PB-219 718

THAILAND MALARIA OPERATIONAL RESEARCH UNIT

William Chin

Center for Disease Control
Atlanta, Georgia

30 June 1972

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 219 718

RESEARCH FINAL
PROJECT REPORT

JUNE 30, 1972

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

THAILAND MALARIA OPERATIONAL RESEARCH UNIT

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
Health Services and Mental Health Administration
Center for Disease Control
Atlanta, Georgia 30333

1. Report No.	2. TH-614.532-C397a	3.	4. Publication Date PB-219718
5. Title and Subtitle "Thailand Malaria Operational Research Unit", ~~Research Final Project Report~~			6. Report Date Jun[redacted], [redacted]72
7. Author(s) William Chin,			8. Performing Organization Rept. No.
9. Performing Organization Name and Address Center for Disease Control Atlanta, Georgia 30333			10. Project/Task/Work Unit No.
			11. Contract/Grant No. PASA TCR (HA) 2-66; PASA RA (HA) 1-70
12. Sponsoring Organization Name and Address Department of State Agency for International Development Washington, D.C 20523			13. Type of Report & Period Covered Final Report 8/69 - 7/72
			14.

15. Supplementary Notes

16. Abstracts

Drug studies began initially with surveys for chloroqu[illegible]sistant falciparum malaria in six different areas of Thailand which showed resistance rates ranging from 69-94%. To develop an alternative treatment regimen against these strains, the evaluation of sulfalene-trimethorprim and sulphormethoxine-pyrimethamine was accomplisned which showed no significant difference between the two treatment regimens. Subsequent studies of sulphormethoxine-pyrimethamine showed that cure rate were at least 90% and that primaquine was required to counter the demonstrated lack of sporontocidal effect of the combination. Based on these results, the NMEP adopted the use of sulphormethoxine-pyrimethamine as the standard treatment for falciparum malaria in Thailand.

The evaluation of alternative treatment regimens against falciparum strains "resistant" to sulphormethoxine-pyrimethamine based on quinine alone or in combination with other drugs was also accomplished. The results showed possible quinine resistance (to seven days treatment) and suggested that quinine in combination with tetracycline may provide additive effect.

One significant finding was that CDC light trap with dry ice as a monitoring device of absolute vector density was as reliable as human bait catches.

17b. Identifiers/Open-Ended Terms

*Malarial fever, ~~*Tha~~ ~~*Thailand~~)

17c. COSATI Field/Group 614

18. Availability Statement		19. Security Class (This Report)	21. No. of Pages 38
		20. Security Class (This Page)	22. Price $3.00

RESEARCH FINAL
PROJECT REPORT

JUNE 30, 1972

THAILAND MALARIA OPERATIONAL RESEARCH UNIT

under Participating Agency Service Agreement Number RA(HM) 1-70
PIO/T Number 931-17-511-503-72-3117706 & 3127703 with U.S. Agency for International Development

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
Health Services and Mental Health Administration
Center for Disease Control
Atlanta, Georgia 30333

2

REPORT SUMMARY

A.1. *Project Title:* Thailand Malaria Operational Research Unit

Contract Numbers: PASA TCR (HA) 2-66
PASA RA (HA) 1-70

2. *Principal Investigator:* William Chin, M.D.

Contractor: Malaria Program
Center for Disease Control
1600 Clifton Road, NE
Atlanta, Georgia 30333

3. *Contract Period:* September 1967 to June 1972

4. *Period covered by Report:* September 1967 to June 1972

5. *Total A.I.D. Funding of Contract to 30 June 1972:* $ 424,998.49

6. *Expenditures and Obligations through 30 June 1971:* $ 283,796.49

7. *Expenditures and Obligations for FY 1972:* $ 141,202.00

8. Estimated Expenditures for Next Contract Year: NA

B. Narrative Summary of Accomplishments and Utilization

The life span of the project, from signing of ProAg on 30 April, 1970 to the AID/W decision to terminate the project in October 1971, was less than 18 months. In fact, the time-interval between full implementation and the decision to terminate the project was only six months.

Drug studies began initially with surveys for chloroquine-resistant falciparum malaria in six different areas of Thailand which showed resistance rates ranging from 69-94%. To develop an alternative treatment regimen against these strains, the evaluation of sulfalene-trimethoprim and sulphormethoxine-pyrimethamine was accomplished which showed no significant difference between the two treatment regimens. Subsequent studies of sulphormethoxine-pyrimethamine showed that cure rates were at least 90% and that primaquine was required to counter the demonstrated lack of sporontocidal effect of the combination. Based on these results, the NMEP adopted the use of sulphormethoxine-pyrimethamine as the standard treatment for falciparum malaria in Thailand. To detect emergence of resistance to sulphormethoxine-pyrimethamine, a monitoring study, with financial assistance from the Roche Far East Foundation, was initiated in Pakchong. The evaluation of alternative treatment regimens against falciparum strains "resistant" to sulphormethoxine-pyrimethamine, based on quinine alone or in combination with other drugs was accomplished. The results showed possible quinine resistance

(to seven days treatment) and suggested that quinine in combination with tetracycline may provide additive effect.

Entomologic studies were limited due to late arrival of senior entomologist. One significant finding was that CDC light trap with dry ice as a monitoring device of absolute vector density was as reliable as human bait catches.

A. General Background

Both the malaria control program and the early stages of the malaria eradication program in Thailand were instrumental in dramatically reducing the number of malaria cases from an estimated 3.6 million in 1950 to 118,100 detected in 1965. Since 1965, when the program first achieved country-wide spraying coverage, progress has been much more gradual. In 1967, after two years of comprehensive house-spraying, it was recognized that technical problems may be responsible for the lack of substantial progress. Further, it was felt that operational research was needed to evaluate the following factors as possible impediments to successful malaria eradication and to develop remedial measures:

1. Exophilic and exophagic habits of the two principal vectors, *Anopheles balabacensis balabacensis* and *A. minimus*, which enable the two vectors to avoid contact with DDT deposits on sprayed surfaces and therefore to transmit malaria.

2. Chloroquine-resistant strains of *Plasmodium falciparum* which, because of lack of effective and operationally feasible alternative treatments, allow a buildup of a large reservoir of infection.

3. Mass migration of transitory laborers to and from meso- and hyperendemic malarious areas.

On September 1, 1967, the Royal Thai Government (RTG) and United States Operations Mission Thailand (USOM) signed an agreement to finance a research activity for two years, thus creating the Thailand Malaria Operational Research Unit (TMORU).

In December 1967, a Participating Agency Service Agreement (PASA) was signed by the Public Health Service (PHS) of the U.S. Department of Health, Education, and Welfare and the Agency for International Development/Washington (AID/W) under which the PHS would assume responsibility for staffing and implementation of the research studies. The unit chief was designated later in December 1967. The activity was aborted in January 1968 by the initiation of the Balance of Payments (BALPA) exercise. As a result of subsequent USOM considerations, the activity was reduced (from a proposed staff of five) to one PHS team member who would remain until the end of his tour (June 30, 1969). In August 1968, a PHS Malaria Advisor to Region II (Chiang Mai), whose position was eliminated by the BALPA exercise, was transferred to TMORU to work as an entomologist for the duration of his tour (June 30, 1969).

