AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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Batch 72

1. SUBJECT CLASSIFICATION

A. PRIMARY
Serials — Y-NS00-0000-G352

B. SECONDARY
Health--Tropical diseases--Central America

2. TITLE AND SUBTITLE
Activities for the year 1969/1970

3. AUTHOR(S)
(101) U.S. Ctr.for Disease Control,Atlanta,Ga. Central Am.Malaria Research Station

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1970	90p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
HEW/PHS/CDC

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(Research summary)

9. ABSTRACT

10. CONTROL NUMBER
PN-RAB-609

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Central America
Malaria

13. PROJECT NUMBER

14. CONTRACT NUMBER
PASA RA(HM)2-67 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

ACTIVITIES FOR YEAR ENDING JUNE 30, 1970

central america malaria research station

under

Participating Agency Service Agreement
Number RA(HA) 2-67
PIO/T Number 931-17-511-474-72-3106758
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
MALARIA PROGRAM
ATLANTA, GEORGIA 30333

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INTRODUCTION

The Central America Malaria Research Station, a joint effort of the U.S. Agency for International Development and the U.S. Public Health Service, was inaugurated in February 1968, and has, as of June 30, 1970, completed a second full year of operation.

During the year, additional animal facilities, installation of emergency water and electrical supplies, and air conditioning of the chemistry and epidemiology laboratories and the room containing the climate simulation cabinets were completed. Station buildings are in excellent condition, and the laboratories and offices are now well equipped for any planned function of the Station. Three new vehicles (Jeep Commandos) received during the year have assured adequate transport for the staff and for field investigations.

Additions to the Station library during the year included an extensive collection of texts on malaria and other tropical medical topics and a collection of reprints--some 12,000 in number--which represents perhaps the most complete collection on malaria in the world.

Staff

During the year, the total staff of nine U.S. technicians was realized, and several changes were effected. The full complement of 35 authorized national employees was on hand at the end of the year. The staffing of the nine U.S. positions during FY 1970 was as follows:

Position	Staff, 1970	Dates	
		Assumed	Vacated*
Chief	Donald J. Pletsch	Jul 67	Nov 69
	Geoffrey M. Jeffery	Nov 69	-
Epidemiologist	Robert S. Lawrence	Aug 67	Jul 69
	Paul C. White	Oct 69	-
Parasitologist**	McWilson Warren	Jan 70	-
Entomologist***	Lawrence L. Lewallen	Aug 67	Jul 69
Research Entomologist	Samuel G. Breeland	Nov 67	-
Research Entomologist	Joseph R. Austin	Jan 69	-
Research Biologist	Charles W. Miller	Oct 68	-
Public Health Educator	Anna M. Obert	Sep 68	-
Biological Laboratory Technician	Morris H. Price	Oct 68	-
Administrative Officer	Kenneth E. Willey	Aug 67	Jun 70
	Allen J. Norton	Jun 70	-

*No date vacated indicates that the position was filled on June 30, 1970.
**Position established January 1970.
***Position abolished January 1970.

During the year, consultants at the Station included a health educator for a total of approximately five weeks (two visits), a specialist in the chemotherapy and epidemiology of malaria for two weeks, an entomologist for three weeks, and a mosquito-abatement specialist for two weeks. A number of staff members from the Malaria Program and other CDC components came to the Station for periods of temporary duty. These included an administrative officer for approximately four weeks (two visits); an entomologist for approximately four months (two visits); a medical epidemiologist for approximately two months during the period when none was assigned to the Station as permanent staff; an enteric disease epidemiologist (EIS) for two weeks; a research entomologist and a research biologist (TDL) for a month; and a number of Malaria Program headquarters staff members for periods varying from a few days to two weeks.

Working Relationships with Various Groups

National Organizations

Cordial and mutually beneficial associations have been maintained with the Ministry of Public Health and Social Welfare of El Salvador, with the National Antimalarial Campaign (CNAP), and with other Salvadoran government agencies. The Director and staff of CNAP have continually offered and provided indispensable assistance and encouragement to the CAMRS. They have been most generous in providing epidemiologic and entomologic background data, in making available investigational areas and situations which could be used by CAMRS studies, and in cooperative activities in drug and insecticide field studies.

Similarly cordial and useful relationships were maintained with the National Malaria Eradication Services of all of the countries of Central America and Panama, both directly by visits to and cooperative studies within these countries and through representation at the several regional meetings of the Program Directors and of the Working Groups for Coordination of Eradication Programs. At the 9th Semiannual meeting of the Working Group in San Jose, Costa Rica, CAMRS was represented by the ROCAP Malaria Advisor. The 16th meeting of the Directors of Malaria Eradication Programs of Mexico, Central America, Panama, and the Caribbean Area was held in Santo Domingo and was attended by the former and present Chiefs of the CAMRS. In these meetings, resumés of the programs and progress of CAMRS were presented.

International Organizations

The staff continued to work closely with the Pan American Health Organization (PAHO) and the United Nations Children's Fund (UNICEF) officials in problems of mutual interest in the area. The PAHO advisors assigned to various countries hosting staff visits or projects were always helpful in accomplishing the desired purposes.

Collaborative studies were done with the AMRO-0216 (PAHO) group working in El Salvador in evaluating the usefulness of OMS-33 (Baygon) as a residual house spray. CAMRS personnel conducted chemical studies on the fumigant action of the insecticide to correlate with the entomologic findings in sprayed premises and also assisted with the epidemiologic evaluations.

A number of visits were made to and received from personnel of the PAHO Headquarters in Washington and the Zone Headquarters in Guatemala to discuss the progress of current joint efforts and to explore future cooperative projects.

U.S. Embassy and USAID

Attendance at the monthly staff meetings of USAID/San Salvador aided in maintaining a mutually helpful relationship with this Mission. The Joint Administrative Services, the USAID Controller's Office, and other Embassy-based offices continued

to serve very well many administrative needs of the Station. A continuing liaison was maintained with the Mission through the office of the Public Health Advisor.

Continuing encouragement and visits from the Ambassador, the Deputy Chief of Mission, and other Embassy officials have been most welcome and helpful in fulfilling the mission of CAMRS.

In visits to other countries where projects were underway or being explored, necessary arrangements and contacts have been ably provided by USAID and Embassy personnel. The Chief Malaria Advisor at ROCAP (Guatemala) has been kept informed of CAMRS activities and has provided close liaison with national and international malaria eradication activities in the region.

Administrative assistance and support were provided the USAID/Washington and the CDC representatives during the evaluation of the Malaria Eradication Program of El Salvador.

PHS (CDC) Personnel and Programs

The Station served as a local contact for developing cooperative efforts between several CDC programs and national groups of El Salvador. During an encephalitis outbreak early in the year, CAMRS participated in a coordinated Mission effort to provide assistance and facilitated contacts with CDC to obtain equine vaccines and other technical assistance.

During the latter half of the year, at the request of the Ministry of Health, CAMRS provided liaison with CDC for providing epidemiologic assistance in the continuing Shigella dysentery epidemic. A base of operations and essential services were provided for an epidemiologist from CDC to study the epidemic, and arrangements were made through CAMRS to provide epidemiologic training for a Ministry of Health physician at CDC Atlanta.

Laboratory support and field assistance were provided for an extended visit of two staff members from Technical Development Laboratories (TDL), Savannah, to study improved light traps and possible pathogens for biological control of anophelines.

Visitors to CAMRS

During the year the Station received 75 visitors, for varying lengths of time, representing a wide range of interests--such as research, academic, diplomatic, and commercial.

Development of Research Projects

Many of the research projects under study during the current year represent continuation of those initiated previously. Newer studies have been introduced as seemed desirable along the lines dictated by the original mission of the Station. With the changing strategy of malaria eradication programs and the probable return of greater emphasis on a diversity of control methods, the program of the Station has been further directed toward seeking basic information on vectors and parasites to provide rationale for the selection of control measures, toward the reevaluation of past methodology (such as larviciding and engineering), and toward the development of new weapons (such as new methods of house spraying, new insecticides, ULV spraying). Particular attention has been directed toward a combined approach, where multiple disciplines are used to evaluate the problems and multiple measures are used in logical combinations or succession to achieve the desired measure of control or eradication. Included in all of these studies has been the evaluation of the element of human population attitudes toward the measures used, their acceptance of these

measures, and the likelihood of these responses exerting a significant influence on the success of these measures, with exploration of educational techniques which might alter the acceptance patterns.

The continuing studies on the ecology of vectors begin to reveal patterns which will be highly significant in designing control programs. These studies have, at the same time, demonstrated that our state of knowledge concerning mosquito vectors is very incomplete and that there is much to be learned concerning their habits and the relationships of these habits to control methodology.

The characterization of the Central American strains of the human malarias is similarly important. Determination of relapse patterns, pathogenicity and normal response to various antimalaria chemotherapeutic agents promises to yield results highly important in the future planning of collective drug programs and other control efforts.

No available or potential control method has been ruled out as a possible subject for investigation by CAMRS. Only restrictions of time, staff, and budget necessarily serve to restrict the program. The Station hopes that the concepts of multifaceted investigations and of multifaceted control programs can be well demonstrated through its efforts.

Most of the studies undertaken by the Station have been located within El Salvador. Here, there have been many areas particularly well adapted to our basic program, there has been excellent cooperation from the National Program staff, and transportation and communication problems have been at a minimum. Brief international disturbances early in the year aborted efforts to mount several planned larviciding projects in Honduras and briefly upset the conduct of some studies within El Salvador. Cooperative studies have been done in regard to the evaluation of a new insecticide (OMS-33) as a house spray in Nicaragua, and these studies are continuing. An effort was made to initiate an extensive project in the Mato Grosso area of Brazil for the comparison of various doses of DDT and a DDT fraction (p-p' DDT) for effectiveness as residual insecticides. However, several on-the-spot evaluations of the malaria situation in the area revealed such a low incidence as to make invalid any epidemiologic evaluations of spray programs. The preliminary studies provided good baselines for future studies on the immuno-epidemiology of malaria in such an area, and these will be continued in collaboration with other CDC components.

Following is a more detailed summary of the major areas of investigation of interest to CAMRS during the year 1969-70.

Use of trade names is for identification purposes only and does not constitute endorsement by the Public Health Service or U.S. Department of Health, Education, and Welfare.

VECTOR ECOLOGY STUDIES

Anopheline Breeding Habitats and Seasonal Distribution

Sufficient data have been accumulated by CAMRS to interpret the extent and significance of anopheline habitats and seasonal breeding in El Salvador. The following discussion is based on more than 1,000 field collections, many on a continuing basis through several seasons in coastal areas involving most types of available habitats. A general schematic diagram of breeding habitats by season is shown in Figure 1.

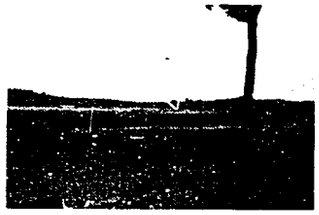
Dry Season Breeding

Studies between January 1968 and June 1970 indicate that dry season (January-May) populations of Anopheles mosquitoes in the malarious areas of coastal El Salvador are plentiful. Rivers and estuaries are the main sites; additional sources are irrigation channels with occasional overflows and permanent bodies of water with surface or marginal vegetation (Figure 1, lines 5-8).

Rivers cease to flow shortly after rains diminish in November, and by January, pooled and vegetated portions support substantial anophelism. River breeding reaches a peak in March and April and generally ceases in June when dry season water reserves begin to decrease. However, some breeding may continue into the month of June when rains have begun to inundate neighboring pastures and lowlands which become early rainy season habitats. Rivers are unique among coastal breeding areas because high levels of both A. albimanus and A. pseudopunctipennis breeding occur. During the 1969 dry season, the overall percentage was 45.2 percent albimanus and 54.8 percent pseudopunctipennis in samples representing 10,369 larvae in 6,315 dips, or 1.64 larvae per dip. However, during 1970, the ratio was overwhelmingly in favor of pseudopunctipennis which made up 81.9 percent of the identification samples which represented 18,572 larvae from 6,840 dips, or 2.7 per dip. Despite shifting population ratios from season to season and river to river, collectively the two species coexist in river situations. The role of river breeding, especially that of pseudopunctipennis, in the overall malaria picture must be evaluated. Also, observations indicate the need for ecological studies to elucidate the reasons for distribution patterns within a single river whereby portions of certain rivers support only albimanus, other portions support both species together, and still other portions only pseudopunctipennis. Also, some rivers tend to harbor only one of the two species to the virtual exclusion of the other. To date, the most reliable measure of adult anopheline densities resulting from river breeding has been diurnal counts of resting mosquitoes under bridge and from natural resting sites close to the breeding areas.

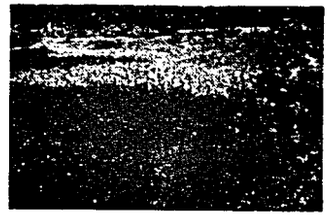
Estuaries (esteros) are important dry season breeding places and essentially support only albimanus populations. Estuaries vary greatly from one to the other, and apparently, mosquito breeding characteristics must be determined on an individual basis. However, certain patterns seem to be evident. For example, breeding reaches a peak during the dry season and continues unabated until the estuary is opened to the sea either by rainy season flushing or by mechanical means. Rainy season breeding in estuaries is negligible, although associated marshes and flats may provide anopheline habitats. For reasons unknown, Estero Majagual has produced very few mosquitoes during the time it has been under CAMRS observation. By contrast, Estero Ticuiziapa has produced moderate breeding, and Estero San Diego has supported heavy breeding. All of these estuaries share the same geographic area in the vicinity of La Libertad in the central coastal area. If the breeding pattern in Estero San Diego repeats itself on an annual basis, control efforts could be restricted to a period of only several weeks during the dry season. On the other hand, Estero Ticuiziapa shows some rainy season breeding in addition to dry season peaks

Line 8. Wet Weather Lake



Line 9. Floodwater Aedine Habitat

Line 9. Floodwater Aedine Habitat



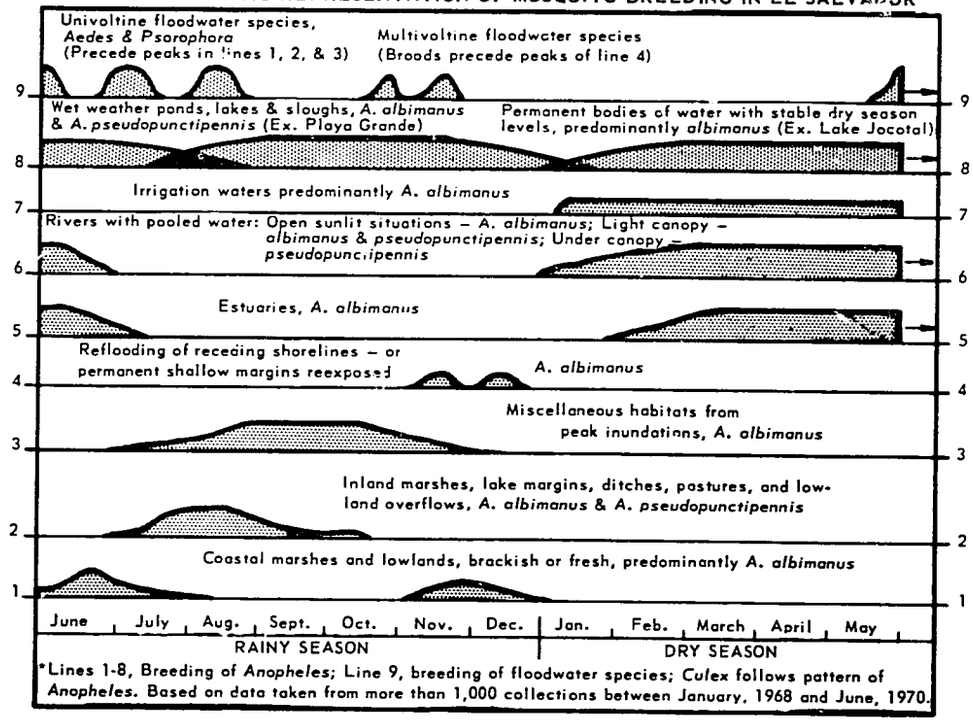
Line 8. Dry Season Lake

Line 3. Peak Rainy Season Habitat

Line 2. Lake Margin Overflow

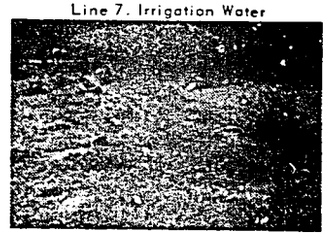
Line 1. Coastal Marsh

GENERAL SCHEMATIC REPRESENTATION OF MOSQUITO BREEDING IN EL SALVADOR*



*Lines 1-8, Breeding of *Anopheles*; Line 9, breeding of floodwater species; *Culex* follows pattern of *Anopheles*. Based on data taken from more than 1,000 collections between January, 1968 and June, 1970.

