	0
			Oct 22	3	1	0	499	50	0
7	Dengue	Jun 22	Jun 25—26	17	2	1	167	17	5
			Oct 31	0	0	0	500	50	0
8	Probable Dengue	Jun 30	Jul 5—6	25	3	0	135	14	0
			Oct 21	0	0	0	361	37	0
9	Dengue	Jul 7	Jul 9—10	3	1	1	480	49	1
			Oct 17	1	1	0	427	43	0
10	Dengue	Jul 6	Jul 10—11	11	2	0	122	13	0
			Oct 16	0	0	0	227	23	0
11	Dengue	Jul 8	Jul 11—12	1	1	0	536	54	4
			Oct 15	0	0	0	420	42	0
12	Dengue	Jul 12	Jul 16—17	23	3	3	443	46	3
			Oct 2—3	16	2	0	259	26	0
13	Probable Dengue	Jul 13	Jul 21—22	2	1	0	300	30	3
			Oct 30	0	0	0	120	12	0
14	Dengne	Jul 15	Jul 20—21	17	2	0	32	5	0
			Oct 1—2	0	0	0	322	33	0
15	nt	Jul 15	Jul 21—22	27	4	0	93	10	0
			Sep 30—Oct 1	1	1	0	264	27	0
16	Dengue	Jul 18	Jul 22—23	5	2	2	63	7	1
			Sep 28—29	0	0	0	223	24	0
17	Dengue	Jul 18	Jul 24—25	5	2	1	31	4	0
			Sep 23—24	2	1	0	193	20	0
18	Dengue	Jul 21	Jul 24—25	4	2	1	74	8	1
			Sep 17—18	0	0	0	380	38	0

TABLE 4. Continued

Case	Diagnosis[1]	Illness Onset Date	Mosquito Collection Date	Mosquito Species					
				A. aegypti			A. albopictus		
				No. of Mosq.	No. of pools		No. of Mosq.	No. of pools	
					Tested	Pos.		Tested	Pos.
19	Dengue	Jul 26	Jul 28—29	24	4	3	225	23	0
			Oct 29	4	1	0	350	35	0
20	Dengue	Jul 26	Jul 29—30	1	1	0	53	6	0
			Nov 11	1	1	0	120	12	0
21	Negative	Jul 20	Jul 30—31	15	3	0	0	0	0
			Nov 12	7	1	0	140	14	0
22	Dengue	Jul 28	Jul 31—Aug 1	1	1	0	132	14	1
			Nov 8	0	0	0	120	12	0
23	nt	Jul 30	Aug 1—2	0	0	0	154	16	2
			Sep 16—17	1	1	0	259	26	0
24	Negative	Jul 27	Aug 2—3	1	1	0	42	5	0
			Sep 4—5	0	0	0	170	17	0
25	Probable Dengue	Jul 27	Aug 3—4	13	2	1	140	15	0
			Nov 19	11	2	0	120	12	0
26	Dengue	Aug 1	Aug 5—6	0	0	0	134	14	0
			Nov 5	0	0	0	130	13	0
27	Dengue	Jul 30	Aug 4—5	3	1	1	54	6	0
			Sep 3—4	0	0	0	117	12	0
28	Dengue	Aug 2	Aug 7—8	0	0	0	299	30	2
			Sep 2—3	1	1	0	225	23	1
29	Probable Dengue	Aug 3	Aug 8—9	6	1	0	78	8	0
			Aug 28—29	2	1	0	97	10	1
30	Dengue	Aug 5	Aug 20—25	0	0	0	909	92	1
31	nt	Aug 7	Aug 12—13	0	0	0	557	56	0
			Ang 27—28	1	1	0	398	40	1
32	Probable Dengue	Aug 12	Aug 16—17	0	0	0	412	42	0
			Aug 26—27	1	1	0	483	49	0
33	Dengue	Aug 26	Sep 1—2	6	1	1	267	27	2
			Nov 4	6	1	0	110	11	0
34	Negative	Nov 18	Nov 20	0	0	0	755	76	3[3]

1. Serologic diagnosis of dengue infection: Dengue = 4 fold or greater rise in HI antibody titer or recovery of dengue virus from serum. Probable dengue = high fixed HI antibody titers. Negative = no or low levels of HI antibody

2. nt — not tested

3. Three isolates from A. albopictus pools of Nov 18 are as yet unidentified. They are not dengue viruses.

A total of 63 dengue virus strains were isolated from in and around the homes of suspect cases of dengue infection. Table 4 depicts the results of testing all mosquitoes collected in the vicinity of these homes for the duration of the study. While more isolates were obtained from pools of A. albopictus than from A. aegypti, the recovery rate from the latter species was much greater. Twenty—five dengue viruses were isolated from 89 pools of A. aegypti (representing a total of 478 female mosquitoes), while 38 strains were recovered from 1832 pools of A. albopictus, representing 18017 female mosquitoes. Less than 1% of the A. albopictus were collected indoors, while over 90% of the A. aegypti were collected indoors. In each case where serologic studies were performed, virus strains were isolated from mosquitoes in and around homes of patients with a serologic diagnosis of either definite or probable dengue.