At the conclusion of the BALPA exercise, the USOM recommended to AID/W that the activity be funded from other than the USOM budget and that the project be regionalized. Negotiations between PHS and AID/W were initiated in August 1968. On May 14, 1969, after the negotiations were successfully concluded with Research Advisory Committee (RAC) approval for an operational research unit for a projected five year duration, USOM received a request from AID/W to concur in a centrally funded regional malaria research project to be staffed by five PHS members and 15 in-country nationals. Because of a

series of unforeseen administrative determinations by USOM, concurrence for implementation of the TMORU was delayed. On April 30, 1970, a Project Agreement (ProAg) was signed between the USOM and the RTG providing for an American staff of four instead of the originally proposed five positions. With the arrival of both the fourth U.S. technician and the four vehicles by April 1971, full operational status of the unit was finally achieved. After only three months of attaining full operational status, the TMORU was assessed by a team representing the RAC. Based in part on the review team's findings, AID/W decided in October 1971 to terminate the project. As a result of this decision, three of the TMORU U.S. technicians departed post by the end of 1971, leaving the Chief to complete drug studies and to completely phase out the project by June 1972.

B. Statement of Project Objectives as Stated in the Contract

Probable causes of persistent transmission of malaria, despite total coverage house-spraying in Thailand include the following:

1. Outdoor transmission, due to outside biting and resting habits of the vectors and habits of the people living in the affected areas.

2. Unrecognized changes in the habits of known vectors.

3. Presence of unrecognized vectors with breeding, biting, and resting habits not sufficiently affected by residual house-spraying to bring about interruption of malaria transmission.

4. Presence of chloroquine-resistant strains of falciparum malaria that do not yield to the conventional antimalarial drugs now in use by the NMEP, giving rise to a large reservoir of infections.

5. Mobility of a significant proportion of the rural population whose movements to highly malarious areas not only expose them to malaria infections outside treated villages but, due to frequency of such movements, also make them unavailable for the relatively long-term administration of the currently used antimalarial drugs required for radical cure.

The specific cause of program failure in any given locality is apt to be a combination of two or more of the probable factors listed above, but may be due to only one. On-site investigations will be necessary to define the exact nature of the cause or causes of failure and the extent of each area affected. Once the cause or causes of failure are defined, on-site field operational research may be implemented to develop new or improved methods or combination of methods in order to achieve the ultimate program goal of malaria eradication.

C. Continued Relevance of Objectives

In its report of the TMORU review (July 21-23, 1971) conducted by a team representing the RAC, the following is stated:

> "In terms of the practical aim of speeding up the progress of malaria eradication in Thailand by identifying and solving its technical and operational obstacles, we feel that the TMORU deserved the highest priority. The need for it still remains."

While significant progress was made in documenting the problem of chloroquine-resistant falciparum malaria and in the development of an alternative treatment regimen against such strains, the other major technical problems have been studied incompletely or have not been studied at all. Additionally, since the technical problems found in Thailand are common to other countries in this region, solution of these problems would have widespread benefit. Logic therefore would dictate continued strong support for research aimed at the resolution of such problems but regrettably, the project has been terminated.

D. Accomplishments to Date

Drug Studies

Significant progress was made not only in the documentation of the problem posed by the existence of chloroquine-resistant falciparum malaria but also in the development of an alternative treatment against such strains. Based on the TMORU findings, the NMEP of Thailand decided and thus became the first malaria program to change the standard radical treatment against falciparum malaria from chloroquine, used the past 20 years, to a single oral dose treatment with sulphormethoxine-pyrimethamine.

Studies of chloroquine-resistant falciparum malaria

The period from early 1968 until after the signing of the ProAg in April 1970 was one characterized by uncertainty of TMORU status and lack of permanent technical staff. Microscopes were the only available equipment. Under these circumstances, it was logical to concentrate on documentation of the problem of chloroquine-resistant falciparum malaria. Such studies required minimal staff temporarily assigned from the NMEP. During this period, a series of surveys were performed in six different areas of Thailand. In addition, in collaboration with the Epidemiology Section of the NMEP, a seventh survey which included the use of questionnaires and which encompassed the entire country, was performed. The surveys reported are based on a one-month follow-up after treatment with 1.5 gm. chloroquine (base) adult equivalent dosage. The results of the surveys are indicated in the following table.

Survey for Chloroquine-resistant Falciparum Malaria
TMORU and NMEP, Thailand

Place of study	No. cases treated	Failures	
		No.	%
Pakchong	17	16	94
Chumphon	16	14	88
Sakon Nakhon	61	45	74
Trad	29	27	93
Throughout Thailand*	32	28	88
Sakaerat	85	69	81
Kabinburi	42	29	69

*Questionnaire survey in collaboration with NMEP

In general, these survey results showed that the incidence of chloroquine-resistant strains of falciparum malaria is high in all areas studied and that the relatively lower drug failure rates of 69% and 74% were encountered in areas of stable malaria (Kabinburi and Sakon Nakhon) with little migration of the inhabitants and a resulting high immunity to malaria among the villagers.

These findings fully confirmed other such studies in which one-month follow-up was utilized. Among these, the series of three studies reported by Dr. Tranakchit Harinasuta from the Bangkok School of Tropical Medicine are most noteworthy. She reported successive failure rates of 19%, 95% and 100% in 1962, 1965 and 1967, respectively.

With attainment of full operational status by early 1971, the evaluation of alternative treatment regimens and standard presumptive treatment was possible. These studies included the following:

1. A comparative evaluation of sulfalene-trimethoprim (SF-T) and sulphormethoxine (Fanasil)-pyrimethamine (S-P) against falciparum malaria was performed in the Trad Provincial Hospital. This study was made possible through the active collaboration between the TMORU, U.S. component of the SEATO Medical Research Laboratory, Bangkok, and the staff of the Trad Provincial Hospital.

   Thirty-one patients were given a single dose of SF-T (1 gm. sulfalene and 0.5 gm. trimethoprim) and 34 patients were given S-P (1 gm. sulphormethoxine and 0.05 gm. pyrimethamine). Radical cures were observed in 84% given SF-T and in 91% given S-P. Clearance of fever and asexual parasitemias were similar. Factors of prior immunity and antimalarial intake showed no apparent influence on the treatment outcome. No significant toxic side reactions were observed in subjects administered

either one of the combination treatments.

Based mainly on the findings cited above, the NMEP in October 1971 adopted the use of sulphormethoxine-pyrimethamine as the standard radical treatment against falciparum infections. At the same time, since the study also showed significant emergence of gametocytes following treatment with sulphormethoxine-pyrimethamine, the NMEP senior staff decided to retain primaquine 15 mg. daily for five days as part of the new radical treatment.

2. The evaluation of the presumptive and radical treatments against falciparum malaria in Thailand was performed in Pakchong and in Siracha. The need for such a study was based on the fact that the presumptive treatment of falciparum malaria had remained static in the face of emergence of drug-resistant parasites. Further, since the adoption of new standard treatment for falciparum malaria was a pioneering effort, it was agreed by both the NMEP and TMORU that more precise information on its effectiveness as gained from controlled operational use was required.

   The results showed that the effectiveness of the presumptive treatment (chloroquine 600 mg. (base) and pyrimethamine 50 mg.) has been greatly reduced. The infection in 28 of 43 subjects (65%) given this treatment were still patent at day seven or had recrudescence of asexual forms of falciparum malaria by day 14 and, 7 of 12 (58.3%) of the *A. b. balabacensis* lots fed on the patients exhibiting gametocytemia post treatment resulted in infection to the sporozoite stage.

   Radical treatment, single oral dose of sulphormethoxine 1 gm. and pyrimethamine 50 mg., resulted in cure rates of 52 of 58 (91.2%) cases treated in Siracha and 49 of 51 (96.1%) of cases treated in Pakchong. *Anopheles b. balabacensis* feedings on cases with gametocytemia following radical treatment resulted in infection to the sporozoite stage in 7 of 15 (46.6%) of the lots. Use of primaquine for the first five days of radical treatment resulted in effective suppression of gametocytemia.

3. An evaluation of quinine alone or in combination with tetracycline and pyrimethamine against falciparum malaria was initiated because previous studies of sulphormethoxine-pyrimethamine against falciparum malaria have shown that its use can result in an expected failure rate of about 10%. This study represents an attempt to develop an operationally feasible alternative drug regimen for use against such cases of treatment failure so that the buildup of resistant strains against sulphormethoxine-pyrimethamine may be minimized.