Line 7. Irrigation Water



Line 6. Pooled River

Line 5. Estuary Margin

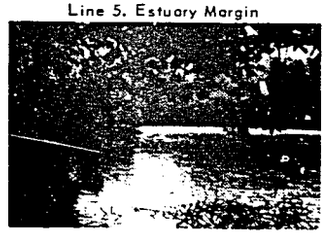


FIGURE 1.

because of pasture habitats associated with Ticuiziapa, while in San Diego, breeding is restricted to the Estero proper. New Jersey light traps have been particularly useful in measuring albimanus adult densities associated with estuary breeding. Additional methods are counts from animal stables, natural diurnal resting sites, and human bait collections. Another study described elsewhere in this report relates estuary breeding to certain physical properties of the aquatic environment.

Permanent bodies of water, such as Lake Jocotal, which have favorable levels and light conditions during the dry season, offer good anopheline breeding conditions. Not all lakes and ponds are suitable even though water is present. Small ponds with abrupt margins tend to recede below the vegetation line. Other bodies of water with larger surface areas are so shallow that rapid recession and turbidity combine to prevent heavy anopheline breeding, or rank growths of vegetation provide excess shade which hinders breeding. Adult densities around permanent pools of water can usually be adequately measured by counts from cattle stables, houses, natural resting sites, or light traps.

Irrigation channels and associated overflows offer other dry season habitats; although not major breeding sources, they may become locally important. For example, during the 1969 larviciding project west of La Libertad when rivers and estuaries were larvicided weekly, high adult counts resulted from undetected irrigation sources. Prompt location and treatment of the sources reduced the counts accordingly.

Rainy Season Breeding

Figure 1 shows that early season inundations in late May or early June in coastal pastures and lowlands result in a brood of univoltine (one generation per year) flood water mosquitoes in the genera Aedes and Psorophora, whose eggs have been dormant in the soil until the flooding (line 9). This is followed by anopheline production in the same areas within a matter of days with some overlap (line 1). Anopheline breeding continues for a while in the principal dry season habitats (estuaries, line 5; rivers, line 6). This insures continuing propagation during the dry/rainy season transition. As rains continue, accumulations of water fill marsh areas, ditches, and other lowlands; additionally, water basins overflow to increase habitat availability in marginal areas (line 2). These areas are augmented by still more flooded sites of many types, such as roadside ditches, potholes, wheel ruts, stream margins, inland pastures, and fields (line 3). Meanwhile, wet weather ponds, lakes, and sloughs fill and, in some cases, overflow the associated margins (line 8). Toward the end of the rainy season, receding water lines are likely to expose marginal vegetation, or refloodings occurring from intermittent flash rains may produce breeding of the type represented by line 4.

Floodwater mosquito broods are produced as new sites are inundated. For example, the second brood, line 9, precedes new inundations represented by line 2; the third brood, line 9, precedes the new inundations of line 3; and the fourth and fifth broods of line 9 precede the corresponding inundations of line 4. These floodings produce small broods of multivoltine floodwater species.

As rains diminish and water levels recede in November or December, dry season habitats become available again. Permanent bodies of water, such as Lake Jocotal, which overextend their basins during the rainy season to produce mosquitoes in overflow marginal areas (lines 2 and 3), recede to basin conditions in the dry season and begin to produce mosquitoes in marginal vegetation under more stable conditions as illustrated by line 8. Meanwhile, river flow decreases, and the rivers become available as dry season habitats.

A review of Figure 1 indicates that the rainy/dry season transition represents the annual low point of anopheline breeding in El Salvador.

In any given malarious area of El Salvador, there are certain rather specific types of major breeding sources. A thorough knowledge of these sources would permit sound planning of malaria suppression through vector control.

Relationship of Estuary Conditions and Anopheline Breeding

In a continuation of the study initiated in February 1969, the chemical and physical properties of Estero San Diego were investigated in relation to anopheline breeding. A general outline of the Estero and the station locations used for sampling both for salinity and presence of larvae is presented in Figure 2. Generally, this Estero is open to the ocean soon after the beginning of the rainy season (May-October), when it is subjected to two tidal flushings a day. The large volume of water being discharged into the Estero by the three rivers during these months is sufficient to maintain the opening to the ocean. As the end of the rainy season approaches, the volume of water in the rivers decreases and the Estero soon is closed off by wave action. The result is a large impounded body of saline water which is slowly diluted and enlarged by the continual discharge of river water, eventually to become an excellent breeding area for anopheline larvae.

During last year's investigation, the Estero closed in January, and larvae were found at various stations in February. In March, April, and May the stations were 100, 89, and 83 percent positive for larvae. In early June, the Estero opened and the entire area was flushed clean. No larvae were found in the area during the next eight months (July-February). Although at times the salinity values were favorable for larval breeding, the constant tidal flushing kept the area clear. The Estero closed off in mid-February; again, scattered larvae were found the following month. Population buildup this year, however, has been more gradual than last year in that only 60 percent of the stations were positive in April; 80 percent in May; and 100 percent in June. Inasmuch as the salinity values are almost identical, no explanation can be given for this change. Salinity values and absence or presence of larvae for the past year in Estero San Diego are presented in Table 1.

Previously, larvae have only been found in waters of the Estero at a salinity of 18.0 or less (parts per thousand). This year, three separate collections were made of larvae at salinities of 22.8, 22.9, and 23.6. This suggests that a higher salinity tolerance exists for the species as a whole than had been suspected or that a physiological strain of the species may be present. Further studies are planned to investigate this possibility. All larvae collected during this study have been identified as *Anopheles albimanus*. No *A. pseudopunctipennis* have been found in this Estero.

During the past year, a mosquito-abatement consultant visited the laboratory and advised on the possibility of opening the Estero to the ocean on a year-round basis. Because of the unavailability of suitable ditching equipment and the cost of installing and maintaining tide gates, dynamite has been proposed. A study is planned for next year to evaluate this possibility and develop the timing sequences certain to be necessary. This year the Estero was opened the last week in June by manual efforts of the residents in the area.

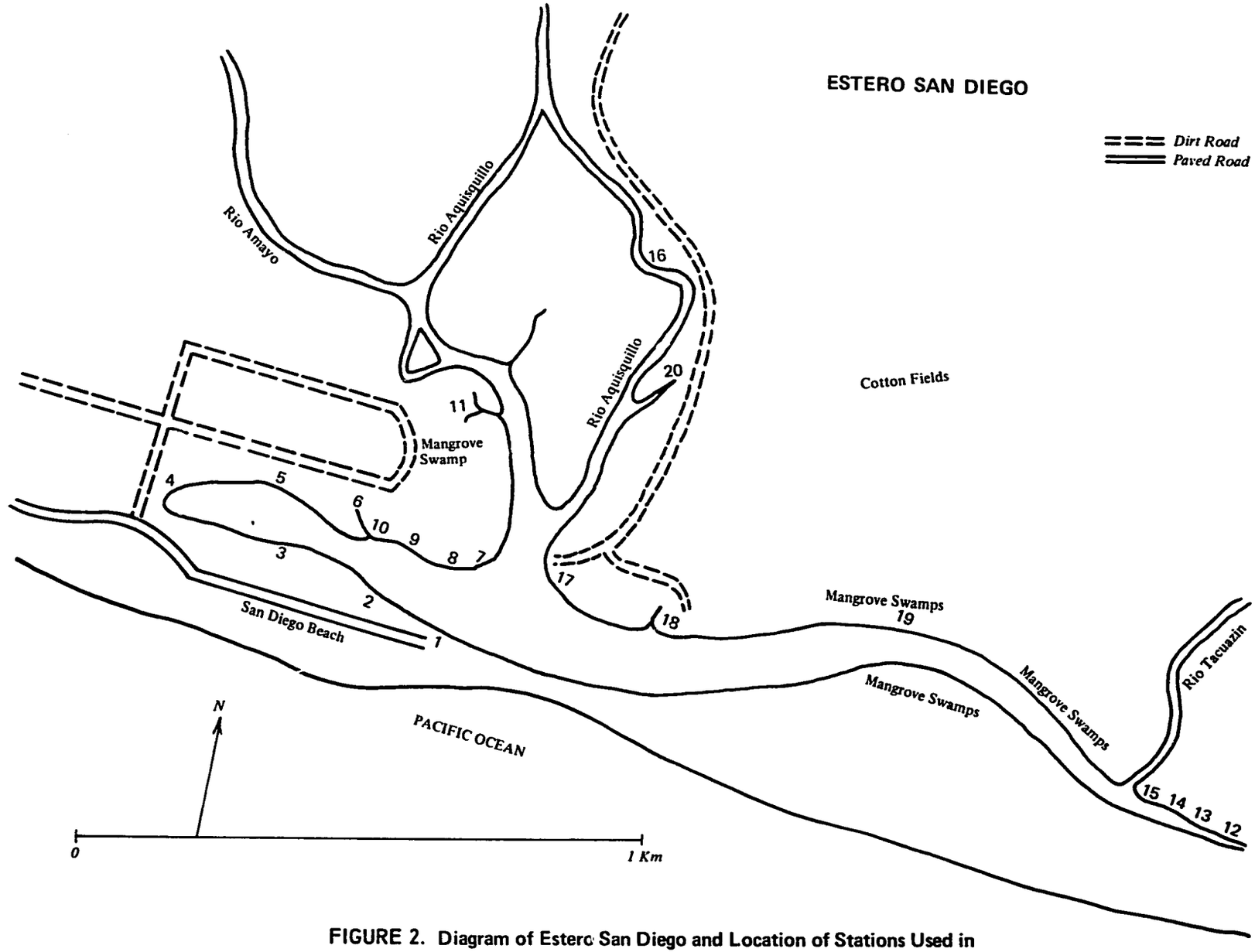


FIGURE 2. Diagram of Estero San Diego and Location of Stations Used in Water Sampling and Larval Dipping.

TABLE 1

Relation of Salinity in Estero San Diego and Presence of Larvae from July 1969 to June 1970 1/

Station No.	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
1	28.7	27.4	4.9	29.6	29.8	30.6	30.5	16.8	20.0	16.6	13.6	0.6 +
2	29.0	27.1	5.9	29.3	30.4	30.6	29.7	23.4	19.6	18.2 +	13.6	1.4 +
3	26.3	22.6	5.6	19.9	29.4	32.4	29.4	27.4	23.6 +	22.9 +	17.2 +	1.0 +
4	22.4	17.6	4.8	0.7	18.4	6.4	-	-	25.2	7.2 +	20.2	-
5	15.2	19.6	3.8	0.5	-	12.6	-	-	14.4	19.1 +	16.0 +	-
6	29.6	26.2	3.9	0.6	8.4	26.2	18.3	17.8	22.8 +	19.2	13.8 +	0.3 +
7	29.2	28.0	6.0	29.6	30.9	32.8	32.6	19.4	19.8	15.6	13.2 +	0.8 +
8	29.0	27.8	6.4	31.7	31.0	30.8	31.8	18.6	19.6	16.6	12.8 +	1.0 +
9	29.1	27.6	6.2	29.8	31.1	31.4	30.8	17.8	19.8	-	13.2 +	1.1 +
10	-	-	-	-	-	-	-	-	-	-	3.6 +	1.0 +
11	19.9	-	-	-	8.4	12.0	14.0	13.8	17.4 +	12.0 +	12.7 +	0.2 +
12	-	-	-	-	-	-	33.6	30.0	25.6	-	17.4 +	-
13	-	-	-	-	-	-	34.1	29.2	25.0	13.0 +	15.4 +	-
14	-	-	-	-	-	-	32.5	31.2	23.2	13.4 +	18.2 +	-
15	-	-	-	-	-	-	33.1	29.4	23.4	-	13.0 +	-
16	6.5	1.0	1.3	2.2	9.5	4.4	19.5	17.6	11.6 +	5.2 +	16.8 +	0.3 +
17	7.4	2.8	-	4.7	23.1	21.6	24.6	25.2	19.8	15.4 +	11.0 +	0.8 +
18	18.4	22.6	0.6	-	31.5	31.0	30.4	27.8	21.6	15.4	12.0	0.7 +
19	-	-	-	-	-	-	31.2	-	21.8	-	14.0 +	1.1 +
20	14.3	5.2	0.2	11.1	30.6	27.4	26.5	25.4	18.8	7.4 +	10.2 +	1.2 +
Avg.	21.8	19.7	4.1	15.8	24.0	23.6	28.4	23.2	20.7	14.5	13.9	0.8

1/ Salinity based on total halide halogen content.
+ Larvae present.
- No sample taken.

Anopheline Densities

The Station's interest in anopheline densities has been largely associated with measuring mosquito populations with regard to such specific control projects as larviciding, ultra low volume (ULV) applications, village fogging, and residual wall treatments. Difficulties encountered and experience gained have greatly increased our ability to measure anophelism, and during the current year efforts have been made to monitor a coastal population continually.

Since most CAMRS pilot control projects have involved concepts different from residual spraying of houses, it has been necessary to use other means of measuring mosquito densities than those associated with domiciles. This report summarizes CAMRS experience in measuring general anopheline densities and discusses seasonal anophelism in the La Libertad area of coastal El Salvador.