Plaque—forming agents were isolated from three of 76 pools of A. albopictus collected on Nov. 18 in the vicinity of the home of case 34. This child failed to develop dengue HI antibody during convalescence, and while the three viruses are as yet unidentified, it has been shown that they are not dengue viruses.

Mosquito collections are listed in Table 5 by collection period to illustrate the striking reduction noted in adult A. aegypti following the institution of the control program. During the two—month period from 5 Aug to 3 Oct, only 37 adult females were collected for testing. In spite of the continued large number of A. albopictus available for testing, and the increased numbers of A. aegypti collected in Oct—Dec, only one pool of either species was positive for dengue virus, that being a pool of A. albopictus collected on 13 Nov near the home of Case A, a child who experienced an apparent dengue infection in May, prior to the beginning of the surveillance program. Infected pools of both A. aegypti and A. albopictus had been collected at the same house in May.

TABLE 5

RECOVERY OF DENGUE VIRUSES FROM AEDES MOSQUITOES ON KOH SAMUI, 1968

Collection Date	Mosquito species					
	A. aegypti			A. albopictus		
	No. of Mosq.	No. of pools		No. of Mosq.	No. of pools	
		Tested	pos.		Tested	pos.
May 29—Jun 2	26	4	2	474	48	2
Jun 10—18	101	12	8	450	47	4
Jun 22—26	58	7	1	376	39	7
July 4—6*	43	5	0	395	40	0
July 9—17	38	7	4	1581	162	8
July 20—Aug 5	119	27	9	1394	49	8
Aug 5—13	6	1	0	1068	108	2
Aug 16—29	4	3	0	2299	233	3
Sep 1—5	7	2	1	779	79	3
Sep 16—24	3	2	0	832	84	0
Sep 29—Oct 3	17	3	0	1078	110	0
Oct 15—24	52	17	0	3365	360	0
Oct 25—Nov 21	442	82	0	5831	546	1
Nov 22—Dec 19	249	39	0	5263	73	0
Dec 20—Jan 10	17	3	0	1733	25	0
Jan 13—Feb 17	67	5	0	2047	38	0
Feb 18—Mar 22	210	26	0	344	25	0
Collections retated to patients homes	478	89	25	18017	1832	38
Collections unrelated to patients homes	981	156	0	11291	334	0
Total	1459	245	25	29308	2166	38

* Shipment thawed enroute to lab.

