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to treatment with chloroquine and other synthetic drugs were isolated from members of the study team during the year, and the malaria rate among team members showed that these drugs were ineffective in preventing malaria at Khao Mai Kaeo. Observations on family incidence of malaria indicated a very uneven distribution of infections among households, and among members of a single household. The possible relationship of these observations to immunity is under study. A considerable number of people were seen in the study area who had circulating asexual parasites without evident symptoms, an element of possible importance in the transportation of the disease into new areas. Shorter surveys were made in Khao Yai National Park and environs, and on the Malaysian border. In those areas, P.vivax infections were relatively more abundant than in the principal study area.

tabulated. These data are to be compiled over a two year period to provide baseline data for more detailed studies on malaria transmission and control to be conducted in the area. Other study areas were visited whenever time permitted, to observe differences in the pattern of malaria transmission.

Progress: The field study was initiated in the Khao Mai Kaeo area of Southeastern Thailand in May, 1963. The results of malarimetric studies are presented below, and the results of Anopheles collections in the area will be found in the report of Study Number 51. Two short surveys were conducted in other parts of Thailand; one near Pakchong on the Korat Plateau and one in the forest settlement at Waeng on the Malaysian border. Each will be discussed separated below:

a. Khao Mai Kaeo

(1) The Land and Community: Khao Mai Kaeo is the name of an administrative group of hamlets (Tambon) in the Ban Lamung District of Choburi Province, Thailand. It is situated in the southeastern region of Thailand ($12^{\circ} 56'N$ and $101^{\circ} 05'E$) comprising an area of approximately 156 square kilometers. Some 15 years ago this area was a hilly tropical evergreen forest, as classified by the Royal Forestry Department of Thailand. Now the forest has been almost completely cleared and replaced by tapioca plantations. Only 15 percent of the area remains as a remnant of the forest which is continuously invaded for more land for cultivation. Most of the area is about 110 feet above the sea level, while the hilly part is as high as 250 to 300 feet. Rainfall usually is abundant at Khao Mai Kaeo, averaging from 1270 to 2794 mm. annually, with most of this concentrated in the rainy season from May to November. During the rainy season numerous small and seepages are found in the sandy soil. Many of these dry up completely or partially during the summer months, from February to April, except where the soil surface is protected by remnants of forest. Transportation is poorly developed in the region with rough jeep and truck tracks leading inland from the coastal highway. During the rainy season these tracks become muddy and flooded and often impassable, even for four wheel drive vehicles. Only four old hamlets are present, having been settled in the forests which originally covered the area some decades in the past. New small hamlets, generally consisting of a few houses each, have been scattered through the area in the last ten years. These hamlets are still in a transitory state, and new temporary shelters are built yearly, most frequently during the rainy season, due to an influx of temporary workers on the tapioca plantations. There were 206 households covered in May 1963, This number increased to 345 as recored in March 1964. The number of persons occupying the houses varied from one to as many as 25. Tha latter are inhabited chiefly by temporary field workers engaged in tapioca cultivation. There is one Wat(Buddhist temple)

in the district, with an attached primary school about sixty five students. There is no government health facility of any kind within this 156 square kilometer area. Malaria is well known to both the old and new residents of the district, being the most serious disease affecting the health and wealth of the community.

(2) Malarimetric Study

(a) Spleen Index. Two series of spleen examinations were made, one during May 1963 and the other in March 1964. Children attending the primary school were examined there and pre-school children were included during house-visits. Hackett's method was applied, and the physician in charge of the team was the examiner. The results of the two spleen examinations are shown in table 1 and 2. There was only a slight difference in the spleen rates shown in the two tables. The reduction of the spleen rate in the age group above 10 years may be due to the fact that many of the examinees in this group during March were new migrants. Malaria in Khao Mai Kaeo can be considered as hyperendemic according to commonly accepted criteria.

(b) Parasite Index. Blood films for malaria parasite examination were obtained from the school children at the time of the spleen examination, and smears from other age groups were collected during house visits. In order to have a comprehensive picture of the prevalence of malaria, efforts were made to collect blood films from all inhabitants in the area. Every house, either permanent or temporary was thoroughly searched for and visited. Members present in the households at the time of visits were included in the blood collection. Thick and thin films on the same slide were made from each person. Giemsa stain was used in a dilution of 1:20 with a buffer solution of pH. 7.2, stained for 60 minutes.