   Of the limited antimalarials presently available for consideration as an alternative regimen, quinine remains the drug of choice. Data on the sensitivity of Thai strains of *P. falciparum* to quinine is limited and conflicting. Studies on at

least two Thai strains of falciparum malaria performed in the United States, utilizing induced infections in non-immune prisoner volunteers, indicate that a 100% cure rate can result from five days of quinine treatment (in a dosage of gr X tid). Conversely, Harinasuta and her colleagues reported four and five days quinine treatment in Thai falciparum patients resulted in failure and that seven days of quinine treatment was required for consistent cures. A regimen of quinine treatment requiring seven days has obvious disadvantages of prolonged duration and accompanying side reactions. Such limitations render impractical its field use by a malaria operational program.

Recently, the SEATO Medical Research Laboratory reported that tetracycline was found to be an effective antimalarial against Thai strains of *P. falciparum*. While the side reactions to tetracycline are minimal, the requirement of ten days treatment also limits its usefulness for administration in the field. The need therefore to develop a treatment regimen based on quinine which is both effective and operationally feasible is obvious.

It is not known whether the addition of tetracycline to quinine would produce synergistic effect enabling reduction in total duration of treatment. A potentiation effect between quinine and pyrimethamine against Vietnam strains of falciparum malaria has been reported, but it is unknown whether these two drugs would have the same effect against Thai strains.

A study in collaboration with the staff at Propokklao Hospital, Chantaburi was undertaken to assess these factors in order that an alternative regimen may be developed for field use by the NMEP against those strains of falciparum malaria found to be "resistant" to sulphormethoxine-pyrimethamine.

In this study 59 adult subjects, including males and females, were treated on an alternating basis with five respective treatment regimens. Completion of one-month follow-up was accomplished in 39 subjects with the following cure rates:

Treatment:

I:	Quinine gr X tid x 7 days	75.0%
II:	Quinine gr X tid x 5 days	37.5%
III:	Quinine gr X tid x 4 days	42.9%
IV:	Quinine gr X and tetracycline 250 mg. tid x 3 days	71.4%
V:	Same as treatment IV plus pyrimethamine 50 mg. with 1st quinine-tetracycline dose	66.7%

The results indicate that none of the five treatment regimens evaluated can be recommended for field use as an alternative treatment for strains of falciparum malaria "resistant" to sulphormethoxine-pyrimethamine. Since the combination of quinine-tetracycline showed an apparent additive effect, the possibility exists that adjustment of the length of treatment of this combination to four days may result in an acceptable cure rate (at lease 90%). Further study along such lines is obviously needed.

Another significant finding was the possible existence of quinine-resistant strains in the two cases of treatment failure given the seven-day course of quinine. This observation should be confirmed by additional studies in which exposure to infection can be controlled and the possibility of reinfection eliminated.

4. A study to monitor the continued effectiveness of sulphormethoxine-pyrimethamine-primaquine against falciparum malaria was implemented in Pakchong. Since one of the major limitations of the sulphormethoxine-pyrimethamine combination as an antimalarial is the potential for resistance development by the malaria parasite exposed to such a treatment, it was felt necessary to devise a method for early detection of resistance emergence. Through the generosity of the Roche Far East Foundation, a fund has been provided on an annual basis to implement such a study. An assessment of cure rate in 50 falciparum cases is planned twice yearly during the two peak transmission seasons from May-July and October-December. It is hoped that this series of study will receive continued support both from the NMEP and the Roche Far East Research Foundation. At the writing of this report, the initial study was well underway.

Entomology

Perhaps the most difficult technical problem confronting the NMEP is the displacement of *Anopheles minimus* by *A. b. balabacensis* as the principal malaria vector. Information on biting and resting habits of *A. b. balabacensis* is meager or conflicting; data on host preference, flight range, and response to insecticides is practically non-existent.

With the arrival of the TMORU senior research entomologist in April 1970, plans were conceived to study *A. b. balabacensis* over a span of several years. As a primary step, base-line data was gathered during the peak density period of August-December 1970 in two study sites (Kabinburi and Thamai). With the availability of such base-line data, plans were projected for similar peak periods in 1971 to study the effect of control measures on this vector as well as for laboratory insectary studies on the response of *A. b. balabacensis* to insecticides. Termination of the TMORU precluded fruition of these plans.

Base-line data accumulated during the months of August-December 1970 indicate that peak biting activity of *A. b. balabacensis* occurred between 2200-2400 hours at Thamai, while at the Kabinburi study site biting peak was

later, occurring almost equally between time periods of 2400-0200 and 0200-0400.

The study on the use of the CDC light trap (supplemented with dry ice) in the two study sites during the peak density months of 1970 indicated that the proportion of times when the light trap yielded positive catches and man-biting resulted in negative catches, was 0.114. Considering the relatively great variation in man-biting collections from one collector to another, and the effects of weather conditions on both methods, the data indicates that the light trap is at least as sensitive as the man-biting method for the monitoring of *A. b. balabacensis* density.

Epidemiology

The most perplexing problem confronting the NMEP is the persistence of malaria transmission in areas where DDT house-spraying has been utilized for several years. An example of such an area is Kabinburi. DDT house-spraying in the study site began in 1961 and since 1968 the area has been placed in the late attack phase with ACD visitations of inhabitants. In spite of this history, the Slide Positivity Rate (SPR) as recorded by the NMEP for this area was 28.8% in 1968 and 33.6% in 1969. To sort out the possible causes, be they operational or technical, a long-term epidemiologic study in this area was conceived.

During the second quarter of CY 1971, two villages in this area were mapped and the households interviewed. Blood smears were taken from every inhabitant for detection of malaria parasites and for determination of antibodies to malaria. At the same time, monitoring of vector species was continued. TMORU staff members visited this study area on a monthly basis to update epidemiologic and entomologic data. Plans were made to study the effect of various methods of antimalaria control once base-line data was established. The supplemental methods to be applied singly or in combination included mass drug treatment, ULV application of malathion and ULV application of Abate as a larvicide.

Premature termination of the TMORU aborted these plans.

Consultations

During FY 1969, at the invitation of the Chief Malaria Advisors (CDC/AID) to the malaria eradication programs of the Republic of the Philippines and the Republic of Vietnam, respectively, the Chief, TMORU visited these two countries to offer recommendations on chemotherapy of malaria and to render assistance in formulation of research protocols for the study of drug-resistant falciparum malaria.

E. Dissemination and Utilization of Research Results

Research results were disseminated by TMORU in the following ways:

1. NMEP Contacts. Research results were presented continually to NMEP senior staff on informal, ad hoc basis. Special effort was made at the conclusion of specific studies to present a

complete report to the interested malaria program staff.

2. Seminars and Lectures. A lecture "Malaria in Thailand" was presented to the medical staff of the U.S. Army hospital, Bangkok on two occasions, December 11, 1969 and February 11, 1971. A seminar on "Chloroquine-resistant falciparum malaria" was conducted for graduate students in microbiology, Mahidol University, Bangkok on September 9, 1971.

3. Reports. (a) Quarterly reports were forwarded to AID/W, WHO/Geneva, CDC/AID regional malaria officer and country malaria advisors in Southeast Asia. (b) Annual reports received the same distribution but also included malaria research workers in other agencies and institutions around the world.

4. Formal presentations. (a) A paper "The impact of Chloroquine-resistant falciparum malaria on malaria eradication in Thailand" by William Chin and Sombat Chayabejara, was presented at the annual American Society of Tropical Medicine and Hygiene meeting in 1968. (b) A paper "A comparative evaluation of sulfalene-trimethoprim and sulphormethoxine-pyrimethamine" by William Chin, David M. Bear, Edward J. Colwell and Sanong Kosakal, was presented at the annual American Society of Tropical Medicine and Hygiene meeting in December 1971.