TABLE 6

NEUTRALIZING ANTIBODY TITERS OF REFERENCE DENGUE ANTISERA AGAINST DENGUE STRAINS FROM KOH SAMUI, 1968

Strain Number	Source	MK2 Passage Level	Titer* of prototype antisera			
			D1	D2	D3	D4
Homologous Strain			200	420	320	500
KS68—AM16	A. albopictus	1	<10	<10	<10	190
KS68—AM25	A. aegypti	1	"	"	"	120
KSAM2138	A. albopictus	3	10	10	10	230
KS68—DM30	A. albopictus	1	"	"	"	110
KS68MDM36	A. aegypti	1	"	"	"	85
KS68—1M75	"	1	"	"	"	125
KS68—1M77	"	1	"	"	"	74
KS68—2M82	A. albopictus	2	"	40	"	500
KS68—2M85	A. aegypti	1	"	<10	"	250
KS68—3M88	"	1	"	"	"	120
KS68—3M89	"	1	"	"	"	70
KS68—4M106	A. albopictus	1	"	"	"	320
KS68—4M111	"	1	"	"	"	100
KS68—4M112	"	1	"	"	"	60
KS68—4M115	A. aegypti	1	"	"	"	64
KS68—4M116	"	1	"	"	"	160
KS68—4M117	"	1	"	"	"	>640
KS68—5M130	A. albopictus	1	"	"	"	200
KS68—5M136	"	1	"	"	"	80
KS68—7M150	"	1	"	"	"	150
KS68—7M151	"	2	"	"	"	400
KS68—7M160	"	1	"	"	"	80
KS68—7M161	"	1	"	"	"	>640
KS68—7M166	"	1	"	30	"	120
KS68—7M168	A. aegypti	1	"	<10	"	30
KS68—9M220	A. albopictus	1	"	"	"	70
KS68—9M226	A. aegypti	2	"	"	"	640
KS68—11M288	A. albopictus	2	"	"	"	120
KS68—11M290	"	3	"	"	"	640
KS68—11M319	"	1	"	40	"	40
KS68—11M332	"	1	"	<10	"	350
KS68—12M363	"	2	"	"	"	350
KS68—12M366	"	2	"	"	"	200
KS68—12M374	"	4	17	"	20	160
KS68—12M368	A. aegypti	2	<10	"	<10	640
KS68—12M387	"	1	"	"	"	45
KS68—12M388	A. aegypti	1	"	"	"	120
KS68—13M402	A. albopictus	4	17	<10	<20	320
KS68—13M412	A. albopictus	2	<10	"	"	160
KS68—13M433	"	1	"	"	"	55
KS68—16M449	"	2	"	"	"	640
KS68—16M451	A. aegypti	1	"	"	"	60
KS68—16M456	"	1	"	"	"	40
KS68—17M469	"	1	"	"	"	50
KS68—18M460	A. albopictus	2	"	"	"	160
KS68—18M474	A. aegypti	1	"	"	"	25
KS68—19M489	"	2	"	"	"	550
KS68—19M490	"	2	"	"	"	220

Table 6. (Continued)

Strain Number	Source	MK2 Passage Level	Titer* of prototype antisera			
			D1	D2	D3	D4
KS68—19M503	A. aegypti	1	<10	<10	20	320
KS68—22M517	A. albopictus	1	"	"	"	120
KS68—23M539	"	2	"	"	"	190
KS68—23M541	"	1	"	"	"	70
KS68—25M576	A. aegypti	1	"	"	"	320
KS68—27M583	"	1	"	"	"	>640
KS68—28M615	A. albapictus	2	"	"	"	150
KS68—28M617	"	3	"	"	"	140
KS68—28M990	"	2	"	"	"	110
KS68—29M935	"	1	"	"	"	100
KS68—30M835	"	2	"	"	"	100
KS68—31M895	"	2	"	"	"	100
KS68—33M959	"	1	"	"	"	50
KS68—33M971	"	1	"	"	"	40
KS68—33M972	A. aegypti	1	"	"	"	90
KS68—34563	Serum Case 22	4	"	"	"	230
KS68—34567	Serum Case 26	1	"	"	"	70

* Reciprocal of 50% plaque reduction titer.

Between 17 Oct 68 and 22 March 69, 156 pools of A. aegypti (981 mosquitoes) and 334 pools of A. albopictus (11, 291 mosquitoes) collected at Entomology surveillance sites (see section on Mosquitoes) were tested for virus isolation, and all were negative. These mosquitoes were collected in and around houses unrelated to recognized cases of clinical dengue.

All dengue isolates were identified by means of the plaque reduction test in LLC—MK2 cells, described in previous reports. All were tested against type—specific monkey antisera. With the exception of the three viruses mentioned above, all strains were found to be dengue—4. Neutralization data are shown in Table 6. As had been observed in previous years, there is considerable variation among strains in their capacity to be neutralized by a single antiserum. While some are neutralized by high dilutions of serum, similar to the homologous reaction, others are neutralized only by low dilutions of serum.

Two strains of dengue 4 were isolated from the acute phase sera of patients with clinical dengue (cases 22 and 26.)

SUMMARY

An attempt was made to interfere with transmission of dengue viruses on an island by means of an A. aegypti control program. Program effectiveness was monitored by means of observations on mosquito population indices, sero—conversion rates in a sample of school children, and the incidence of clinically apparent dengue infections. A striking, temporary reduction was noted in the population of A. aegypti adults, as well as a more prolonged reduction in Stegomyia larvae in domestic water containers. While serologic data from school children were inadequate to measure trends in dengue virus transmission, no cases of clinically apparent dengue were observed after 25 August. Comparison with the slopes of the epidemic curves of 1966 and 1967 (see previous reports) suggests that the 1968 outbreak was truncated. This apparent truncation was associated in time with the A. aegypti control program, the last case occurring two weeks after the program was completed. However the observation that dengue 4 was prevalent during this period, as it had been in 1967, suggests that the abortive nature of the outbreak may have been the result of a relative refractoriness of the population to homotypic reinfection.