The blood collections were carried out four times between May 1963 through March 1964, in order to study the prevalence and trends of Malaria at different times of the year. It should be noted that the month of May marked the beginning of the monsoon season, after an absence of significant amounts of rain since December, almost six months. The dry season was at its height by the end of March and the streams were almost completely dry. Malaria transmission was considered to be at its lowest point for the year. Table 3 shows the results of the examination for malaria parasites during these four intervals.

The results of the blood examinations (Table 3) showed that during the dry season while transmission was at the lowest point the parasite rate was as high as 32.1%. By the end of the transmission season the parasite rate was 35.0%. The low percentage of the malaria rate during

TABLE 1. Spleen examination of children. Khao Mai Kaeo, Bang Lamung District, Cholburi Province, Thailand during May 1963.

Age group	No.exam.	Total Posit. spleen	Spleen rate	Spleen size					
				0	1	2	3	4	5
Under 1 year	27	18	66.6 %	9	0	13	5	0	0
2 - 9 years	104	93	89.4 %	11	18	34	37	4	0
10 years	58	35	60.0 %	25	6	14	9	3	1

TABLE 2. Spleen examination of children. Khao Mai Kaeo, Bang Lamung District, Cholburi Province, Thailand during March 1964.

Age group	No.exam.	Total Posit. spleen	Spleen rate	Spleen size					
				0	1	2	3	4	5
Under 1 year	36	21	58.3 %	15	0	17	4	0	0
2 - 9 years	127	109	85.9 %	18	27	48	31	3	0
10 years up	92	41	44.6 %	51	17	22	1	1	0

TABLE 3. Blood film examinations, Khao Mai Kaeo, Cholburi Province, Thailand, May 1963 to March 1964.

Months	No.exam.	No. Positive	Parasite rate	<u>P.falciparum</u>		<u>P.vivax</u>	
				Asex.	Sex.	Asex.	Sex.
May to June	757	191	30.0 %	184	37	7	4
July to Sept.	656	135	20.5 %	131	23	4	1
Oct. to Dec.	810	284	35.0 %	245	30	35	5
Jan. to March	1,239	398	32.1 %	340	26	48	12

the month of July to September 1963 might be attributed to the DDT residual house spraying which took place during the period of 15 May to 11 June 1963.

Among the three Plasmodium species known to exist in the malarious area of Thailand, only P. falciparum and P. vivax were observed in the slides examined. P. falciparum far out-numbered P. vivax, the former making up as much as 97 percent of the total positive. There was no P. malariae. One case was found during examination, but the person did not reside in the study area. It was observed also that sexual malaria parasites were always present in the blood films. With the presence of large numbers of Anopheles species capable of completing the sporogonic cycle, transmission of the disease in the area should be at a high level.

(3) Age Group Distribution of Positive Blood Films. When the examinees were classified into age-groups it was clear that the infant parasite rate was as low as 9.6% and as high as 23%. The presence of malaria parasite in infants strongly indicated that transmission existed throughout the year in the area, especially intra-domicillary transmission. Table 4, 5, 6, and 7 showed the age group distributions of malaria parasites during the four examination periods. In order to find the youngest age among the infants infected with malaria parasites, especially during the March examination, the infants data were retabulated to show the age in months of the infants infected with malaria (Table 8). As the blood films were made during February and March and the youngest age found positive was within the first month of life, malaria transmission must have been taking place in the area by the end of March. The absence of positive infants between the age of 4 to 6 months is probably due to the small number examined, rather than absence of malaria in this group.

(4) Infections Within Households. As noted in table 3, the parasite rate among inhabitants of the study area was as high as 35% during the peak season of October to December, 1963. When the parasite data were examined in terms of the number of households with malaria infected members, it was found that there were 138 households where at least one member had a positive smear. Two hundred thirty eight households were examined during this period, giving a rate of 57.9% infected households. During the January to March 1964 examinations 155 out of 345 households (44.9%) had at least one member positive by peripheral blood smear. These figures illustrate the high rate of malaria infection in the study area, but perhaps more importantly, they raise certain questions relating to immunity to malaria in a hyperendemic area. These include:

(a) Why did so many households in the limited and rather homogeneous study area escape malaria entirely during the year?