The following manuscripts are currently undergoing CDC review prior to proposed publication:

"A comparative evaluation of sulfalene-trimethoprim and sulphormethoxine-pyrimethamine against falciparum malaria in Thailand" by William Chin, David M. Bear, Edward J. Colwell and Sanong Kosakal.

"The evaluation of the presumptive and radical treatments against falciparum malaria in Thailand" by William Chin and Manop Rattanarithikul.

"The evaluation of quinine alone or in combination with tetracycline and pyrimethamine against falciparum malaria in Thailand" by William Chin and Ravivan Intraprasert.

Utilization of research results:

As a result of the research data presented, the NMEP changed the standard radical treatment for falciparum malaria from chloroquine-primaquine to sulphormethoxine-pyrimethamine-primaquine. In addition, the NMEP is considering changing the presumptive treatment by substituting primaquine for pyrimethamine and has taken under advisement the possibility of continuing research on the modification of a quinine-tetracycline combination in order to develop an acceptable alternative treatment regimen for those falciparum strains found to be "resistant" to the recently adopted standard radical treatment.

F. Statement of Expenditures and Obligations and Contractor Resources

TMORU Funding Summary

	Fiscal Year					
	1968	1969	1970	1971	1972	Total
U.S. Input						
Personnel Costs	16,647.83	22,167.91	66,929.77	100,217.51	99,141.45	305,104.45
Travel & Transportation	2,006.30	34.46	13,805.86	12,500.25	35,457.43	63,804.30
Supplies	1,577.91	1,738.91	3,702.40	2,882.03	949.53	10,850.78
Equipment	1,625.62	-	23,409.44	3,835.39	89.61	28,960.06
Overhead	1,035.64	1,197.06	3,753.03	4,729.17	5,564.00	16,278.90
Total	$22,893.30	$25,138.34	$111,600.50	$124,164.35	$141,202.00	$424,998.49
RTG Input						
Project Account (May 1970-April 1972)					$324,147.10 =	$ 15,562.00
Trust Fund (May 1970-May 1972)					$707,087.00 =	$ 33,954.00
Total						$ 49,516.00
TOTAL						$474,514.49

Project Account includes funds for local purchase of commodities, salary, and travel for NMEP personnel and alteration and repair of facilities.

Trust Fund pays salaries and travel of Thai staff.

G. Work Plan and Budget Forecast for Coming Year

At a time when final solutions to technical problems confronting antimalaria effort in Southeast Asia remain essentially unresolved, the TMORU is being phased out. The rapid emergence of operational problems directly related to faltering financial support by both the respective host countries and USAID compounds the problems of the country malaria programs of this region. Therefore, the need is greater than ever for continued operational research in malaria in order to develop the most efficient operational procedures to economize on the dwindling resources.

It is important to emphasize again that the technical problems are not limited to any one country but are regional in scope. While *A. b. balabacensis* may only show "behavioral" resistance to DDT, other vectors, principally those found in India and Indonesia show physiologic resistance to DDT. The problem of drug-resistant malaria is becoming more widespread. Some three years ago, chloroquine-resistant falciparum malaria was reported from only Thailand, Cambodia, Malaysia and Vietnam; Laos and the Philippines have now been added to this list. Common to all the countries in this region is the problem of uncontrolled population migration into and out of meso- and hyperendemic malarious areas.

H. Appendices

1. Activities Report, July 1, 1970-June 30, 1972

2. Activities for the Year Ending June 30, 1970
   Activities for the Year Ending June 30, 1969
   Activities for the Year Ending June 30, 1968
   (available on request)

APPENDIX I

Activities Report, July 1, 1970 June 30, 1972

ACTIVITIES REPORT

JULY 1,1970 thru JUNE 30,1972

THAILAND MALARIA OPERATIONAL RESEARCH UNIT

under Participating Agency Service Agreement Number RA(HM) 1-70
PIO/T Number 931-17-511-503-72-3117706 & 3127703 with U.S. Agency for International Development

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
Health Services and Mental Health Administration
Center for Disease Control
Atlanta, Georgia 30333

TABLE OF CONTENTS

INTRODUCTION 1

ACTIVITIES

Research Plans 2

Drug Studies

Evaluation of sulfalene-trimethoprim/sulphormethoxine-pyrimethamine against falciparum malaria in the districts of Kabinburi and Pak Thong Chai 2

Evaluation of sulfalene-trimethoprim/sulphormethoxine-pyrimethamine against falciparum malaria at the Trad Provincial Hospital 4

Evaluation of presumptive and radical treatments against falciparum malaria in Pakchong and Siracha 7

Evaluation of quinine alone or in combination with tetracycline and pyrimethamine against falciparum malaria at the Prapokklao Hospital, Chantaburi 7

Monitoring of sulphormethoxine-pyrimethamine-primaquine effectiveness against falciparum malaria in Pakchong 8

Kabinburi Epidemiologic Study of Malaria Transmission and Control 10

Entomologic Studies

Relative abundance of malaria vectors and other anophelines 14

Behavior patterns of malaria vectors 15

Comparison of malaria prevalence and vector densities 15

Vector population monitoring methods 17

Insectary 17

Other Activities 19

INTRODUCTION

On September 1, 1967 the Royal Thai Government and the U.S. Operations Mission, Thailand signed a project implementation agreement to finance malaria research activities, thus creating the Thailand Malaria Operational Research Unit (TMORU). The Chief, TMORU was designated in December 1967. However, not until a Project Agreement was signed on April 30, 1970 was it possible to implement the unit.

The personnel and the dates of service at TMORU are as follows:

Chief	Dr. William Chin	Dec. 67-Jun. 72
Senior Research Entomologist	Dr. John W. Kliewer	Apr. 70-Feb. 72
Research Entomologist	Mr. Carl A. Vickery	Jun. 69-Dec. 71
Medical Epidemiologist	Dr. David M. Bear	Mar. 71-Dec. 71

With the arrival of both the fourth U.S. technician and the four vehicles and the completion of the insectary building, the TMORU finally became fully operational during the third quarter of FY 1971.

ACTIVITIES

Research Plans

During the period July 1970-June 1972 the TMORU revised study plans to meet the expected phase-out schedule by June 1972 and gave priority to the following:

1. The development of an effective and acceptable treatment for use by the National Malaria Eradication Project (NMEP) as an alternative to quinine against chloroquine-resistant falciparum malaria and,

2. The study of the principal vector, *Anopheles balabacensis balabacensis*: its ecology, behavior and response to insecticides including DDT.

Drug Studies

Evaluation of sulfalene-trimethoprim/sulphormethoxine-pyrimethamine against falciparum malaria in the districts of Kabinburi and Pak Thong Chai

The features of the study site in the districts of Kabinburi and Pak Thong Chai have been reported previously. In brief, the study procedure comprises the following:

1. At the time Active Case Detection (ACD) smears were taken, each suspected case was given a presumptive dose of chloroquine-primaquine.

2. Approximately two weeks later, follow-up smears were taken again.

3. Those cases still positive for falciparum malaria were considered for inclusion in the study if they indicated availability for weekly follow-up during the next month.

4. The subjects for study were then divided into two groups. Adult males (15 yrs. and over) were given a single oral dose of SF-T (sulfalene (1 gm.) and trimethoprim (0.5 gm.)). All the females and males less than 15 years were given the regimen used by the NMEP for treatment of chloroquine-resistant falciparum malaria, S-P (sulphormethoxine and pyrimethamine) according to the following dosage schedule:

Age*	Sulphormethoxine (0.5 gm. tabs)	Pyrimethamine (25 mg. tabs)
≥ 15 yrs.	2	2
9-14 yrs.	1 1/2	1 1/2
4- 8 yrs.	1	1
1- 3 yrs.	1/2	1/2
6-12 mos.	1/4	1/4

*Not to be given to pregnant females beginning in their 8th month of pregnancy and patients with history of kidney disease.