Diurnal Density Measurements

Early searches in the coastal area of El Salvador, generally south of the littoral highway (Figure 3), revealed rather extensive populations of both A. albimanus and A. pseudopunctipennis in diurnal resting places (first, under bridges, culverts, in outbuildings, and other man-made structures and later in a variety of natural resting places such as rock crevices, tree cavities, and ground holes). It soon became apparent that such sites were the daytime resting places of these two species throughout their range in El Salvador. Subsequent spot observations in Nicaragua, Honduras, and Costa Rica indicate similar resting places for albimanus. Summaries of interim observations on mosquito densities in natural diurnal resting places, descriptions of sites, and their relative importance appeared in both the 1968 and 1969 CAMRS Annual Reports. To date, 181 collections representing all months of the year and many localities in El Salvador have been made and the results recorded in detail. A numerical summary of this information by type site, collected between February 1968 and June 1970, is given in Table 2. The 181 collections yielded 7,670 albimanus adults (76.6%) and 2,344 pseudopunctipennis adults (23.4%). The sex ratio of albimanus was 24.5 percent males and 75.5 percent females; of pseudopunctipennis, 34.0 percent were males and 66.0 percent, females. A. pseudopunctipennis showed a decided preference for darker and more protected places than did albimanus.

After learning the daytime whereabouts of the two common anopheline species, their 24-hour pattern of movement into and out of the diurnal resting situations had to be determined. The procedure involved counting resting mosquitoes in a given site at hourly intervals throughout a 24-hour period. Counts were made at more frequent intervals during dawn and dusk when mass movement would have been expected to occur. The observations were made concurrently at six sites made up of three red-box shelters each and at a completely natural site. The movement pattern was as follows:

A. albimanus (Figure 4)--The resting population was stable from about 10 a.m. to 5 p.m. when the mosquitoes became restless and a little movement began. Mass migration out of the resting places occurred between 6 and 6:30 p.m. During the early evening hours immediately after darkness, there was little or no resting. Between 8 and 12 p.m., there was minimum movement into the natural resting sites, but there was steady and increasing movement into such places between 12 p.m. and 3 a.m. Between 3 and 5 a.m., the population in natural resting places reached a peak. Between 5 and 6 a.m., there was some "resettling" and a gradual decline in numbers in a given place until around 10 a.m. when the number stabilized and remained essentially constant until around 5 p.m.

A. pseudopunctipennis (Figure 4)--The resting population was stable between 10 a.m. and 5 p.m. At approximately 5 p.m., the specimens became restless, but mass migration out of the diurnal resting sites took place between 6 and 6:30 p.m. During

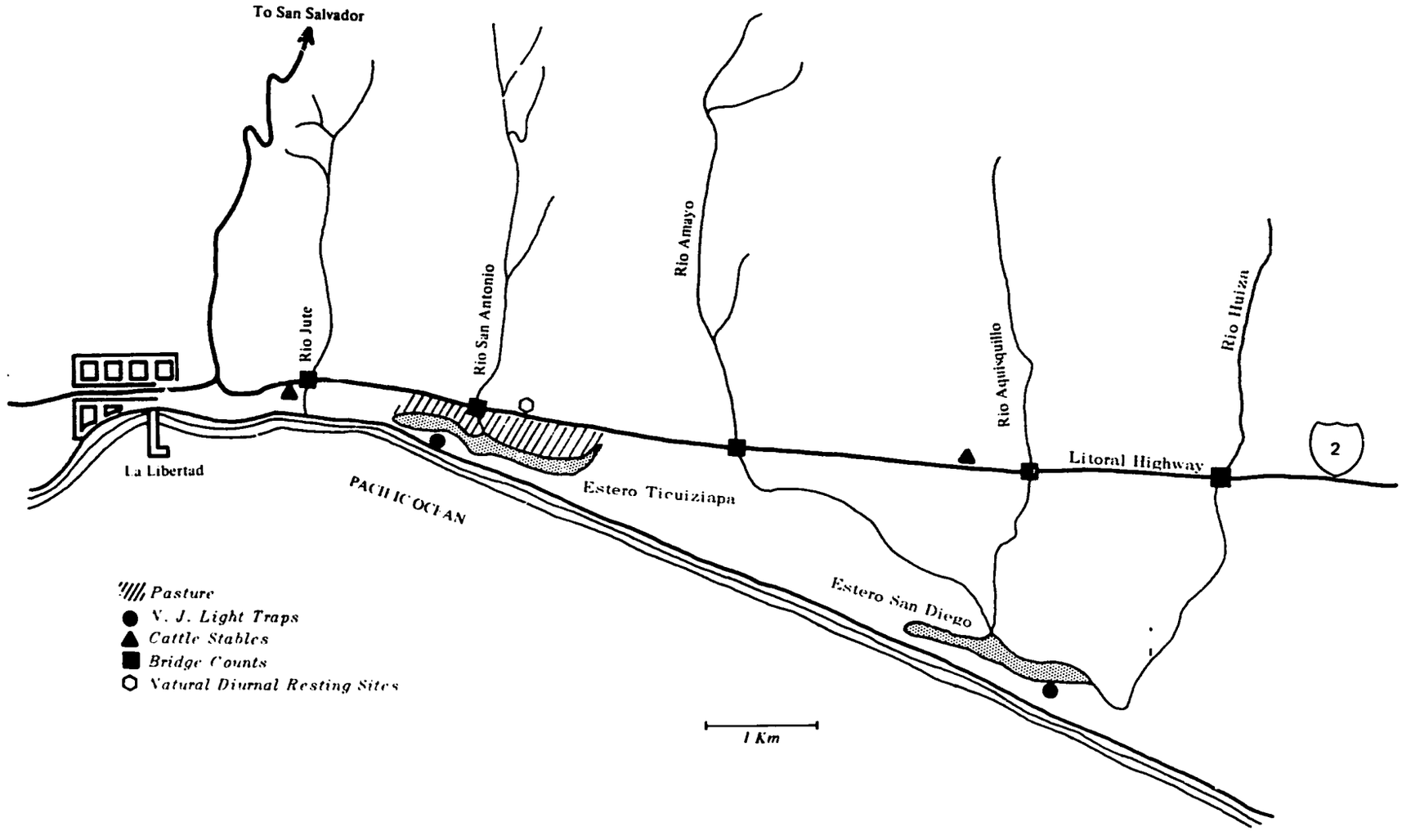
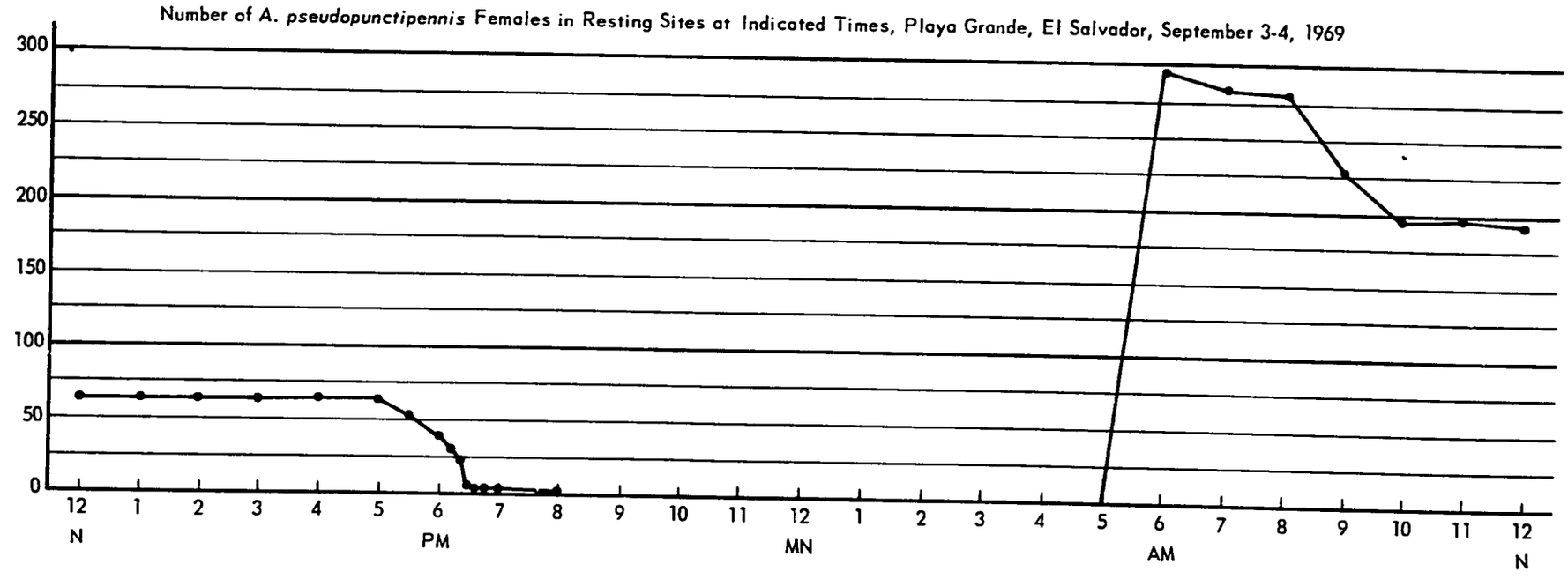


FIGURE 3. Sketch Map of La Libertad Study Area Showing Collecting Sites

TABLE 2
Summary of Captures of Diurnally Resting Adult Anophelines from Natural Situations
El Salvador, February 1968-June 1970

Type of Site	No. of Collections	*A. albimanus*				*A. pseudopunctipennis*			
		Male	Female	Total	Average	Male	Female	Total	Average
Large Rocks	104	1184	2526	3710	35.7	707	1257	1964	18.9
Rock Walls	27	318	2441	2759	102.2	0	185	185	6.9
Small Rocks and Tree Trunks	11	100	317	417	37.9	4	48	52	4.7
Tree Hollows and Roots	10	31	40	71	7.1	23	10	33	3.3
Ground Holes	13	115	166	281	21.6	37	29	66	5.1
Lava Deposits	2	63	189	252	126.0	0	0	0	0
Large Shaded Cliffside	2	9	9	18	9.0	8	3	11	5.5
Tree Trunks and Fallen Logs	6	24	18	42	7.0	0	0	0	0
Small Rocks, Shaded Margins of Stream Beds	3	0	12	12	4.0	3	0	3	1.0
Miscellaneous Sites									
Horizontal Cliffside Cavity behind Waterfall	1	0	0	0	0	16	14	30	30.0
Base of Century Plant	1	3	14	17	17.0	0	0	0	0
Animal Burrow	1	34	57	91	91.0	0	0	0	0
Total	181	1881	5789	7670	42.4	798	1546	2344	13.0



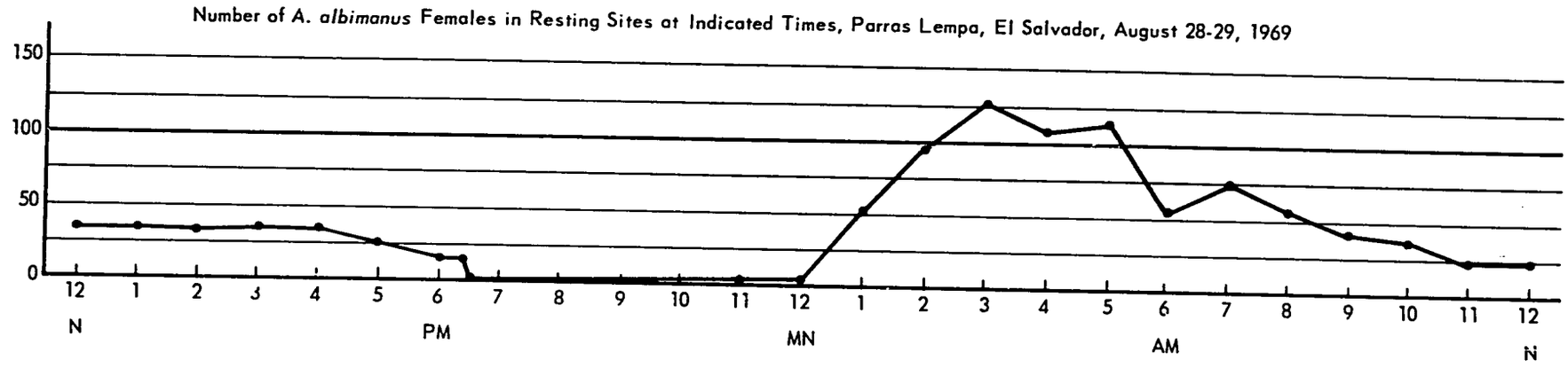


FIGURE 4. Resting Cycle of Anophelines, El Salvador, 1969

the darkness of night (6:30 p.m.-5 a.m.), no mosquitoes were found in the natural resting or box stations. Between 5 and 6 a.m., there was mass movement into the daytime resting places with peak numbers being found between 6 and 8 a.m. Between 8 and 10 a.m., there was some "resettling," but numbers were stable between 10 a.m. and 5 p.m.

The reciprocal of the resting curve, established in the foregoing manner, represents the activity pattern of the two species. Thus, albimanus departs its daytime resting places at dusk and is most active between dusk and midnight. These are the hours when maximum activity would be expected regarding its flight and feeding habits. A. pseudopunctipennis, on the other hand, has an activity pattern lasting throughout the hours of darkness.

The implications of these studies are many. For example, the ULV treatments of 1968 and 1969 were at a time of the day (5-6 a.m.) when anophelines were in protected niches of daytime resting places (Figure 4). Obviously, the first several hours after dark should be optimum from the standpoint of exposed mosquitoes. Also, current host preference studies show that a high percentage of blooded females found in houses during the early morning hours have fed on something other than man. This implies that a portion of blood-fed mosquitoes enters houses solely for shelter and not for blood. Therefore, the terms, endophilic and endophagic, commonly used to describe inside-loving and inside-feeding anophelines, respectively, may truly represent distinct entities. Perhaps the truly endophagic portion of the population is that portion which enters a dwelling during the early evening hours seeking blood while the shelter-seeking females already having had a blood meal from outside sources are not endophagic at all and are endophilic only by the chance of being nearer to a dwelling than a tree cavity or some other natural or artificial shelter. This aspect is being pursued by studying blood-meal sources of A. albimanus collected from dwellings during various periods of the night.

Therefore, daytime density measurements for certain purposes are not only feasible, but desirable. The collection of specimens from diurnal resting sites for host preference studies would seem to result in less bias than collections from night host-specific situations such as cattle corrals.

For A. albimanus, counts from natural diurnal resting places near breeding areas reflect adult populations equally as well as some of the more commonly used nighttime methods such as stable counts and human bait captures, depending, of course, on the purpose of the collection. For density measurements, considering the added cost and drain on manpower of night captures, plus the effect of climatic conditions such as wind, rain, and moon phase on albimanus during the nighttime activity period, it is probable that daytime counts of resting mosquitoes in protected niches might be even more reliable as a population index. Experience has shown that when albimanus is found in a cattle corral during a night capture period, it is correspondingly present in daytime niches in the vicinity of the same corral and much more conveniently counted. Recent observations near Lake Jocotal showed, for example, approximately 2,000 albimanus females counted during the day in a brick shed near the corral. The count was made in 20-30 minutes. Additionally, crevices in rock walls nearby harbored hundreds of albimanus which could be easily counted during any hour of daylight. The same pattern applied to natural resting sites near the corral at Playa Grande and at a third location near Jocotal. This method has been used at many corral sites throughout El Salvador with equally good results. Counts from natural resting sites in the vicinity of Estero Ticuiziapa show the same peaks as light trap data for the same place and period.