(b) Within infected households, why did so many individuals, apparently exposed to the same risk of infection, escape malaria infection during the year?

Table 4. Age group distribution, May to June 1963.

Months	No.exam.	No. Positive	Parasite rate	<u>P.falciparum</u>		<u>P.vivax</u>	
				Asex.	Sex.	Asex.	Sex.
Under 1 year	48	11	23.0%	10	3	1	1
2 - 9 years	195	47	24.0%	44	9	3	1
10 years up	514	133	25.8%	130	25	3	2
Total	757	191		184	37	7	4

Table 5. Age group distribution, July to Sept. 1963

Months	No.exam.	No. Positive	Parasite rate	<u>P.falciparum</u>		<u>P.vivax</u>	
				Asex.	Sex.	Asex.	Sex.
Under 1 year	29	2	6.8%	2	0	0	0
2 - 9 years	144	33	23.0%	30	8	3	0
10 years up	483	100	20.0%	99	15	1	1
Total	656	135		131	23	4	1

Table 6. Age group distribution, October to December 1963

Months	No.exam.	No. Positive	Parasite rate	<u>P.falciparum</u>		<u>P.vivax</u>	
				Asex.	Sex.	Asex.	Sex.
Under 1 year	52	5	9.6%	4	0	1	0
2 - 9 years	212	75	35.0%	61	6	14	1
10 years up	546	200	37.0%	180	24	20	4
Total	810	284		245	30	35	5

Table 7. Age group distribution, January to March 1964

Months	No. exam.	No. Positive	Parasite rate	<u>P. falciparum</u>		<u>P. vivax</u>	
				Asex.	Sex.	Asex.	Sex.
Under 1 year	116	23	19.8	17	2	6	2
2 - 9 years	339	97	28.9	75	9	19	5
10 years up	784	278	35.4	248	15	23	5
Total	1,239	398		340	26	48	12

Table 8. Malaria parasites in infants with current fever and with history of fever.

Age in Months	Current fever		With history of fever	
	Examined	Positive	Examined	Positive
12 - 11	2	2	0	0
11 - 10	2	1	4	1
10 - 9	4	1	8	1
9 - 8	4	2	6	0
8 - 7	5	1	9	1
7 - 6	5	1	8	3
6 - 5	3	0	6	0
5 - 4	3	0	3	0
4 - 3	4	1	5	0
3 - 2	2	1	6	2
2 - 1	2	2	7	2
Under 1 month	1	1	6	0
Total	40	13	68	10

Studies are presently under way to look into these questions in more detail. The micro-distribution of the vectors may play a role in the first phenomenon listed (malaria-free households), but the most readily acceptable explanation for the second is the existence of some form of immunity to P. falciparum infection in many inhabitants of the study area.

(5) Asymptomatic Parasitemias. The presence of individuals in a population with circulating malaria parasites of P. falciparum in the absence of concurrent clinical illness may play an important role in the introduction of the disease into new areas. The introduction of rapid international air transportation makes this danger a real problem in global medicine. In our early studies in the Khao Mai Kaeo area a number of people showed parasitemia without evident clinical signs, and an attempt was made to assess the extent of this component of the Malaria infected population. The examinees were asked about their history of illness at the time blood films were made. Information concerning febrile attacks was recorded under three categories:

(a) Actual fever at the time of visit, or with history of fever during the preceding three days.

(b) History of fever during the preceding 2 weeks, but without feverish sensations at the time of visit.

(c) No history of febrile attack for more than 3 weeks.