TABLE 1
Summary of ACD Results in Kabinburi District during November 1970

Age (Years)	Sex	Total Smears	Positive				
			Total	*P.f.*	*P.f.* + g	g	*P.v.*
≤ 1	M	0	-				
	F	1	0				
1-4	M	17	5	4	1		
	F	9	4	4			
5-9	M	14	6	6			
	F	12	1	1			
10-19	M	9	1	1			
	F	15	3	3			
≥ 20	M	39	19	18		1	
	F	31	9	8			1
Total	M	79	31	29	1	1	
	F	68	17	16			1
	All	147	48	45	1	1	1
			(SPR=32.7%)	(97.9%)			

P.f.: *P. falciparum* rings g: *P. falciparum* gametocytes *P.v.*: *P. vivax*

TABLE 2
Summary of ACD Results in Pak Thong Chai District
During December 1970

Age (Years)	Sex	Total Smears	Positive				
			Total	*P.f.*	*P.f.* + g	g	*P.v.*
≤ 1	M	0					
	F	1					
1-4	M	4	2	2			
	F	4					
5-9	M	14	7	2	4		1
	F	14	5	4			1
10-19	M	52	19	12	4		3
	F	27	11	7	1	2	1
≥ 20	M	123	23	16	4	1	2
	F	33	9	9			
Total	M	193	51	32	12	1	6
	F	79	25	20	1	2	2
	All	272	76	52	13	3	8
			(SPR=27.9%)	(89.5%)			

5. WBC counts were performed along with each smear taken.

A total of 97 falciparum cases detected by ACD activity (Tables I and 2) were potentially available. At the end of the study, a total of 43 cases were given treatment, 14 cases with SF-T and 29 cases with S-P. One month follow-up was possible in only 23 cases. Of those, 21 were cured.(Table 3)

TABLE 3
Percentage Cured after SF-T and S-P Treatment
Kabinburi and Pak Thong Chai Districts

	No. cases	Cured*	
		No.	%
SF-T	8	7	(87.5)
S-P	15	14	(93.3)

*Blood smears remaining negative during weekly one-month follow-up.

There were no detectable toxic side reactions to either combination. No significant depression of the leukocyte counts was observed.

In addition, it was possible to follow closely the effect of SF-T on a chloroquine-resistant falciparum infection in a TMORU staff member. In this case, SF-T treatment followed by 1.5 gm. (base) chloroquine plus three doses of intravenous quinine (10 gr. each) both of which he received in a hospital and finally a second course of SF-T failed to cure the infection. This subject's infection was finally cured with quinine 10 gr. three times daily for five days.

The results from this limited study, while apparently encouraging, should not be accepted with undue significance since about 50% of the test subjects were asymptomatic at the time of treatment, reflecting a possible high degree of immunity to malaria.

Evaluation of sulfalene-trimethoprim/sulphormethoxine-pyrimethamine against falciparum malaria at the Trad Provincial Hospital

In collaboration with the SEATO Medical Research Laboratory, a comparative evaluation of sulfalene + trimethoprim (SF-T) and sulphormethoxine + pyrimethamine (S-P) against falciparum malaria was completed at the Trad Provincial Hospital.

Thai males 15 years or older, infected with falciparum malaria and with no obvious evidence of renal, cerebral or hepatic complications, were selected for the study. Following a physical examination, the subjects were alternately assigned to one of two single oral dose treatment schedules as follows: (1) SF-T (sulfalene 1 gm. and trimethoprim 0.5 gm.) and (2) S-P (sulphormethoxine 1 gm. and pyrimethamine 0.05 gm.)

Prior to treatment the following determinations were performed:

1. Routine lab tests of leukocyte count (WBC) and hematocrit (Hct.) determination.

2. Special lab tests for assessment of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency and malaria fluorescent antibody titers.

Following treatment, thick-thin blood smears were read, WBC and Hct. determinations were made daily for the first week, and at the end of the 2nd, 3rd and 4th weeks. Patients were hospitalized for seven days whenever possible or were discharged after three consecutive daily smears were found to be negative. Following discharge, the subjects were asked to return once weekly for the completion of the one-month follow-up.

A total of 94 patients were examined and selected for study. Due to failure of completing initial follow-up or because of mixed infections, six cases were eliminated from the study, yielding a total of 88 cases for further consideration. Of the 88 cases, 11 (12.5%) were found to be G-6-PD deficient by the fluorescent spot test method using the Hyland G-6-PD screening kit. The respective cure rates for the two treatments determined after one month follow-up were 83.9% and 91.2%, with no significant difference. (Table 4).

TABLE 4
The Results of Sulfalene-trimethoprim (SF-T) and Sulphormethoxine-pyrimethamine (S-P) against Falciparum Malaria
Trad Provincial Hospital, Thailand

	Treatment	
	SF-T	S-P
Ages of Subjects (Yrs.)		
Range	15-50	15-51
Average	26.1	25.5
Initial Parasitemia (mm^3)		
Range	28-120,054	271-125,400
Average	16,313	15,683
Average days to clear parasitemia	2.2	2.6
Average days to clear fever	2.4	2.5
Immediate Response		
Cases	45	43
Parasitemia cleared $\leq$ 7 days: No.	43	42
%	95.6	97.7
After one month follow-up		
Cases	31	34
Cured:* No.	26	31
%	83.9	91.2

*Parasitemia became subpatent within 7 days after treatment and weekly follow-up smears were negative. No significant difference (Fishor's Exact Test P=0.4)

The effect of treatment on gametocytemia in 62 cases was observed for 28 days. In the majority of cases, gametocytes were first observed about six days after treatment (Table 5). Since the insectary was not operational at the time of this study, the possible sporontocidal effect of the two regimens was not assessed.

TABLE 5

The Effect of Sulfalene-trimethoprim and Sulphormethoxine-pyrimethamine on Gametocytemia in 62 Cases of Falciparum Infections Observed for 28 Days

		Treatment	
		SF-T	S-P
Cases observed 28 days		29	33
Gametocytes present on initial smear		3	1
No gametocytes observed in 28 days		7	4
Gametocytes found on smears after treatment		19	28
Days detected:	Range	1-14	1-23
	Average	5.9	6.2

The WBC count and hematocrit values of all study subjects, including the 11 with G-6-PD deficiency, were well within limits found in patients with malaria.

With the exception of post treatment vomiting by two patients following administration of sulfalene-trimethoprim, all subjects tolerated medication well; no other adverse side reactions were observed.

Of interest are the histories elicited from 75 of the study subjects concerning prior treatment with antimalarials before coming to the hospital. These may be summarized as follows:

Type of treatment and source of antimalarials	No. cases
Injections (from 1-10) with or without oral medication from "quacks" or illegal practitioners	28
No prior antimalarials	20
Oral medication purchased from drug store	16
Oral medication from other Royal Thai Government (RTG) agencies (highway dept., forestry dept., etc.)	8
Oral medication from NMEP	3
Total	75

During the period of this study from April 20, 1971 to June 16, 1971, the combined SEATO and TMORU personnel took smears from 1,181 individuals (Trad Hospital outpatients). Of this number 434 (36.7%) were found to be positive.

Based partly on the findings cited above, the NMEP in October 1971 adopted the use of sulphormethoxine-pyrimethamine as the standard radical treatment against all falciparum infections. At the same time, since the study also showed significant emergence of gametocytes following treatment with sulphormethoxine-pyrimethamine, the NMEP senior staff decided to retain primaquine 15 mg. daily for five days as part of the new radical treatment.