Nocturnal Density Measurements

Several good methods of measuring nighttime anopheline densities are available and the choice of one or more is usually one of convenience or purpose.

Cattle corrals (establos) are common throughout the malarious areas of El Salvador and usually offer a reliable index of anophelism for the locality. Female A. albimanus are strongly attracted to the animal concentration, but A. pseudopunctipennis, only slightly so. The attractiveness to pseudopunctipennis seems to vary from one corral to the other and is seemingly not related to the absolute numbers of the species in the vicinity. The two establos in the La Libertad study area serve to illustrate this point. In Establo El Coco, between February 1969 and June 1970, only 45 females (1.2%) of pseudopunctipennis were counted from 43 collections, while albimanus was represented by 3,801 (98.8%) specimens in the same collections. In Establo Melara, on the other hand, 62 (6.5%) pseudopunctipennis were collected as compared to 887 (93.5%) albimanus, and the former was more consistently present in Melara despite the fact that larval surveys showed a higher percentage of pseudopunctipennis in Rio Jute (94.9%), near Establo El Coco, than in Rio Aquisquillo (90.7%), which is situated near Establo Melara. Also, the larval densities of anophelines was higher in Rio Jute (3.4 per dip) than in Rio Aquisquillo (1.5 per dip).

Establo collecting is best during the first several hours after darkness corresponding to the albimanus activity peak previously described. Although large numbers of specimens are sometimes collected in short periods of time in establos, numbers fluctuate widely with the number of cattle. Specimens collected from cattle corrals serve limited purposes and are used mainly to indicate anophelism or as a source of specimens for certain types of biological studies.

Collecting anophelines attempting to bite humans is a realistic approach to measuring some of the more important aspects of anopheline behavior in relation to malaria transmission. This method has been used by CAMRS in connection with ULV studies and as a source of specimens for longevity studies and for studies on susceptibility to malaria parasites and insecticides. Human bait collections were used to determine density changes among wild populations during ULV trials. During the same trials, it was discovered that biting collections around concentrations of light, such as service stations, offer a good indexing situation because of the positive response of albimanus to the combination of light and food.

The New Jersey-type light trap has demonstrated a high degree of efficiency in measuring A. albimanus densities. It has been practically useless in measuring A. pseudopunctipennis densities. The CDC trap has not proven effective for either species. With respect to the New Jersey trap, thousands of Anopheles trapped at various locations during 1969 and 1970 included only a handful of pseudopunctipennis. Light traps have been operated continually at two locations (San Diego and Ticuiziapa Beaches) since early 1969. Results show that 21,966 albimanus were collected in the San Diego trap in 145 trap nights for an average of 151.5 per trap night. The Ticuiziapa trap yielded 5,386 albimanus in 188 trap nights for an average of 28.7 per trap night. Only 7 pseudopunctipennis were captured in the San Diego trap and only 4 in the Ticuiziapa trap. Females made up 98.4 percent of the San Diego light trap collections and 95.6 percent of the Ticuiziapa trap collections. Light traps were useful in measuring densities during the 1969 ULV trials. In recent tests in a high density area near Lake Jocotal, a total of 3,563 albimanus (99.0% females) were captured in one trap night of operation.

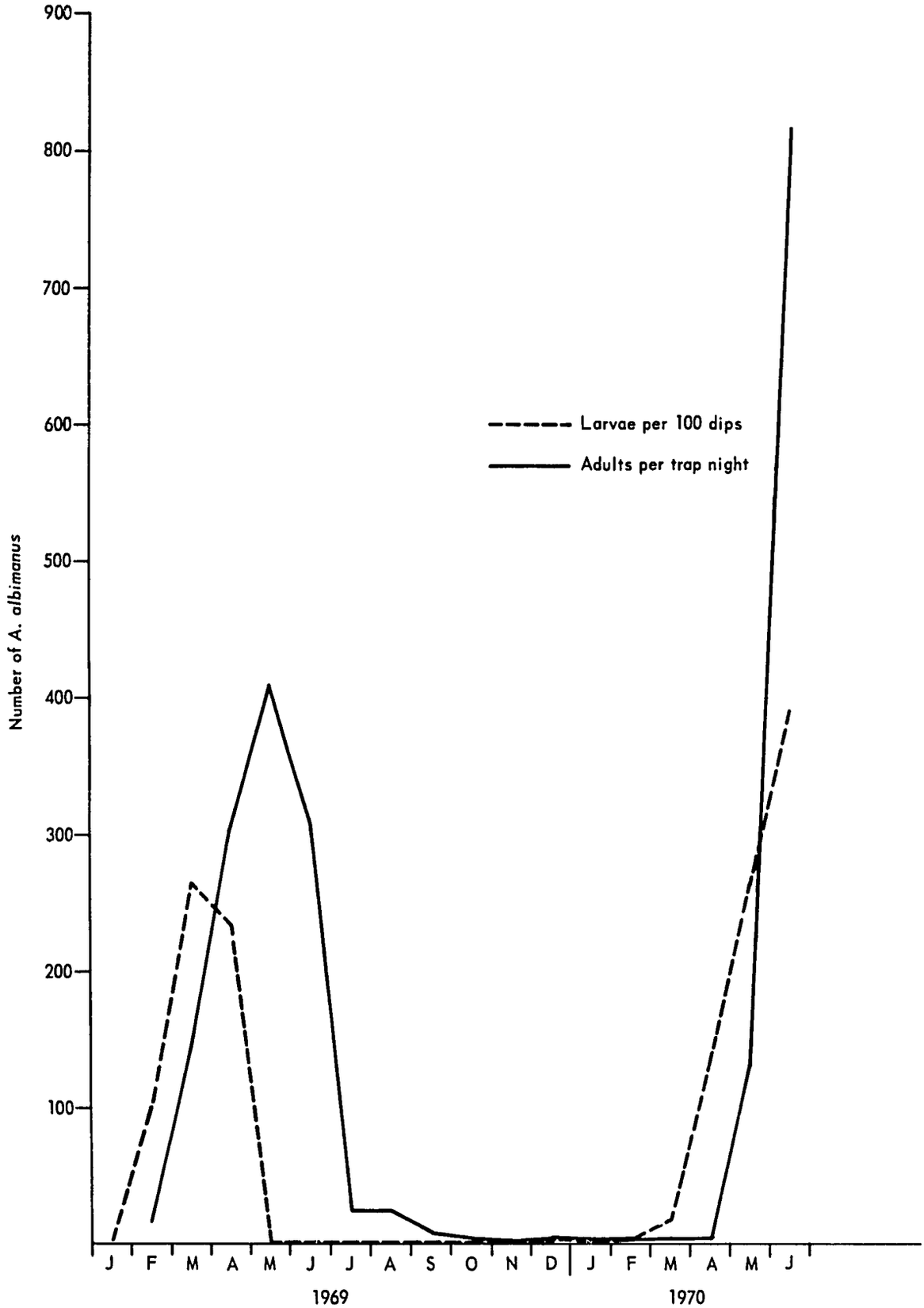
Seasonal Distribution of *Anopheles*

The determination of *Anopheles* adult densities on a seasonal basis depends upon the use of the same tools and methods described for collecting mosquitoes on a 24-hour basis. After several seasons of work in the La Libertad area, certain seasonal patterns are apparent. Essentially, this area produces significant populations of anophelines only during the dry season because of the type of breeding habitats available to and used by the species present. The area includes two large estuaries (esteros): Estero San Diego, which is not complicated by associated breeding habitats and Estero Ticuiziapa (Figure 3), which is complicated by an associated pasture (pantano) which supports considerable mosquito breeding. In addition to the esteros, several rivers support substantial dry season breeding. Rainy season habitats are restricted to flooded pantanos, roadside ditches, and overflows from miscellaneous inundations. The amount of breeding in such sites is heavy during the early months of rainy season but is reduced by mid-rainy season because of standing water becoming polluted and covered with a vegetative film. The types of breeding places include the dry season types of Figure 1 (esteros, line 5; rivers, line 6; and irrigation waters, line 7) and the rainy season types (pantanos, line 1; overflows, line 2; and peak inundations, line 3). There is extremely high correlation between the larval populations of the esteros and adult populations as measured by light traps associated with the esteros. Figure 5 shows these data for Estero San Diego which is uncomplicated by outside breeding sources. In addition to light trap correlation, a similar relationship exists between breeding in the Ticuiziapa complex and natural diurnal resting counts in the vicinity of the pantano. There is also high correlation between river breeding and counts of adult mosquitoes resting under bridges associated with the rivers.

Plotting of seasonal density data such as that of Figure 5 would be useful in planning malaria reduction through mosquito control if such data were available for each malarious area. A glance at this curve shows that the application of an adulticide, for example, between July and February would serve no useful purpose in this particular area, whereas a more timely application after detection of dry season populations might serve for the entire year. Also, opening of the estero, or larviciding, or combinations of these methods might be indicated. At least, the data serve to show that control efforts could be a function of need rather than administrative consideration.

Although much has been learned about rainy season densities, it has been on an opportunistic basis, and no seasonal pattern for specific areas with heavy rainy season breeding can be shown at this time. However, it is evident that continuing density studies are needed for specific areas and that such studies should yield useful information of the type described for the dry season breeding areas near La Libertad. Preferably, these data should be accumulated together with malariometric data, and an effort should be made to include areas that have both rainy and dry season breeding and others that have essentially rainy season breeding. From such seasonal information, it might be possible to extrapolate, for other regions of the country, seasonal patterns of anophelism which might form the basis of more intelligent control practices.

FIGURE 5. Number of *A. albimanus* Larvae Per 100 Dips, Estero San Diego; and Number of Adult *A. albimanus* Per Light Trap Night, San Diego Beach January 1969 – June 1970



Host Preference Studies

Four lots of mosquito blood smears on filter paper have been submitted to the Fort Collins, Colorado, CDC facility for precipitin tests to determine the blood feeding source of individual specimens comprising each lot. Results have been received on three of the four lots totaling 574 A. albimanus and 172 A. pseudopunctipennis blood meal determinations (excluding 44 negative albimanus and 10 negative pseudopunctipennis). Specimens are collected from various sources from various localities in an effort to correlate blood feeding habits with ecological factors important in malaria transmission.

Of 574 albimanus blood meals from eight localities in five Departments, 470 (81.9%) have been from cattle; 45 (7.8%), from humans; 30 (5.2%), from unknown mammals; 14 (2.4%), from dogs; 6 (1.1%), from horses; 5 (0.9%), from pigs; 3 (0.5%), from raccoons; and 1 (0.2%), from an unknown bird (Table 3). Included in these determinations are five double feedings (human/cattle-1, human/raccon-1, and horse/cattle-3). Collectively, man and associated domestic animals make up 94.1 percent (540 of 574) of the feedings. When viewed by locality, the data show that the only human feedings came from Jocotal, La Curruncha, and Condadillo. The ratio of human feeding to that on other animals is higher in Condadillo and La Curruncha with 50.0 percent and 20.8 percent human blood meals, respectively. Jocotal and La Curruncha are interesting because of a relatively large number of feedings on unknown mammals. In this connection, a mammal survey and collection of sera from common animals other than domestic species is indicated. It is perhaps significant that all human feedings have been from the low flat areas of southeastern El Salvador, although much additional data must be accumulated before a hypothesis with implications can be formulated.

Of 172 pseudopunctipennis blood meal determinations by locality, 147 (85.5%) were from cattle; 9 (5.2%), from horses; 9 (5.2%), from unknown mammals; 4 (2.3%), from humans; 2 (1.2%), from pigs; and 1 (0.6%), from a dog (Table 4). These figures include a double feeding from human/cattle and one from horse/cattle. All four human feedings came from a collection of diurnally resting adults under the bridge over Rio Huiza in the Department of La Libertad. Collectively, 163 of 172 specimens (94.8%) of pseudopunctipennis had fed on man and associated domestic animals, a figure remarkably close to the 94.1 percent recorded for albimanus. Many of the pseudopunctipennis were collected from natural or artificial shelters, for the most part removed from human habitation. Unlike albimanus, unknown mammal feedings for pseudopunctipennis were not peculiar to particular localities, although the overall percentage (5.2%) was equal for the two species.

A breakdown of data by type collecting site for A. albimanus shows, as might be expected, that among intradomiciliary collections, human-fed specimens were more frequent than from other types of sites. However, this varied with the locality, and a considerable number of such specimens had fed on animals other than man. Of 167 specimens collected resting on surfaces inside houses, 44 (26.4%) had fed on humans, but 82 (49.1%) had fed on cattle and another 19 (11.4%), on unknown mammals. Collectively, 145 (86.8%) fed on man and domestic animals. There were three raccoon feedings including one double feeding of man/raccon and two double feedings of horse/cattle. These data serve to show that although human feedings are greater among blood-fed specimens collected from inside houses, such specimens are not exclusively human-fed. This fact, coupled with the before midnight activity pattern of albimanus lends credence to a study to correlate time of entry into a house with endophagy as previously discussed under the section on anopheline density studies with respect to the 24-hour habits of the species. Further, intradomiciliary results from Atalaya are quite different from those of three other localities with no human feedings among 30 specimens. Again, the other localities are in southeastern El Salvador whereas Atalaya is in the southwestern portion of the coastal plain. With

TABLE 3
Summary of Blood Meal Determination by Locality
Anopheles albimanus
March 10-April 29, 1970

Locality	Blood Meal Identifications								
	Human	Cattle	Dog	Horse	Pig	Raccoon	Unknown Mammal	Unknown Bird	Total
La Paz									
Río Apante	0	2	0	0	1	0	1	0	4
La Unión									
Condadillo	12	3	5	0	0	0	4	0	24
San Miguel									
Jocotal	18	194	1	2	1	0	11	1	228
La Curruncha	15	29	8	1	3	3	13	0	72
San Vicente									
Apastepeque	0	27	0	0	0	0	0	0	27
Parras de Lempa	0	29	0	2	0	0	0	0	31
Sonsonate									
Atalaya	0	138	0	1	0	0	0	0	139
San Antonio	0	48	0	0	0	0	1	0	49
Total	45	470	14	6	5	3	30	1	574
Percentage	7.8	81.9	2.4	1.0	0.9	0.5	5.2	0.2	

Note: Double feedings included in the table are 1 Human/Cattle; 1 Human/Raccoon; and 3 Horse/Cattle.

TABLE 4
Summary of Blood Meal Determination by Locality
Anopheles pseudopunctipennis
March 10-April 29, 1970

Locality	Blood Meal Identifications						
	Human	Cattle	Dog	Horse	Pig	Unknown Mammal	Total
Ahuachapán							
Rio Naranjo	0	8	0	1	0	1	10
La Libertad							
Rio Huiza	4	39	0	3	0	1	47
Rio Comasagua	0	5	0	3	0	2	10
La Paz							
Rio Apante	0	4	1	0	2	1	8
La Unión							
Condadillo	0	29	0	1	0	0	30
San Miguel							
Jocotal	0	21	0	0	0	2	23
Sonsonate							
Atalaya	0	41	0	1	0	2	44
Total	4	147	1	9	2	9	172
Percentage	2.3	85.5	0.6	5.2	1.2	5.2	

Note: Double feedings included in the table are 1 Human/Cattle and 1 Horse/Cattle.

respect to collecting sites other than houses, results of precipitin tests show an overwhelming majority of the feedings to be cattle. In natural diurnal resting sites, 334 of 347 specimens had fed on cattle and only one on a human, whereas in outbuildings 52 of 56 had fed on cattle and none on humans. These collections are probably heavily biased since most of the collecting sites are close to cattle corrals, although the Jocotal sites were well removed from the corral and were associated closely with human habitation.