Although this information is rather vague and cannot be definitely relied upon, it may indicate the presence of asymptomatic parasitemia cases, in which parasites are circulating without complaints of clinical symptoms on the part of the patients. Only those with asexual malaria parasites in their blood were included in the tabulation as positive cases. The fever records were made during the May to June period and also during January to March visits because the febrile attacks were expected to be at a very low level during these dry months. Table 9 and 10 show the parasite data for the examinees relating a history of febrile attack during the last two weeks but no feverish sensations at the time of investigation. The percentage of 27.0% positive among the examinees without concurrent clinical complaints is high enough to be seriously considered in discussing the problem of imported malaria. Additional studies on this subject are under way at present.

(6) Chloroquine Resistant Strains of Plasmodium falciparum
Several reports had been received indicating the existence of strains of P. falciparum in Thailand before the present field study was begun. The existence of these strains was definitely established at Khao Mai Kaeo when a number of the personnel engaged in the study contracted serious

Table 9. Asexual malaria parasites in febrile and non-febrile cases, May to June 1963.

Age group	Total Exam.	No. history of fever		With history of fever		With febrile attack	
		Exam.	Posit.	Exam.	Posit.	Exam.	Posit.
Under 1 year	48	21	0	18	7	9	4
2 - 9 years	195	61	0	50	24	84	23
10 years up	504	102	0	142	67	270	66
Total	757	184	0	210	98*	363	93

* Rate of asymptomatic parasitaemia 46.9%

Table 10. Asexual malaria parasites in febrile and non-febrile cases, January to March 1964.

Age group	Total Exam.	No. history of fever		With history of fever		With febrile attack	
		Exam.	Posit.	Exam.	Posit.	Exam.	Posit.
Under 1 year	116	8	0	68	10	40	13
2 - 9 years	339	23	0	238	45	88	52
10 years up	784	65	0	491	158	228	120
Total	1,239	96	0	787	213*	356	185

* Rate of asymptomatic parasitaemia 27.07%

malaria cases while taking the currently recommended prophylactic schedules of chloroquine or chloroquine-primaquine. The first of these, an investigator on temporary duty from WRAIR, collected Anopheles attracted to him in a mosquito-net type of human bait trap during several nights in the early part of the transmission season. He had taken the recommended chloroquine prophylaxis, but became ill on his return to Bangkok. A radical cure was achieved by the use of quinine. A blood sample taken from the patient (RW) early in the febrile phase was sent to WRAIR for study, and on subsequent transfer to prisoner volunteers was found to highly resistant to chloroquine. However the strain in volunteers responded well to pyrimethamine. Another member of the investigational team (JMN) spent two nights in the study area at the close of the main transmission season in December. He observed mosquito collections, but did not offer himself as bait. The number of bites he received is unknown, but not long after returning to Bangkok (a malaria-free area) he became seriously ill. It should be noted that this patient took what should have been an adequate dose of chloroquine before leaving Bangkok, and took an additional chloroquine-primaquine tablets on the afternoon of the day he arrived in the study area. A detailed analysis of this case, including serum drug levels, is presented in the report of Study Number 9. During the febrile phase of JMN's illness a sample of his blood was inoculated in a Thai volunteer, and this case is also discussed in Study 9. It should be noted that neither of these infections responded to pyrimethamine, differing from the drug spectrum of the strain isolated RW. Thus, at least two forms of drug resistant P. falciparum parasites may exist in the Khao Mai Kaeo area. By the end of the year all of the team members who had participated in Anopheles collections including the principal investigator with one exception, had experienced at least one episode of malaria, despite various schedules of chloroquine prophylaxis. Additional, although inconclusive, evidence for the existence of chloroquine-resistant malaria was obtained by examination of records of recurrence of clinical malaria (and positive blood films) in persons who received chloroquine treatment while visiting the field station. Analysis of these records is still in progress. Persons returning with clinical malaria within two weeks of treatment are being followed in more detail. It is impossible to completely exclude reinfection in this hyperendemic area without detailed daily examination of blood films.

b. Khao Yai National Park and Vicinity

(1) Description of the Area. During scrub typhus investigations at Khao Yai National Park a number of complaints of malaria infection were received from workers in the Park. The Park was recently established in a previously rather inaccessible mountain range covered with evergreen forest. The region is at the southwestern edge of the Korat Plateau, not

far from the city of Pakchong, an area long notorious for malaria. There are two military installations in the Park, a radar station and a communications center. There are a number of permanent streams, and during the rainy season many depressions and seepages hold water. Almost all of those residing in the Park (other than military personnel who have reported no malaria) are workers of the Royal Thai Forestry Department.