Evaluation of presumptive and radical treatments against falciparum malaria in Pakchong and Siracha

The need for a study of the presumptive and radical treatments was based on the fact that the presumptive treatment of falciparum malaria had remained static in the face of emergence of drug-resistant parasites. Further, since the adoption of a new standard treatment for falciparum malaria was a pioneering effort, it was agreed by both the NMEP and TMORU that more precise information on its effectiveness as gained from controlled operational use was required. The study was performed during the first half of FY 1972.

The results showed that the effectiveness of the presumptive treatment (chloroquine 600 mg. base and pyrimethamine 50 mg.) has been greatly reduced. Blood smears from 28 of 43 subjects (65%) given this treatment were still positive at day seven or showed recrudescence of asexual forms of falciparum malaria by day 14, and 7 of 12 (58.3%) of the *A. b. balabacensis* lots fed to the patients exhibiting gametocytemia post treatment resulted in infections to the sporozoite stage.

Radical treatment with sulphormethoxine 1 gm. plus pyrimethamine 0.05 gm. resulted in cure rates of 52 of 58 (91.2%) cases treated in Siracha and 49 of 51 (96.1%) of cases treated in Pakchong. *A. b. balabacensis* feedings on cases with gametocytemia following this treatment resulted in infection to the sporozoite stage in 7 of 15 (46.6%) of the lots. Concurrent daily use of primaquine for the first five days of radical treatment resulted in effective suppression of gametocytemia.

Evaluation of quinine alone or in combination with tetracycline and pyrimethamine against falciparum malaria at the Prapokklao Hospital, Chantaburi

Since the series of studies of sulphormethoxine-pyrimethamine against Thai strains of falciparum malaria indicate that a failure rate of about 10% can be expected, it was felt important to assess alternative treatment regimens against such "resistant" strains.

Of the limited antimalarials presently available for consideration as an alternative regimen, quinine remains the drug of choice. Data on the sensitivity of Thai strains of *P. falciparum* to quinine are limited and conflicting. Studies on at least two Thai strains of falciparum malaria performed in the United States, utilizing induced infections in non-immune prisoner volunteers, indicate that a 100% cure rate can result from five days of quinine treatment (in a dosage of gr X three times per day). Conversely, Harinasuta and her colleagues reported that four and five days of quinine treatment in Thai patients with falciparum malaria resulted in failure and that seven days of quinine treatment was required for consistent cures.

A regimen of quinine treatment requiring seven days has obvious disadvantages of prolonged duration and accompanying side reactions. Such limitations render impractical its field use by an operational malaria program.

Recently, the SEATO Medical Research Laboratory reported that tetracycline was found to be an effective antimalarial against Thai strains of *P. falciparum*. While the side reactions to tetracycline are minimal, the requirement of ten days treatment also limits its usefulness for administration in the field. The need, therefore, to develop a treatment regimen based on quinine which is effective against those strains of falciparum malaria found to be "resistant" to sulphormethoxine-pyrimethamine, and also is operationally feasible, is obvious.

Such a study was developed and carried out in collaboration with the staff at the Prapokklao Hospital, Chantaburi, during the second half of FY 1972. In this study, 59 adult male and female subjects were treated on an alternating basis with five respective treatment regimens. Completion of one month follow-up was accomplished in 39 subjects with the following cure rates:

Treatment:

I:	Quinine gr X three times/day x 7 days	75.0%
II:	Quinine gr X three times/day x 5 days	37.5%
III:	Quinine gr X three times/day x 4 days	42.9%
IV:	Quinine gr X and tetracycline 250 mg. three times/day x 3 days	71.4%
V:	Same as treatment IV plus pyrimethamine 50 mg. with 1st quinine-tetracycline dose	66.7%

The results indicate that none of the five treatment regimens evaluated can be recommended for field use as an alternative treatment for strains of falciparum malaria "resistant" to sulphormethoxine-pyrimethamine. Since the combination of quinine-tetracycline showed an apparent additive effect, the possibility exists that adjustment of the length of treatment of this com-combination to four days may result in an acceptable cure rate (at least 90%). Further study along such lines is obviously needed.

Another significant finding was the possible existence of quinine-resistant strains in the two cases of treatment failure given the seven day course of quinine. This observation will need to be confirmed by additional studies in which exposure to infection can be controlled and the possibility of re-infection eliminated.

Monitoring of sulphormethoxine-pyrimethamine-primaquine effectiveness against falciparum malaria in Pakchong.

Since one of the major limitations of the sulphormethoxine-pyrimethamine combination as an antimalarial is the potential for resistance development

by the malaria parasite exposed to such a treatment, it was felt necessary to devise a method for early detection of resistance emergence. Through the generosity of the Roche Far East Foundation, a fund has been provided on an annual basis to implement such a study. It is hoped that an assessment of cure rate in 50 falciparum cases will be made twice yearly, during the two peak transmission seasons from May-July and October-December, and that this study series will receive continued support both from the NMEP and the Roche Far East Research Foundation. At the writing of this report, the initial study was nearing successful completion.

Kabinburi Epidemiologic Study of Malaria Transmission and Control

A year-long study of malaria epidemiology in the Thung Pho Canton of Prachinburi Province was begun in April 1971. This study was designed to obtain information related to the following:

1. The relative importance of possible operational deficiencies and/or technical problems which are responsible for continued transmission of malaria.

2. The effect of population stability on malaria transmission.

3. The type of control measure or combination of measures required to interrupt transmission where *A. b. balabacensis* is virtually the sole vector.

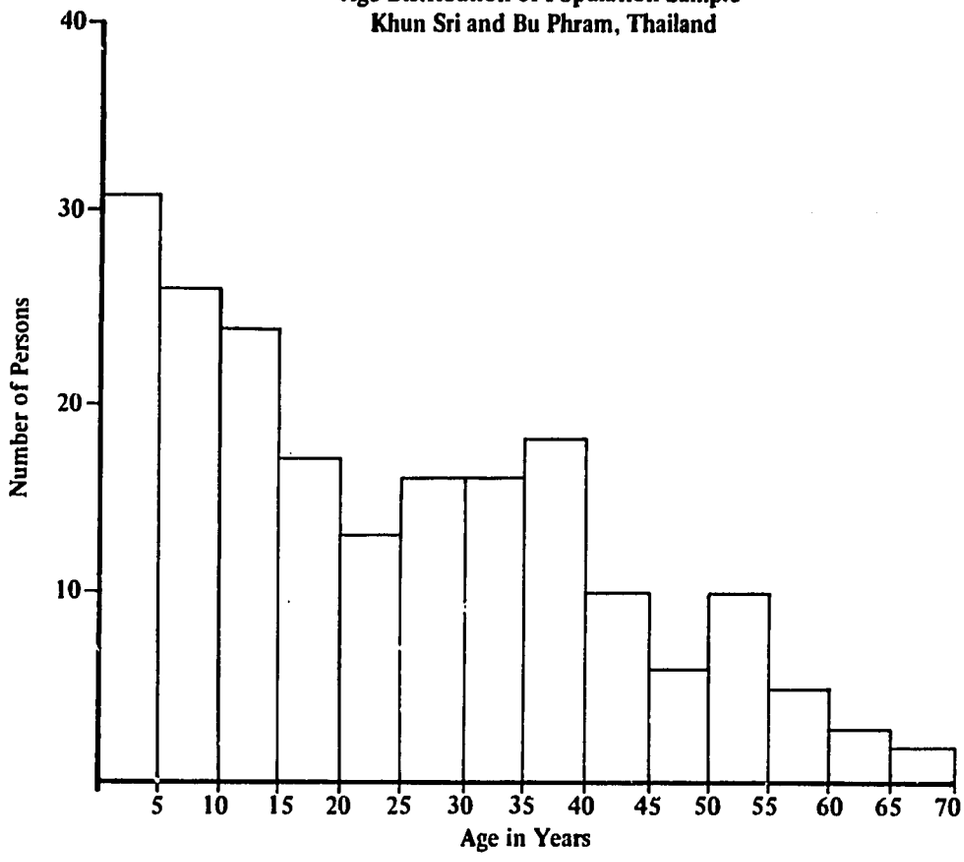
Portions of two villages (Khun Sri and Bu Phram) and the highway connecting them were mapped and found to be comprised of 222 houses. Initially, village meetings were held with the Headman, District Chief, Public Health Officer, and NMEP Zone Chief to introduce the epidemiology team and enlist the cooperation of the villagers. Then a blood slide survey was conducted in the two villages and filter paper blood samples for malaria antibody determination were collected. In Khun Sri 17 of the 122 slides taken were positive for malaria (13.9%). In Bu Phram 8 of 157 slides were positive (5.1%).