A breakdown by collecting sites for A. pseudopunctipennis, as with albimanus, shows that the majority of specimens from natural daytime resting sites exhibited a predominance of cattle-fed specimens (91 of 97), even though one of the sites (Jocotal) was well removed from the immediate vicinity of the stable. Bridges offer extremely favorable diurnal resting sites to A. pseudopunctipennis since this species breeds heavily in associated rivers. Specimens from bridge collections show a high percentage of cattle feedings, 56 of 75 (74.7%), but there is a good qualitative distribution with feedings recorded for man-4, dog-1, horse-7, pig-2, and unknown mammal-5. The relatively high percentage and representation of horses from three of four bridge sites is interesting.

A comparison of results for albimanus and pseudopunctipennis from common collecting sites shows that, of 106 albimanus, 100 were cattle-fed, 3 had fed on unknown mammals, and 1 each on pig, horse, and man. These results were very similar for pseudopunctipennis with 66 human feedings, 5 from unknown mammals, 2 from pigs, and one each from horse and dog. From these data, it appears that among adult populations shared by albimanus and pseudopunctipennis, there is little difference in the host preference of the two species.

Summarizing, test results indicate that both albimanus and pseudopunctipennis are strongly attracted to cattle for blood meals and that the presence of cattle in an area lends strong bias to blood meal preferences, even where collections are made in or near human dwellings, or from natural resting places quite removed from the immediate vicinity of cattle corrals. Except for a single specimen, A. albimanus with human blood have been captured exclusively from inside houses although the percentage varies from house to house. Even when rather high percentages of blood-fed albimanus have been captured from houses, other blood meal sources have equaled or exceeded those of human source. A. pseudopunctipennis has not been captured from houses and is not usually found in numbers near human habitations. Blood meal sources, apart from human habitation, have been quite similar for albimanus and pseudopunctipennis where the two species occupied the same area. Double feedings have been recorded for human/raccoon, human/cattle, and horse/cattle among albimanus, and human/cattle and horse/cattle among pseudopunctipennis. Several clues as to need for future studies are indicated by host preference results.

Physiological Age-Grading

Specimens representing the same groups collected to determine infectivity rates were used for physiological age-grading studies. The hypothesis is that mosquitoes might be longer lived from one place to the other and from one season to the other, factors which would have a direct bearing on malaria transmission potential. The data of Table 5 show that between April and June 1970, essentially dry season, the albimanus populations sampled were extremely young, and, therefore, not particularly dangerous in terms of malaria transmission. Of 297 albimanus females dissected from seven coastal departments, only 43 (14.5%) were parous as determined by dissection and examination of ovaries. The 43 parous specimens included only one specimen with evidence of more than one gonotrophic cycle.

A. pseudopunctipennis, on the other hand (Table 6), showed a higher level of parous females with 59 of 163 (36.2%) having completed at least one gonotrophic cycle. Analysis shows that of the 59 parous specimens, 50 had completed one gonotrophic cycle; seven, two cycles, one, three cycles, and one, five cycles. The remaining females showed 104 (63.8%) nulliparous. This species is essentially a dry season mosquito and might be better able to cope with dry conditions than albimanus, resulting in longer population life during this time of year.

These studies will be continued systematically to detect changes in longevity patterns as they might occur. Meanwhile, there is some laboratory evidence that A. albimanus exhibits gonotrophic dissociation whereby blood ingestion by the female may not result in gonadal development. Since interpretation of age-grading studies of the type being pursued depends upon gonotrophic concordance in the species, laboratory studies are underway to determine the level of confidence that may be placed in age-grading techniques being used.

TABLE 5
Results of Dissections to Determine the Physiological Age
of *Anopheles albimanus* in El Salvador
April-June 1970

Department	No. Dissected	Parous		Gonotrophic Cycles	
		No.	%	1	2
Ahuachapán	13	0	0	-	-
La Libertad	87	20	23.0	20	0
La Paz	15	6	40.0	6	0
La Unión	72	4	5.6	3	1
San Miguel	68	8	11.8	8	0
San Vicente	20	3	15.0	3	0
Sonsonate	22	2	9.1	2	0
Total	297	43	14.5	42	1

TABLE 6
Results of Dissections to Determine the Physiological Age
of *Anopheles pseudopunctipennis* in El Salvador
April-June 1970

Department	No. Dissected	Parous		Gonotrophic Cycles				
		No.	%	1	2	3	4	5
Ahuachapán	3	1	33.3	0	1	0	0	0
La Libertad	104	37	35.6	31	5	1	0	0
La Paz	3	1	33.3	1	0	0	0	0
La Unión	21	6	28.6	6	0	0	0	0
San Miguel	17	7	41.2	7	0	0	0	0
Sonsonate	15	7	46.7	5	1	0	0	1
Total	163	59	36.2	50	7	1	0	1

Susceptibility to Plasmodium

Efforts were begun late in the fiscal year (April 1970) to monitor samples of anopheline populations from coastal areas of El Salvador to determine relative levels of susceptibility to Plasmodium from locality to locality and from season to season. Dissections of mosquitoes from 107 collections from seven Departments (Figure 6: Ahuachapán-8, La Libertad-25, La Paz-14, La Unión-23, San Miguel-19, San Vicente-4, Sonsonate-14) yielded no positive guts from 241 albimanus and 50 pseudopunctipennis dissections and no positive glands from 434 albimanus and 275 pseudopunctipennis dissections. Glands and guts were not dissected from the same specimen; hence, there were 675 albimanus negatives and 325 pseudopunctipennis negatives. Collections were made from various sources, but always near coexisting concentrations of people and mosquitoes in malarious areas of the country. No positives were recorded for 312 albimanus and 315 pseudopunctipennis from natural daytime resting sites; 217 albimanus and 2 pseudopunctipennis from intradomiciliary captures; 103 albimanus and 3 pseudopunctipennis from human bait; and 43 albimanus and 5 pseudopunctipennis from cattle corrals. A. pseudopunctipennis adults, as previously pointed out, are difficult to collect in nighttime collections. These studies will be continued on an opportunistic basis to determine if rainy season results are different. However, efforts will be made to become increasingly more selective about sources of mosquitoes to be dissected and, particularly, to collect from areas of active malaria transmission.

The finding of no positives validates much previous work by others and is not surprising in view of corollary information being accumulated. For example, a summary by Horsfall 1/ shows only 32 positive glands and 70 positive guts recorded for albimanus by 22 workers in 10 countries between 1926 and 1947 in approximately 14,000 dissections. Included in this tabulation are reports of two positive glands from 315 dissections in El Salvador (Sutter and Zuniga, 1942); one positive gut (?) and no positive glands from 559 dissections in Costa Rica (Kumm and Ruiz, 1939); two positive guts and no positive glands from 47 dissections in Honduras (Barber and Komp, 1924); and nine positive guts and 26 positive glands from 5,377 dissections in Panama (various authors). Data discussed elsewhere in this report indicate that albimanus might be more zoophilic than anthropophilic (Table 3), more exophagic than endophagic (Table 2), and not particularly long lived, at least in the dry season (Table 5). These are attributes of a relatively poor malaria vector and help explain the difficulty in finding positive specimens. Evidently, whatever efficiency albimanus has as a malaria vector must depend upon overwhelming numbers in the right place at the right time, but CAMRS must identify the right place at the right time.

1/ Horsfall, W.R., 1955. Mosquitoes, Their Bionomics and Relation to Disease. The Ronald Press. New York. viii plus 723 pp.

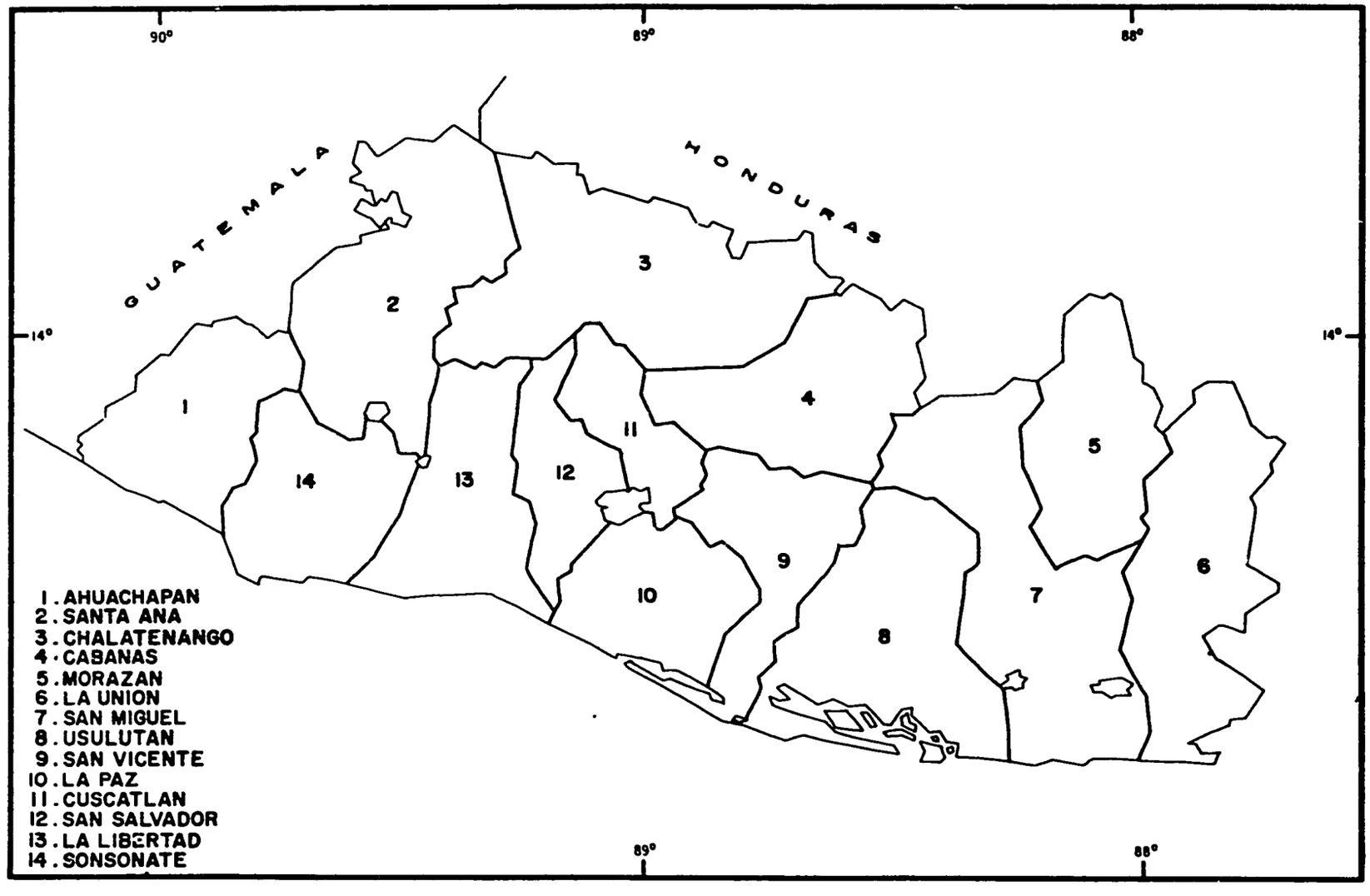


FIGURE 6. El Salvador

VECTOR CONTROL STUDIES

Residual Insecticides

Most of the residual insecticide studies done in 1969-70 were carried out in the eastern part of El Salvador. Figure 7 shows the approximate location and relationship of the different study areas. This eastern part of the country is composed of many nearly level valleys, nestled among hills and low mountains. The valleys are generally below 100 meters, and many have small lakes or lagoons as well as low areas that readily fill with water in the rainy season, making excellent breeding grounds for A. albimanus. Anopheles pseudopunctipennis is found invading dwellings but not in high numbers during the rainy season. A. vestitipennis, which appears only in the heavy rainy season near lake areas, readily attacks man and enters dwellings but is very seldom encountered resting inside.

This is an excellent area for residual insecticide studies because of the many localities which are readily accessible even in the rains. Another advantage of the area is the presence of Laguna El Jocotal where there is a very high density of resistant A. albimanus during most of the year. This density is higher and persists longer than in other parts of the country, assuring a good supply of specimens for bioassays and release tests even if the A. albimanus densities decline or disappear in other localities.

Entomologic methods for evaluation were standard for most of the studies and included the following:

1. Early morning resting densities to determine the intradomiciliary effectiveness of the insecticide.

2. Early evening density captures to determine the effect of the insecticide or density of mosquitoes found biting man in the early evening and through the night in intradomiciliary and peridomiciliary situations. Similar captures were made in cattle corrals to determine possible effect on total mosquito populations in the area.

3. Exit densities and mortalities, using specially designed traps, to determine the entry and exit of anophelines through an entire night in sprayed houses, with determination of survival of those escaping into traps.

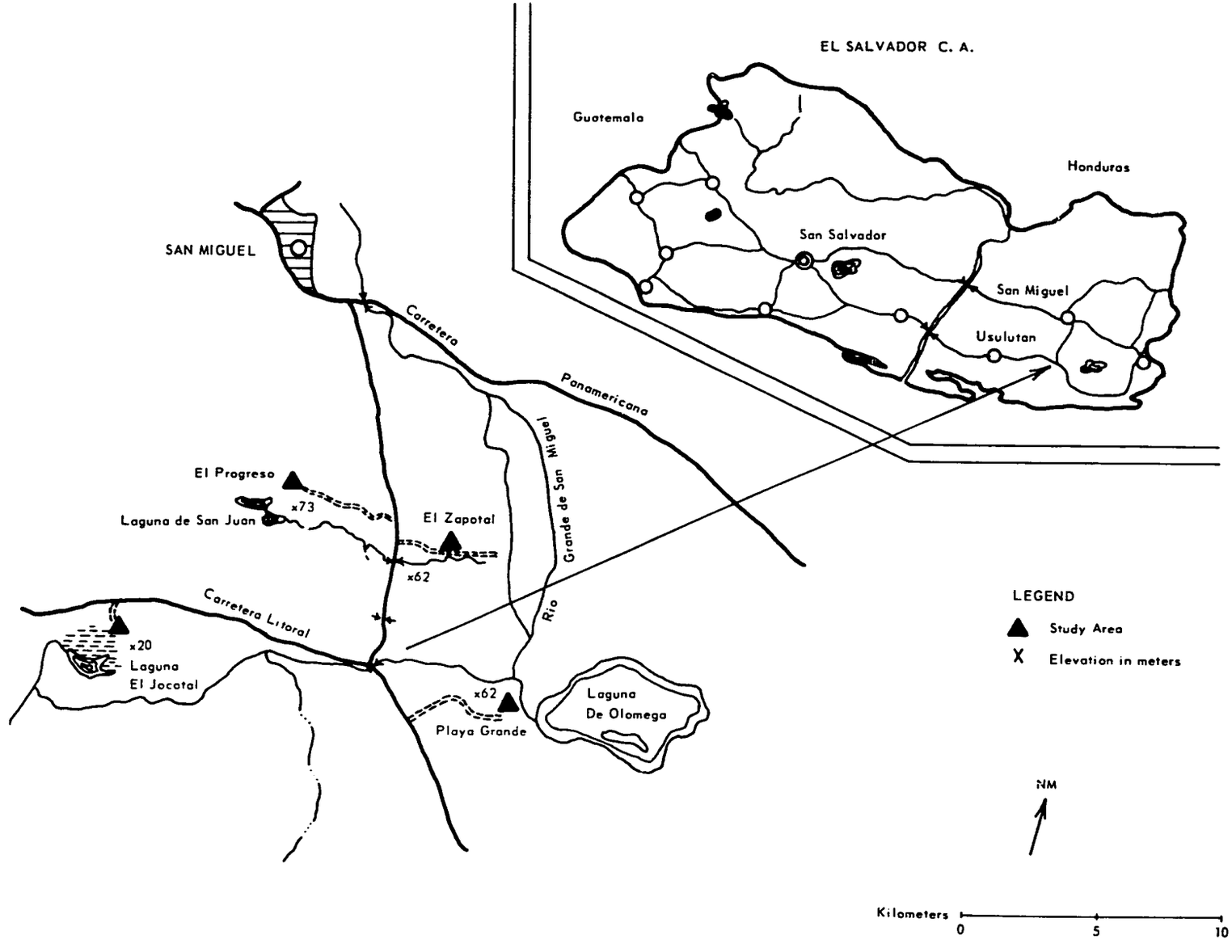
4. Bioassays of sprayed surfaces, using a modified WHO method, to determine the persistence of effectiveness of the insecticide applied in killing mosquitoes. Special testing chambers have been developed to accommodate nearly all types and configurations of wall surfaces (pole, tile, and so on).