(2) Malariometric Data. Blood films were obtained from all of the personnel living in the Park who could be found. Blood films were also obtained from workmen living in the foothills at the base of the mountain outside the Park, and from two new agricultural villages nearby. The results of these examinations are shown in table 11. The percentage of P. vivax infections in the sample was much higher than at Khao Mai Kaeo. During the survey, mid-September 1963, heavy rains were occurring in the area making travel to some of the villages very difficult. During visits to schools and homes spleen examinations were made on 82 children in the Park and surrounding villages. Of 82 children examined, 65 (79.2%) showed various degrees of enlarged spleen. The large percentage of P. vivax among the infections detected makes this area of interest for future study.

C. Waeng District, Narathivas Province.

(1) Description of the Area. This forested area is in the southern most part of Thailand, not far from the Gulf of Thailand. The Department of Public Welfare, Royal Thai Ministry of the Interior, has set up a self-help settlement in the area. The settlement covers 27 kilometers (7000 acres) of undulating terrain in which the original forest is being converted to rubber plantations. Each family settled in the area receives ten acres of land, thus there is usually only one house, generally with five inhabitants, for each ten acres. At the time of the survey, 6-25 January 1964, there were 1921 persons registered as living in the 350 family groups in the settlement.

(2) Malariometric Data. Strong winds and rain in the area throughout the period hampered the work, particularly the mosquito collections reported in Study Number 51. In addition, the Muslim month of fasting was in progress, making it difficult to conduct house visits to the extent desired. The population of this border area is largely Muslim. Fifty two (49.5%) of the 105 children attending the primary school had enlarged spleens, suggestive of a high prevalence of malaria. However, only eight of the smears from these children were positive; 5 for P. vivax and 3 for P. falciparum. House visits were confined to families whose school age children had enlarged spleens. Forty six households were visited. Among the 218 persons examined 7 were positive

Table 11. Malaria parasite examinations, Khao Yai National Park and vicinity, September, 1963.

Locality	No. examined	No. Positive	Parasite rate	P. falciparum		P. vivax	
				Asex	Sex	Asex	Sex
National Park of Khao Yai	102	3	2.9%	2	0	1	0
Foothill Workmen Camp	70	11	15.7%	4	2	7	2
Noeu Ka-does Primary School	38	5	13.1%	4	1	1	1
Bankudkhla Village	39	12	30.7%	2	0	10	4
Wat Musee Primary School	44	2	4.5%	2	2	0	0
Total	293	28		14	5	19	7

for P. falciparum, 2 for P. vivax. Thus, the parasite rate for the district was quite low, despite reports which had reached Bangkok that there was a great deal of malaria in the settlement. The parasite species distribution, based on this small sample, was more like Pakchong than Khao Mai Kaeo.

Summary: Almost one year of study was completed in the Khao Mai Kaeo study area in Southeastern Thailand. The high spleen rates and the high parasites rate indicated that the area was hyperendemic for malaria. The overwhelming preponderance of the infections were due to Plasmodium falciparum, and a number of very severe cases were seen. Many of the people seen at the field station or in their homes had circulating asexual parasites without fever. Many of these people were going about their normal daily work, and could readily have transported the parasites to other districts. The presence of parasitemia in very young infants indicated that transmission was occurring in the area to some extent even during the dry season when vector populations were quite low. All but one of the investigators and technicians engaged in the project contracted falciparum malaria during the study despite the use of prophylactic drugs. At least two strains of proven chloroquine resistant P. falciparum were isolated from members of the investigational team. Shorter field studies were conducted in and near Khao Yai National Park and at Waeng on the Malaysian border. P. vivax infections made up a greater part of the infection picture in those areas.

Conclusions: Malaria, chiefly due to Plasmodium falciparum is hyperendemic in the study area at Khao Mai Kaeo, with some transmission going on all through the year. Drug resistant strains occur in the area, and in general it appears to be well suited for additional detailed studies on immunity and the effect of various drugs.