After all the patients positive for malaria were treated, 40 houses were selected by random number designation to represent the three communities. Within the following six weeks 37 of these houses were visited and all their inhabitants (197 persons) interviewed and examined. The other three houses were found to be temporarily uninhabited or destroyed. The age distribution of the study samples is shown in Figure 1.

Additional findings in the sample included the following averages:

No. of household members	5.3 persons
No. of rooms in house	1.9 rooms
Residence in present house	6.6 years
Usual bedtime	8:00 pm
Sprayed with DDT this year	92 %
Sometimes sleeping away from home	21 %
Using mosquito net every night	79 %
Hematocrit	36.2%
Spleen rate	21.8%
Slide Positivity Rate	16.4%

FIGURE I
Age Distribution of Population Sample
Khun Sri and Bu Phram, Thailand



The filter paper samples containing blood samples from villagers in Khun Sri and Bu Phram were sent to Dr. William E. Collins at the Section on Primate Malaria, Laboratory of Parasitic Diseases, National Institute of Allergies and Infectious Diseases, in Chamblee, Georgia, for assessment of malaria antibodies using the indirect fluorescent antibody (IFA) technique. The IFA titers against falciparum, vivax, and malariae antigens are summarized in Tables 6 and 7.

TABLE 6
IFA Responses of Blood Samples to Three Plasmodium Antigens
Khun Sri and Bu Phram, Thailand

Village	No. of Samples	<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
					Plasmodium falciparum					
Khun Sri	135	43	12	30	12	20	11	5	2	
Bu Phram	106	23	14	22	10	10	16	6	4	1
Total	241	66	26	52	22	30	27	11	6	1
					Plasmodium vivax					
Khun Sri	135	73	28	22	8	2	2			
Bu Phram	106	50	21	27	4	3	1			
Total	241	123	49	49	12	5	3			
					Plasmodium malariae					
Khun Sri	135	80	17	21	9	4	4			
Bu Phram	106	44	10	23	4	14	8	3		
Total	241	124	27	44	13	18	12	3		

TABLE 7
Summary of IFA Responses of Blood Samples
Khun Sri and Bu Phram, Thailand

	Khun Sri	Bu Phram
Number of Samples	135	106
Number and (%) positive IFA (≥1:20) against:		
P. falciparum	80 (59)	70 (66)
P. vivax	34 (25)	36 (34)
P. malariae	38 (28)	53 (50)
Any of the three	89 (66)	78 (74)
Number and (%) with maximum* IFA titer against:		
P. falciparum	73 (82)	58 (74)
P. vivax	8 (9)	6 (8)
P. malariae	20 (23)	27 (35)
Geometric mean positive IFA titer against:		
P. falciparum	1:54	1:71
P. vivax	1:30	1:26
P. malariae	1:34	1:50

*Some samples produced equal responses to more than one antigen.

The level of *P. malariae* antibodies in these villages, especially high in Bu Phram, was surprising considering that for many years the proportion of *P. malariae* positive smears in country-wide surveys has been less than one percent.

Results of supporting entomologic study are included in Entomologic Studies: Vector population monitoring methods.

The vector population in the study site was monitored weekly by the use of CDC light traps supplemented with CO_2. The plan was that when the *A. b. balabacensis* population was sufficiently high, one village would be sprayed by ULV application of malathion from portable back-pack sprayers, while the other would serve as control; when population density returned to "normal", the experiment would be repeated by switching control of the two villages in order to determine whether vector populations could be suppressed by this method and, if so, for how long. The population increase was expected to occur in October 1971 but, because of the October decision to phase out all entomologic activities, back-pack sprayer application of malathion was not attempted.

Entomologic Studies

Relative abundance of malaria vectors and other anophelines

In monitoring vector populations it is important to detect the presence and relative abundance of all known vectors as well as those species which, even though not considered vectors in Thailand, are known to be vectors in other countries. Table 8 lists all anopheline species collected during FY 1971 and FY 1972, and their relative numbers. Of the recognized vectors in Thailand, *A. b. balabacensis* was by far the most abundant at both the Thamai and Kabinburi study sites. Efforts were made to maintain a schedule of visiting each study site twice a month and to make collections on three consecutive nights on each visit. The Kabinburi study was started sooner and the site was visited with more regularity than was the Thamai study site.

TABLE 8
Anophelines Collected by the TMORU Personnel in Two Study Sites
July 1970-September 1971

Anopheline Species	Kabinburi		Thamai	
	No.	Rank	No.	Rank
aconitus	656	8	220	2
annandalei	2	16.5	0	-
annularis	1	18.5	0	-
argyropus	12	15	25	11
balabacensis	1373	6	1557	1
barbirostris	1591	5	41	8
campestris	2	16.5	0	-
karwari	14	13.5	16	12
kochi	6722	1	45	6
maculatus	93	10	1	13.5
minimus	114	9	0	-
peditaeniatus	32	11	42	7
philippinensis	3016	3	55	5
ramsayi	1	18.5	0	-
sinensis	14	13.5	33	10
subpictus	747	7	153	3
tessellatus	1791	4	89	4
umbrosus	0	-	36	9
vagus	6660	2	1	13.5
hyrcanus gp.	18	12	0	-
Total	22,859		2314	

Behavioral patterns of malaria vectors

In an effort to relate vector behavior with human activity patterns, night-biting and light trap (portable CDC light traps supplemented with dry ice) collections were made at the two areas at regular intervals throughout FY 1971 and on three occasions at areas where *A. sundaicus* and *A. aconitus* were relatively abundant. Both of the latter are species of at least potential importance as vectors in Thailand. Beginning in FY 1972, only light traps were utilized for mosquito collection.

Peak biting activity of *A. b. balabacensis* occurred between 2200 and 2400 at Thamai while at the Kabinburi study site biting peak was later, occurring almost equally during the periods 2400-0200 and 0200-0400. In light trap collections at Thamai 84% of the *A. b. balabacensis* were taken before midnight with only 16% from then to 0600. The above figures are based on collections made throughout the breeding season.

Man-biting collections of *A. sundaicus* revealed a peak activity period from 2400 to 0200. Light trap collections, however, were larger for the period preceding midnight.

In light trap collections of *A. aconitus*, 87% were caught from 1800 to 2400 while 13% were taken from midnight on. Man-biting activity was similarly more intense before midnight.

Comparison of malaria prevalence and vector densities

In connection with the study on population monitoring in the Kabinburi area, monthly mass blood surveys were made to obtain information on the relationship between vector densities and slide positivity rates. Results are summarized in Figure 2.