5. Fumigant tests, using special wire cages of mosquitoes placed within and outside of houses, to test the possibility of fumigant activity.

Mosquito populations of the area and those used for the entomologic assays were routinely tested for susceptibility to the insecticides used, using WHO test papers, when available, or locally constructed test panels.

Where possible, entomologic evaluations were supplemented with chemical analyses of residues and fumigant activity associated with some of the compounds tested.

FIGURE 7. Study Areas for Evaluation of Candidate Residual Insecticides
El Salvador, 1969-1970



Village Scale Trials with Fenitrothion (OMS-43)

In June 1969, a small village trial of fenitrothion [OMS-43; o,o-dimethyl 0-(4 nitro-m-tolyl) phosphorothioate] was conducted in El Zapotal, near San Miguel (Figure 7). This village is situated at the foot of low hills in a valley that is approximately 65 meters above sea level. The rural-type houses are mainly of "open"-type construction with grass-thatch or tile roofs and loose, usually pole, walls. Some houses, however, are rather tight, with tile roofs and adobe brick walls. Houses in the fenitrothion test village of El Zapotal, in the control village of Playa Grande, and in a third village, El Progreso, used for similar tests with carbaryl discussed elsewhere, were surveyed to determine the distribution and extent of types of construction. The results are shown in Table 7.

TABLE 7
Percent of Houses with Various Roof and Wall Surfaces
Zapotal, Playa Grande, and El Progreso

Locality	No. of Houses Visited	Percentage of Roofs		Percentage of Walls							
		Tile	Thatch	Poles	Mud	Palm	Adobe	Brick	Thatch	Board	Metal
Zapotal	69	68	32	46	6	8	22	7	-	11	-
Playa Grande	38	16	63	73	4	6	-	-	4	13	-
El Progreso	60	37	63	60	10	9	8	5	4	3	1

Pole wall surfaces dominated in all three villages but a large portion of the houses in Zapotal had tile roofs (68%) and essentially solid walls (54%). In the control village of Playa Grande, 63 percent of the houses surveyed had thatch roofs and only 16 percent had tile roofs (tin roofs predominated in the remaining 21%); most walls were of loose structure (pole, 73%; palm, 6%; thatch, 4%; and board, 13%), adobe and brick being absent among the houses surveyed.

In the test village El Zapotal, 87 houses were treated by the Hudson 10 X-pert hand spray pump, equipped with the Teejet nozzle with a fixed pressure regulator. The 5 percent water suspension of the 50 percent wettable powder fenitrothion was delivered at a target dose of 2 grams per square meter to the sprayed surfaces. The spray had an unpleasant odor during spraying, and several householders (women) complained of dizziness and one complained of nausea and headache. The control village at Playa Grande was sprayed with DDT ($2gr/m^2$) on a regular country schedule and was retreated during the test as part of the regularly scheduled cycle.

Entomologic evaluations of this fenitrothion test were continued for nine weeks after treatment. During this period, according to the human bait and animal bait captures, there were high densities of A. albimanus in both areas, with no reduction of densities attributed to insecticide treatment. Early morning resting densities within fenitrothion-treated houses were reduced during the first and, possibly, the second week after treatment, but by the fourth week and beyond, these densities were back to normal. Wall trap captures yielded 100 percent mortality only once, one week after treatment; in others at weeks 4, 6, and 8, mortality rates were not significantly high. Slight fumigant activity was seen one week after the treatment but not subsequently.

Bioassays of surfaces gave variable results when first conducted three weeks after treatment, depending on the surace tested. Mortalities at 3 weeks were: wood, 80 percent; thatch, 92 percent; tejas (tile), 77 percent; poles, 51 percent; and adobe,

1 percent. By the fifth week, mortalities ranged from negative to 32 percent in all tests and, subsequently, on weeks 7 and 9 showed a further decline in mortality. Similar tests done on DDT-sprayed surfaces in Playa Grande indicated unsatisfactory results on all surfaces with maximum mortalities of about 38 percent on the wood surfaces at the third week after treatment. Mortalities at fifth, seventh, and ninth varied from 0 to 25 percent.

Susceptibility tests conducted using treated panels and/or WHO test papers showed the A. albimanus populations to be highly resistant to DDT. Further, the A. albimanus populations of the El Zapotal area were found to have a considerable degree of resistance to fenitrothion when compared with A. albimanus from the CAMRS colony or from a non-agricultural area of El Salvador. Interestingly, within the test area there is extensive cotton cultivation, with the usual intensive use of insecticides.

In summary, the village scale trials of fenitrothion in El Zapotal indicated a loss of effectiveness in 4 weeks, based on the entomologic evaluations, and a population of A. albimanus relatively resistant to the insecticide. Therefore, this insecticide may not be useful in wide areas of El Salvador where agricultural practices may have led to a decrease in vector susceptibility.

Village Scale Trial with Carbaryl

A small-scale trial of carbaryl (OMS-29; 1 naphthyl N-methylcarbamate) was carried out in the village El Progreso (Figure 7). This village is located on a small rise along the margin of the Lagoon San Juan at an elevation of 73 meters. The houses are scattered through the area and are of the types of construction common to the rural lowlands of El Salvador. A survey of 60 of the houses in this village indicated predominately thatch roofs and pole walls (Table 7). The village of Playa Grande was used as a comparison village. There is extensive cotton-growing in the El Progreso area. Principal anopheline breeding is associated with the Lagoon San Juan and, during the rainy season, the swamps around this Lagoon. The rainy season in this area is generally from May through October; after the rains stop, the non-permanent breeding sites tend to disappear rapidly.

The test village was treated on June 26, 1969, and again about 4 months later (October 20). The Hudson 10 X-pert hand spray pump was used; this was equipped with the Teejet nozzle with a fixed pressure regulator. The carbaryl was used as an 85 percent wettable powder, and the dilution was calculated to give a target dose of 2 grams of technical material per square meter of surface. Sixty houses were treated in the first cycle, and 80, in the second. Practically no odor was apparent after spraying, and no complaints were received from the occupants of the sprayed premises.

Entomologic evaluations were continued over a 15-week period following the first treatment and for 17 weeks following the second. Density studies (human bait and animal bait) showed variable anopheline densities in the control and test areas during the period of evaluation, with a general decrease in the test area in August, probably related to cotton growing and concomitant use of insecticide in the area. In no case could a decrease be associated with the house-spraying tests. Based on early morning captures, carbaryl appeared to have some effect through the first two post-treatment weeks, but little thereafter, even after the second treatment. Using wall trap captures, either with natural populations or with released populations, satisfactory mortalities could be found only in weeks 2 and 4 after the second treatment, with a much less satisfactory result after the first spraying.

Bioassays after the first treatment showed satisfactory kills for up to 13 weeks on wood surfaces and for less than three weeks on tile. Adobe and mud (3 weeks), thatch (9 weeks), and poles (11 weeks) occupied intermediate positions. After the second treatment, there was a general increase in the duration of effect, with wood surfaces showing satisfactory kill when last tested on week 17. Again, mud and tile were poor when first tested two weeks after the second spraying. In the DDT comparison village, bioassays consistently showed unsatisfactory kills on all surfaces treated.

Susceptibility tests on the A. albimanus mosquitoes used in the testing procedures indicated that a relatively high dosage (0.062 g/m^2) of carbaryl and a comparatively long exposure (one hour) were necessary for high mortalities.

In summary, the tests indicated that the insecticide might have some effectiveness for three or four weeks after application. While the bioassay tests suggested an enhancement of activity and persistence after the second application, this was not confirmed by the other entomologic methods.

Exterior Residual Spraying of Houses: Pilot Study

Small-scale pilot studies were done to determine the effectiveness of DDT and a candidate insecticide, carbamate, OMS-33 (Baygon), when used as residual insecticides on exterior surfaces of dwellings. In two small studies, three houses were sprayed with DDT at 4 grams per square meter; three, with OMS-33 at 2 grams per square meter, and three remained unsprayed as controls. All houses had been included in the regular country program with interior application of DDT at 2 grams per square meter at six month intervals. Two of the houses sprayed with DDT and two with OMS-33 were of the "open" types (such as pole or thatch); the other houses were of a closed type (mud or brick, with tile roofs). Exterior walls and eaves were covered in their entirety, using DDT 75% water dispersible powder stock and OMS-33 as a 70% water dispersible powder diluted to deliver the target dose with a Hudson 10 X-pert hand sprayer with the Teejet nozzle with a fixed pressure regulator.

Entomologic evaluation showed considerable effect of OMS-33 for at least five weeks in the open-type houses and eight weeks in the closed type. DDT showed little or no protection in either at any time after spraying. All night biting rates within the OMS-33 sprayed houses were significantly reduced, but there was little change in the peridomiciliary biting rates. In the open-type houses, the outside biting rate was three times the inside in the controls, four times the inside in the DDT-treated; and 18 times the inside in the OMS-33-treated houses. From weeks 6 to 12, these ratios tended to converge, with ratios of 4, 5, and 7 for control, DDT, and OMS-33, respectively.

Bioassay tests of pole surfaces indicated satisfactory kill through week 10 for OMS-33, but unsatisfactory for DDT even at week 2. Wood surfaces with OMS-33 continued to give 100 percent mortality for 13 weeks, but produced low mortality with DDT even at the 4 gram per square meter dosage from the first week on. Mud surfaces produced satisfactory mortality with OMS-33 through 7 weeks but were unsatisfactory at week 13.

These preliminary studies suggested a marked effectiveness of OMS-33 in preventing inside biting for at least five to six weeks when applied to the exterior of houses and confirmed that similar use of DDT, even at 4 grams per square meter would not reduce biting. The results of the OMS-33 study were encouraging for larger scale trials.

Small Village Scale Trial of Exterior Application of OMS-33 (Baygon)

The area of Lagoon El Jocotal was selected for this trial because of the continuing high level of dry season *A. albimanus* populations present. The area contains two small fishing villages, El Borbollón and La Curruncha, situated along the edge of Lake El Jocotal; the lake is located in the eastern part of El Salvador in a valley to the south of the high Volcano Chaparrastique (San Miguel) and is at an elevation of 25 meters (Figure 7).

The El Borbollón village was selected for treatment while nearby La Curruncha served as a control. Both areas had been previously treated in the routine country program with 2 grams per square meter of DDT. Two cycles of treatment with OMS-33 were done. In March, the village was divided into two parts: one (21 houses) was treated exteriorly with the eaves and upper half of each outside wall receiving insecticide, and the other (55 houses) was treated exteriorly with the eaves and the entire outside walls being sprayed. In the second treatment in May, all houses were treated similarly, i.e., eaves and all outside wall surfaces. During the entire observation period, entomologic and chemical evaluations were conducted separately in the two areas to determine any possible subsequent difference because of the variation in the initial treatment.

Spraying was done with the Hudson 10 X-pert hand pump equipped with the Teejet nozzle with a pressure regulator. Because of the "open" character of many of the houses, the operators had to spray at an angle relative to the walls so that excessive spray would not freely pass the openings and enter the houses. The insecticide was OMS-33, 70% water dispersible powder prepared as a 5% suspension; the spray system was calculated to deliver 2 grams per square meter to the surfaces. Delivery of insecticide to the surface was spot-checked by filter paper sampling (6 samples taken) during the second spraying cycle.

With exterior spraying, there was a minimum of inconvenience to the householders. All persons and animals were required to leave during spraying; and food, clothing, and bed covers were covered or removed. No complaints were received from the inhabitants, and no one reported illness in either spray cycle. One of four spraymen in the first cycle and one of three in the second vomited and required rest. No further problems were reported.

Entomologic evaluation included evening and all night captures (peridomiciliary and intradomiciliary), house resting densities, bioassays of outside wall surfaces, and outside fumigant tests. In addition to chemical assay of filter paper samples for actual deposition of insecticide, air sampling was done in and around test houses to determine the amount and persistence of fumigant activity with this insecticide.

After the first spraying, biting rates determined by all-night (12-hour) captures in and around houses indicated a reduction of intradomiciliary biting for one week in the partially sprayed houses and for three to four weeks in the fully sprayed houses. After the second spraying, these groups showed good reductions for four and three weeks, respectively. Additional short, four-hour captures were done following the first treatment. In general, these captures confirmed the results of the longer 12-hour captures. Resting densities of *A. albimanus* within houses were significantly reduced in treated houses for four weeks after both the first and second treatments.

Bioassays were done on a number of sprayed surfaces, two and six weeks after the first spraying and one week after the second. Except for wood surfaces, tests indicated low mortality by the sixth week after treatment.

Testing for fumigant activity on caged adult mosquitoes showed a general loss of effectiveness during weeks 4 and 5 after treatment.

Studies on Fumigant Activity of OMS-33

During the latter half of the year, numerous field trials were made, both by CAMRS and PAHO personnel in El Salvador and SNEM personnel in Nicaragua, to evaluate the suitability of Baygon (OMS-33) as a tool in malaria eradication. In conjunction with entomologic evaluations, attempts were initiated to establish the concentration of this insecticide in the air following application (fumigant effect) and the subsequent fate of this fumigant activity. The apparatus used for air sampling in these investigations is illustrated in Figure 8. The pump was operated by a 6-volt battery and calibrated to draw air through the glass impinger at a rate of 0.3 cubic feet per minute (equivalent to 8.4 liters per minute). Normal sampling periods were for one hour, and all data relative to these investigations (in the tables) are expressed as micrograms per hour.

In all field experiments, a wettable powder formulation of Baygon was used and was applied by conventional house spraying techniques. The target dose was 2 grams per square meter; however, in each of the three locations investigated, the target surface and/or area was different. For example, in the SNEM program in Nicaragua, all interior walls of the house were sprayed as well as any outside house surface protected from rainfall. In the PAHO (AMRO-216) tests in El Salvador, the chemical was applied as two non-overlapping swaths one meter wide to the interior of the house in the area of the roof-wall junction. The CAMRS tests in El Salvador were made with the insecticide applied to the exterior walls of the house and to the under surface of any projecting roof areas.

All trapping impingers were positioned two feet from the sprayed surface at a height of three to four feet. In Nicaragua (total inside coverage), the apparatus was placed solely within the house. In the El Salvador tests, the impingers were placed both inside and outside of the house, as closely as possible opposite one another on either side of the sprayed wall. Figure 9 depicts a typical position of the apparatus during an outside sampling period. Where possible, houses of different construction materials (wood, thatch, pole) were included in the sampling to evaluate the effect of the surface on the release or retention of the insecticide.

To trap the Baygon and simultaneously convert it to its phenol (o-isopropoxyphenol), 30 ml. of 0.05 N NaOH were placed in the impinger. Preliminary tests were done to show that the insecticide was not being lost once it was introduced into the trapping solution during the sampling period.

Samples collected in the field were transferred to teflon-lined, screw-cap flasks and transported back to the laboratory for analysis. The method of analysis involves acidification of the NaOH solution followed by extraction of the o-isopropoxyphenol with benzene, and subsequent formation of a chlorinated derivative using chloroacetic anhydride. In general, all samples could not be extracted on the same day taken; therefore, all samples would be acidified and left in the laboratory until the following day. No observable loss of chemical could be detected in fortified samples so stored when compared with fortified samples stored in the cold for one day or extracted the same day. An important aspect of this analysis is that the chloroacetic anhydride solution be prepared fresh prior to use.

Electron-capture gas chromatography was used for identification and quantitation. Peak heights of equimolar concentrations of Baygon and o-isopropoxyphenol, extracted and derivatized using the above method, were the same, indicating the procedure to be quantitative. To correct for possible response variations in the gas chromatograph and in possible extraction error, a fortified control was included each time a series of samples were analyzed. Control air samples were taken in each test area one day before the first application of the insecticide; in all instances, the samples were free of compounds which might interfere with the chromatographic analysis.

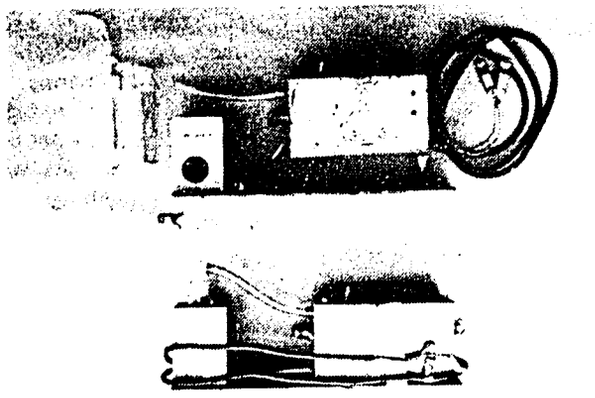


FIGURE 8. Air Sampling Unit Used in Baygon Study. Upper Portion of Photo Illustrates Disassembled Unit. Lower Portion Is Assembled Unit Ready for Field Use

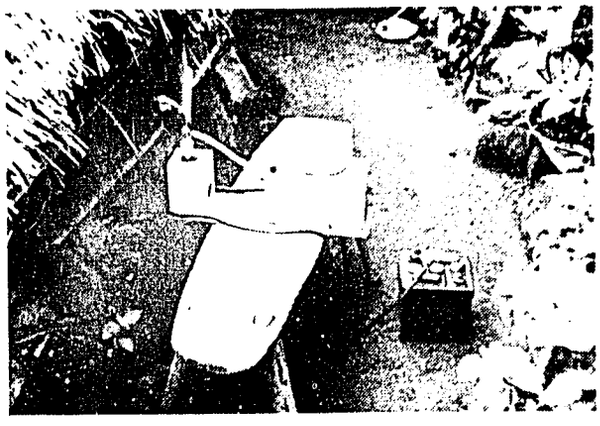


FIGURE 9. Operation of Air Sampler in Typical Field Situation.

Nicaragua Study

The results of samples taken in houses receiving full interior coverage are presented in Table 8. The insecticide was applied during the latter part of the dry season. The average temperature during the sampling period ranged from 34°-37° C. During this period, moderate to gusty desiccating winds were prevalent and no rainfall occurred. In general, this test showed that wood houses have a greater degree of chemical retention than houses of thatch or pole construction. These unpainted, rough, wood walls present a more rigid and porous surface than the loosely constructed, smooth surfaces of the thatch and pole houses, which may at least partially account for the longer fumigant activity. Additionally, when application is to the interior of the house, wood construction affords a greater degree of protection from the possible destructive effects of rain, wind, and sunlight.

However, the table also shows that the greatest degree of fluctuation of concentration occurred in the wood houses. In this type of house, the avenues of air entrance are restricted to the door or doors and windows. Thus, depending on the location of these openings and the wind direction, the air within the house may either be relatively still or highly turbulent. In an open type construction, such as a pole or thatch house, the air would be in constant turbulence regardless of the wind direction, since passage of the air is not restricted to specific openings. Thus, it seems likely that the greater magnitude of concentration variation for the wood house is a function of the air turbulence at the time of sampling.

TABLE 8
Concentration of Baygon in Air Samples
Micrograms per Hour, in Houses* with Full Interior Coverage
Chinandega, Nicaragua (SNEM Program)

Time after Application	Type House Construction				
	Wood	Wood	Thatch	Thatch	Pole
4 Hours	8.9	10.3	3.6	12.6	2.2
1 Day	8.9	10.3	1.8	9.6	4.0
2 Days	5.3	2.4	2.4	3.6	1.4
3 Days	(-)	7.7	3.4	(-)	1.8
6 Days	12.2	1.8	(-)	(-)	1.8
7 Days	5.6	1.8	1.6	(-)	1.2
8 Days	3.6	2.2	1.2	(-)	1.2

* All samples collected from interior of house.
(-) Lost sample due to pump and/or battery failure.

CAMRS Study, El Salvador

Additional evidence, concerning the influence of environmental factors on sampling and retention/liberation of the insecticide from various surfaces was obtained in the evaluation of the CAMRS program involving exterior application. Environmental conditions during this test were somewhat similar to those in Nicaragua with the exception that wind velocity was much reduced and the sampling period extended into the rainy season. In this study, samples were taken both inside and outside of the sprayed houses. On several occasions the concentration of the chemical inside the houses was greater than or equal to that of the outside where the chemical had been applied (Table 9). This finding is best illustrated when the average values of the replicated samples are graphed (Figure 10). The concentrations for the pole houses are quite uniform whereas, again, considerable variation exists for the wood houses. This fact lends support to the previous supposition on the effect of air turbulence at the time of sampling.

TABLE 9
Concentration of Baygon in Air Samples
Micrograms per Hour, in Houses with Exterior Coverage
El Jocotal, El Salvador (CAMRS Program)

Time after	Wood House 1		Wood House 2		Pole House 1		Pole House 2	
Application	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
4 Hours	3.6	7.0	5.6	6.0	6.6	10.7	9.8	13.2
1 Day	1.2	5.8	1.2	7.2	3.6	7.7	2.4	3.6
2 Days	2.5	5.2	(-)	4.0	7.3	6.3	2.4	3.6
5 Days	1.8	1.7	4.3	4.0	2.1	(-)	4.0	3.0
7 Days	1.4	0.1	3.0	1.2	3.0	2.2	1.2	3.0
14 Days	0.4	1.3	0.2	0.6	0.0	0.0	0.0	0.6
20 Days	0.7	1.1	0.6	0.6	0.0	0.5	0.7	1.0
26 Days	0.0	1.1	(-)	0.7	0.0	1.5	0.4	0.2
33 Days	0.6	1.2	1.7	1.3	0.0	0.6	1.3	2.2

(-) No sample due to pump and/or battery failure.

Since the chemical was applied to the exterior of the houses, both wood and pole surfaces were subjected to the same weathering factors. Figure 10 illustrates that under these conditions there is little difference in the retention of the chemical by either material. Between the fourteenth and twentieth day of this investigation, the rainy season began. At this time, a considerable amount of the chemical was being washed from the exposed surfaces, and one would logically expect a sharp decrease in concentration of the chemical to occur. However, a slight increase was detected which persisted throughout the sampling period.

Possibly the increase in humidity resulting from the rains acted as a catalyst, activating that portion of the insecticide not directly subjected to the falling rains, yet possibly reversibly bound to the wall surface.

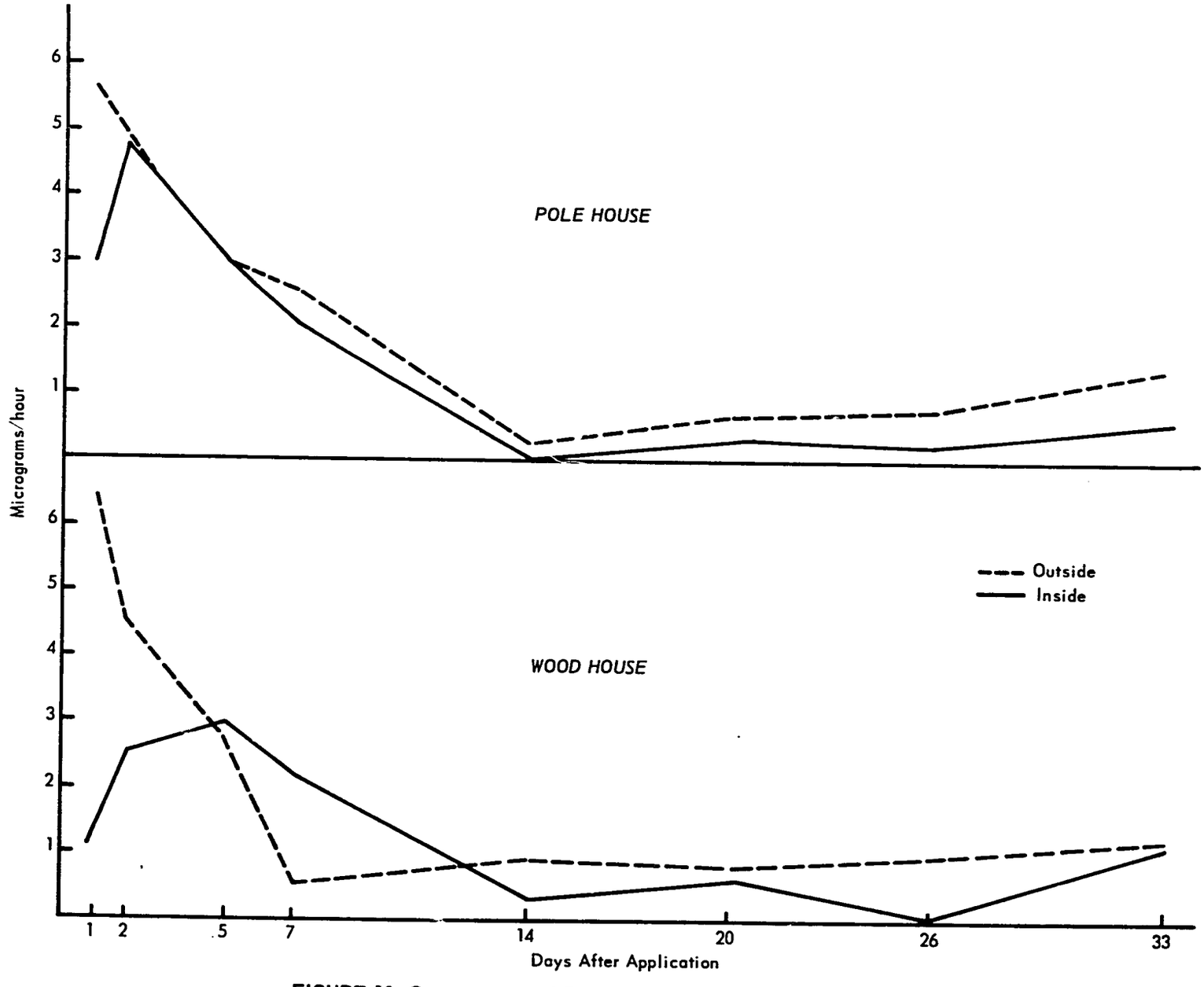


FIGURE 10. Concentration of Baygon in Air Samples Collected Inside and Outside of Houses of Different Type Construction Following an Exterior Application of the Insecticide.

AMRO-0216 Study, El Salvador

The results of a PAHO investigation (Table 10) provided additional support to the theoretical influence of humidity on the release of the insecticide from treated surfaces. This series of tests involved a two-swath application of the chemical to the interior of the house once every 35 days. The duration of this investigation exceeded that of the previous programs. Following the first two applications, environmental conditions were similar to those of the CAMRS program. The second two applications, however, were made during the rainy season.

Following the first application, insecticide concentrations in the air dropped rapidly to zero in all houses after 14 days. Unfortunately, no samples were taken the first day after the second application, but samples collected after seven days closely corresponded to those of the first cycle after the same elapsed period of time. The rainy season began between the seventh and 14th day after the second application; and, as in the CAMRS test, concentration values increased. Following the third application, amounts recovered from within the sprayed houses were the highest of any previous samples, including those taken four hours after application in the SNEM and CAMRS programs. The concentration decreased over the ensuing 28 days, but was still higher than any sample collected from the same houses after the two previous applications. After the fourth application, concentrations again increased; however, the magnitude of the increase was not as great as that after the third application.

Figure 11 represents the average residue values inside houses 1 and 2 for the four applications plotted against time. Because of the absence of samples for the first day following the second application, a theoretical peak range has been plotted. Since rate of application and environmental factors were the same, one could expect an amount at least equal to that found after the first application. The top of the peak is half-way between the values for the first and third applications. This amount could be expected to be present if it is assumed that moisture actively releases bound molecules of the insecticide from the wall surfaces. The triggering mechanism bringing this about could have been the water applied with the second application. This increase would have been short lived since the dry conditions continued. It can be seen that the rate of loss during the third application was not linear, and this may reflect actual changes in humidity; however, no information is available to support this hypothesis.

Apparently, some degree of residual activity is obtained with Baygon, and this activity seems to be dependent upon some level of moisture content in the air. Future field and laboratory investigations are planned under controlled conditions to ascertain the influence that temperature and humidity may have on this compound.

TABLE 10
Concentration of Baygon in Air Samples
Micrograms per Hour
In Houses* with Two-Swath Interior Application at 35-Day Intervals
Las Isletas, El Salvador (PAHO Program)

Applications	Days After	House 1 Inside	House 2 Inside	House 2 Outside	House 3 Outside
1	1	4.8	2.4	4.8	4.8
	7	1.4	0.9	0.0	2.1
	14	0.0	0.0	0.0	0.0
	21	0.0	0.0	0.0	0.0
	28	0.0	0.0	0.0	0.0
2	1	(-)	(-)	(-)	(-)
	7	0.4	1.8	0.1	0.9
	14	1.7	2.2	0.6	0.3
	21	(-)	(-)	(-)	(-)
	28	2.8	1.4	1.2	0.1
3	1	16.8	17.4	8.4	4.8
	7	9.6	15.0	9.8	2.2
	14	7.2	14.4	8.9	1.3
	21	4.8	5.4	1.2	0.9
	28	6.6	(-)	9.0	1.4
4	1	11.8	10.2	18.1	6.0

* All houses of thatch construction.
(-) No sample due to pump and/or battery failure.