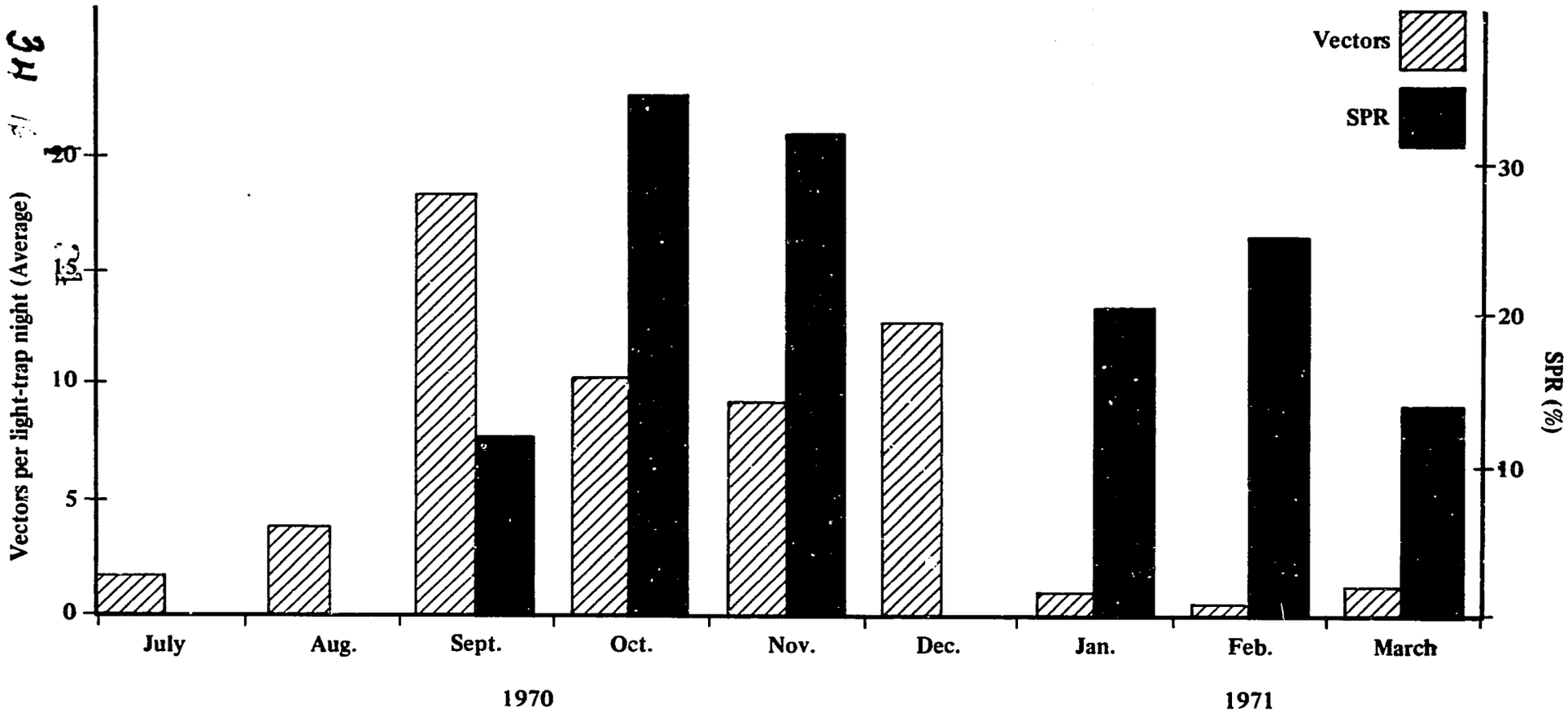
The absence of correlation between vector prevalence and the number of malaria cases, particularly during January, February, and March 1971, is significant. Vectors were virtually absent from our collections while slide positivity rates were substantial. Among the possible explanations for this relationship are: (a) presence of asymptomatic chronic cases which persist in the human population unrecognized and untreated until smears are taken in studies such as ours; (b) presence of an alternate vector or vectors capable of maintaining active transmission, and (c) high prevalence of drug-resistant falciparum malaria resulting in persistence of patent infection in spite of repeated treatment with chloroquine-primaquine.

With regard to the second possibility, species known or suspected to be vectors of malaria in other countries were present in our collections. *A. barbirostris*, the most abundant species in our sample during the first three months of 1971, and thus a prime suspect, is not considered to be a vector of malaria except in Celebes, and there is doubt about the identification of the Celebes form.

It was because of these observations that a more refined epidemiologic study of the area was undertaken in FY 1972.

FIGURE 2

Density of Vectors *(A. b. balabacensis* and *A. minimus)* and Monthly Slide Positivity Rates (SPR) at Kabinburi



Vector population monitoring methods

Reliable measurement of vector population densities is a major problem in epidemiologic evaluation of malaria eradication programs. The human bait collection technique, generally accepted as the most important means of assessing anopheline populations, requires close supervision and, in Thailand, because of extreme risk to infection with drug-resistant falciparum malaria, is hazardous to those making such collections. A simple and reliable population monitoring method is urgently needed.

A comparative study of man-biting catches and light trap collections was undertaken at the Kabinburi and Thamai study sites during the 1970-71 anopheline breeding season.

Summarized in the table below are simultaneous light trap and man-biting collections. These were started each working night at 1800 and terminated at 0600 the following morning. Man-biting collections were made on the verandas of huts or buildings at the study sites. Light trap collections were made with 6-volt CDC portable light traps supplemented with carbon dioxide in the form of dry ice. About two kilograms of dry ice were wrapped in several layers of newspaper and suspended next to the trap during operation. Light traps were placed in nearby vegetation though far enough from the man-biting station so that, while collections were of the same population, the effects of each method on the other were negligible.

Presence of *A. b. balabacensis* as measured by CDC portable light trap and by man-biting collections

Man-biting	Light trap +	Light trap -	Total
+	28	3	31
-	4	0	4
Total	32	3	35

It would appear that the light trap, because of its obvious advantages of safety and ease of operation, could be a useful tool in monitoring anopheline populations. Considering the relatively great variation in man-biting collections from one collector to another, and the effects of weather conditions on both methods, the data indicates that the light trap is at least as sensitive as man-biting methods for monitoring *A. b. balabacensis* populations.

Insectary

Insectary facilities were completed for TMORU use in May 1971. A colony of *A. b. balabacensis* was started from SEATO colony stock and had been built up sufficiently to allow its use in the assessment of sporontocidal effect of drugs.

Attempts were made, beginning in August 1971, to colonize *A. b. balabacensis* from the TMORU study site in Thamai. A new colony was needed, particularly

for studies on the response to insecticides, since the colony from the SEATO stock, having been maintained for many years, has undoubtedly evolved characteristics quite different from those of *A. b. balabacensis* found in nature. Efforts to colonize a new strain were only partially successful. When entomologic activities were suspended in October 1971 the number of available mosquitoes from the Thamai strain were too few to permit further studies.

Other Activities

1. The Chief, TMORU presented a lecture entitled "Technical problems impeding malaria eradication in Thailand" to Peace Corp Volunteers on January 11, 1971.

2. The Chief, TMORU presented a seminar entitled "Malaria in Thailand" to the medical staff of the U.S. Army Hospital, Bangkok on February 11, 1971.

3. The TMORU staff attended the First International Seminar on Medical Research, sponsored by the SEATO Medical Research Laboratory from February 22-25, 1971.

4. TMORU research activities were presented at a meeting of all NMEP epidemiologist-entomologists in Phrabudhabat on May 3, 1971, by Dr. Kliewer.

5. A seminar on chloroquine-resistant falciparum malaria was conducted for graduate students in microbiology, Mahidol University, Bangkok on September 9, 1971.

6. A paper, "A comparative evaluation of sulfalene-trimethoprim and sulphormethoxine-pyrimethamine" by William Chin, David M. Bear, Edward J. Colwell and Sanong Kosakal, was presented at the annual American Society of Tropical Medicine and Hygiene meeting in December 1971.

7. Much time and effort were devoted, during the last quarter of FY 1972, to secure reemployment of TMORU Thai staff and to arrange the transfer of TMORU supplies and equipment.