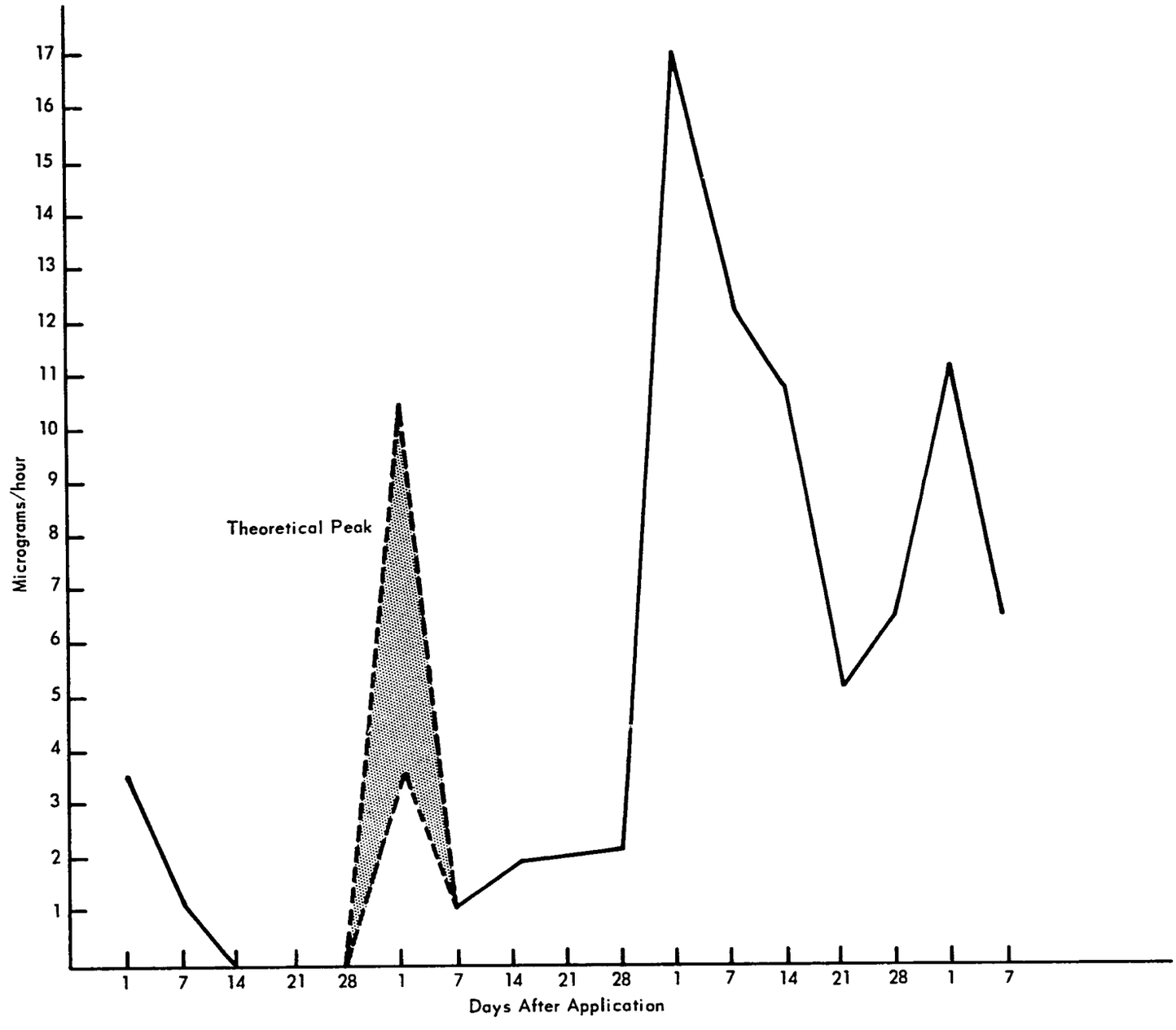


FIGURE 11. Average Concentration of Baygon Inside Houses Receiving a Two-Swath Interior Application Once Every 35 Days – Las Isletas, El Salvador

Malathion Resistance in *A. albimanus*

The finding of significant malathion resistance in *A. albimanus* during field testing of ULV malathion in coastal El Salvador during the previous year has dictated a continuing interest in this problem. An insectary colony of resistant *albimanus* has been maintained, and eggs and larvae provided from time to time to the TDL in Savannah and to several other groups for the establishment of experimental colonies.

CAMRS is collaborating with the University of California at Riverside in a study of the genetics of *A. albimanus* resistance to organo-phosphates. The role of CAMRS is to supply, on a timely basis, insect material from areas of agricultural insecticide exposure and to determine the qualitative and quantitative extent of insecticide use, both currently and historically. Hopefully, genetic experiments being conducted at Riverside will result in information about the rate of development of resistance in the species with the possibility of being able to predict the useful life of a given class of insecticide against the species. This project was inaugurated at the very end of the fiscal year, and further details must await developments.

EPIDEMIOLOGY OF MALARIA

Characterization of Malaria Parasites of Central America

Efforts are being made to characterize malaria parasites of Central America according to their life patterns (including relapse) and their sensitivity to commonly used antimalarial compounds. After locating active infections in various parts of the region during surveys or passive case detection, blood samples are obtained and isolates are inoculated as soon as practicable into one or more *Aotus trivirgatus* monkeys, the common "night" or "owl" monkey of northern South America. A colony of these monkeys is being maintained at CAMRS. Cooperative arrangements have been made with the NIH/NIAID Laboratory at Chamblee, Georgia, for the continuing study of these parasite strains in monkeys and in human volunteers.

During the current year, six strains have been isolated in monkeys and sent to the Chamblee laboratory for further study. These have included three strains of *P. vivax* from El Salvador, one strain of *P. vivax* from Panama, and one strain of *P. falciparum* each from Honduras and El Salvador. One of the Salvadoran *P. vivax* isolates was believed to be mixed with *P. malariae*; unfortunately, however, this isolate was lost soon after its receipt in Chamblee.

One of the El Salvador *P. vivax* isolates has been established in man and its life patterns are being studied. No definitive results are yet available.

The Panama isolate of *P. vivax* has undergone a considerable amount of study, and some patterns of relapse have begun to emerge. In ten volunteers exposed to infection by bites of infected mosquitoes, prepatent periods ranging from 12 to 14 days were observed. First relapses have been observed in three of the ten volunteers to date, and second relapses in two of these three. First relapses occurred 143, 153, and 164 days after treatment of the primary attack with either chloroquine or quinine. Second relapses occurred 55 and 59 days after treatment of the first relapse. These are the first firm data to indicate the probable life pattern of the strains of *P. vivax* common to the Central American region. These kinds of data should be very useful in planning future chemotherapy studies or in planning for effective control of the disease in Central America.

The station plans to collect strains of parasites from the Caribbean Coast of Central America, from inland areas of several of the countries, and from other special situations to determine possible strain differences. An alert is being maintained for the occurrence of chloroquine resistance in falciparum parasites of the region since isolation and confirmation of such strains would be highly important.

Epidemiology of Malaria in Mato Grosso Area of Brazil*

During the year, plans were made for a test of the relative merits of technical grade DDT when applied as a residual spray at 1 and 2 grams per square meter and the p-p' isomer of DDT when applied in an equivalent amount. After preliminary exploration, the Mato Grosso area of Brazil was selected as an appropriate site for this study. This large area had been subjected to only sporadic spraying previously, and locality records seemed to indicate sufficient malaria for good epidemiologic evaluation of the effectiveness of the insecticide. Actually, little definitive information existed on the endemicity of malaria in the area, on the biology (or even the idenity) of the vector, or of the human population characteristics which might be of importance. Therefore, it was necessary for a group from CAMRS to investigate in some detail the epidemiologic factors which would be important in evaluating the test.

The results of these investigations ultimately indicated the unsuitability of the area for the proposed test, but in the course of these investigations much useful information has been collected on the endemicity of malaria in the area, on the factors affecting transmission, and on the methods for appraisal of levels of endemicity.

Four study areas had been selected--two in the Cuiabá sector in the northern part of the state and two in the Campo Grande sector, a considerable distance to the south. Because time and personnel were short, and because this area is almost twice as large as Texas, it was necessary to selectively choose the localities to be examined and also the methodology of the initial malariometric surveys. Multiple approaches were used in limited areas to acquire as much information as possible on malaria endemicity during a single visit. Time did not permit a longitudinal study or even a second visit to the area. Thick films were to be collected to determine the current parasite rates. However, recognizing the limitations of this approach, serological techniques were also used to determine, insofar as is possible with such methods, the levels of prior malaria experience in the population. The two methods available for such serological evaluation are the indirect fluorescent antibody (IFA) test and the indirect hemagglutination (IHA) test. These two tests have been used rather widely in seroepidemiologic studies and have fairly well-known capabilities and limitations in the assessment of malaria experience in individuals and populations. However, the two have not been fully evaluated in simultaneous surveys, and collaboration toward this end was afforded by Dr. Irving Kagan, and his associates, CDC, who provided the IHA testing, and Dr. William E. Collins, NIH/NIAID, who provided the IFA testing. The major known difference between the two tests is in the ability of the IFA test to differentiate among species, while the IHA test is not species-specific. The IHA test is somewhat more automated and large numbers of specimens can be examined in a relatively short time when compared to the IFA test.

Areas for investigation were selected on the basis of locality records of past years, showing malaria cases reported through passive case detection mechanisms. Thus, there was some bias in the selection of areas known to have had malaria cases rather than a random sampling of the population concerned. However, since the aim was to utilize these areas for evaluation of a control program, this bias was acceptable. These pre-selected localities were visited, as were others encountered en route if they appeared to have the necessary attributes of endemicity. Thick blood films were made and whole blood collected in duplicate heparinized capillary (hematocrit) tubes. This measured amount of blood was immediately expressed onto a marked area on a strip of Ropaco #1024-0.38 filter paper, allowed to dry, and stored in tightly closed plastic bags with a hygroscopic material until processed in Atlanta. Blood films were stained within 5 days or less; examination of 100 oil immersion fields was required to verify the negativity of the blood film.

*Report based on preliminary tabulations.

The surveys were done during May 1970. Table 11 presents a brief summary of the size and population densities in the two sectors, Cuiabá and Campo Grande. The population sampled, totally 1,559 persons, was mainly rural, since malaria transmission in urban areas was reported to be very low or nil. Thus, while only 0.6 percent of the total population in the Cuiabá sector was examined, nearly 1.0 percent of the rural population was included. In the Campo Grande sector there is less urbanization and a larger proportion of the population is rural; here again it is estimated that approximately 1.0 percent of the rural population was included.

Most of the people surveyed lived in small communities of scattered homes, usually associated with a stream or small river. Larger villages had schools, but smaller schools could also be found scattered through the more rural study areas. All roads were unpaved except those in the larger towns and much of the population lived along very rough tracks which would become impassable to motor vehicles during periods of heavy rain. Clinics, hospitals, and physicians were available only in the larger towns.

Although a countrywide malaria eradication program had been planned for Brazil by 1958, the areas involved in this study had not been brought under the attack phase by early 1970. Sporadic use of residual insecticides in the region had been in effect for several years, with fairly complete coverage during the second half of 1968 and the first half of 1969. Little or nothing had been done before or since that time (Table 12).

Voluntary collaborator posts had been established and records of blood film examinations through these sources were available from 1966 through 1969. Febriles reporting to the voluntary collaborator were given presumptive treatment. Some impressions, but few conclusions, can be gained from the results of blood film examinations from the municipios investigated for the years 1966-1969 (Table 13). If accepted without qualification, the percentage of positives would appear, in many cases, to be impressive. Yet, no general surveys have been conducted in Mato Grosso. Therefore, information is only available on that part of the population which had febrile illness. A positivity percentage of 28.0 for Cuiabá municipio in 1966 (Table 13) is less formidable when it is considered that the positives are only 0.7 percent of the entire population of this municipio.

The state of Mato Grosso is a very large one, with a total land area of 1,231,549 square kilometers; the population in 1967 was estimated at 1,261,778. Very important, from the point of view of malaria ecology and endemicity, is the north-south length of the state, extending for about 1,200 miles from 7° to 24° south latitude. At least two vastly different ecologies existed in the northern (Cuiabá) and southern (Campo Grande) sectors. Therefore, in discussions of the epidemiology to follow, it is necessary to deal with these two regions separately. The relationship of the areas studied can be seen in Figure 12.

In the Cuiabá sector, the area sampled was within a rough triangle with the base angles at the cities of Cuiabá and Barra do Bugres and with Nortelandia occupying the apex. The northern part of this area is quite definitely of amazonian character, with the more southern areas characterized by arid hilly country.

In the Campo Grande sector, the northern municipios represent more or less an extension of the environment found in the southern Cuiabá sector. This environment gradually becomes more arid and more sparsely populated as one moves to the south. In the southern extremities, the area may even be subjected to an occasional frost--obviously a considerable contrast to the amazonas type of environment around Nortelandia.

TABLE 11
Summary of Size and Population Densities of Municipios in Study Areas
Mato Grosso, Brazil

Municipio	Area Km^2	Total Population	No. Persons Examined
	Cuiabá Sector		
Acorizal	2,123	9,095	22
Alto Paraguaí	2,738	12,513	53
Barra do Bugres	15,117	4,332	275
Cuiabá	12,790	88,666	298
Nortelandia	808	6,250	195
Rosario Oeste	7,870	25,598	62
Varzea Grande	682	16,949	52
	Campo Grande Sector		
Anastacio	5,288	11,219	18
Bonito	5,285	6,416	49
Camapuã	15,000	14,205	92
Coxim	15,783	14,972	106
Miranda	8,795	14,533	202
Pedro Gomes	4,199	7,407	135

TABLE 12
Houses Sprayed by Year and Cycle
Mato Grosso, Brazil

Municipios	1967 Cycle 1	1967 Cycle 2	1968 Cycle 1	1968 Cycle 2	1969 Cycle 1	1969 Cycle 2	No. Houses Existing
Cuiabá Sector*							
Acorizal	-	-	-	1362	1168	-	1476
Alto Paraguaí	19	142	140	1586	1945	-	2731
Cuiabá	-	-	-	688	3619	-	4274
Nortelandia	745	109	535	939	1002	-	1052
Rosario Oeste	-	-	-	2194	1180	-	2799
Varzea Grande	-	-	-	1115	1721	-	1966
Campo Grande Sector							
Anastacio	23	-	-	1115	2045	-	2460
Bonito	-	-	-	234	309	-	1237
Camapuã	-	-	-	598	965	-	965
Coxim	-	-	.	1644	1751	-	1751
Miranda	-	115	860	3127	2006	592	3372
Pedro Gomes	-	-	-	1024	514	-	1024

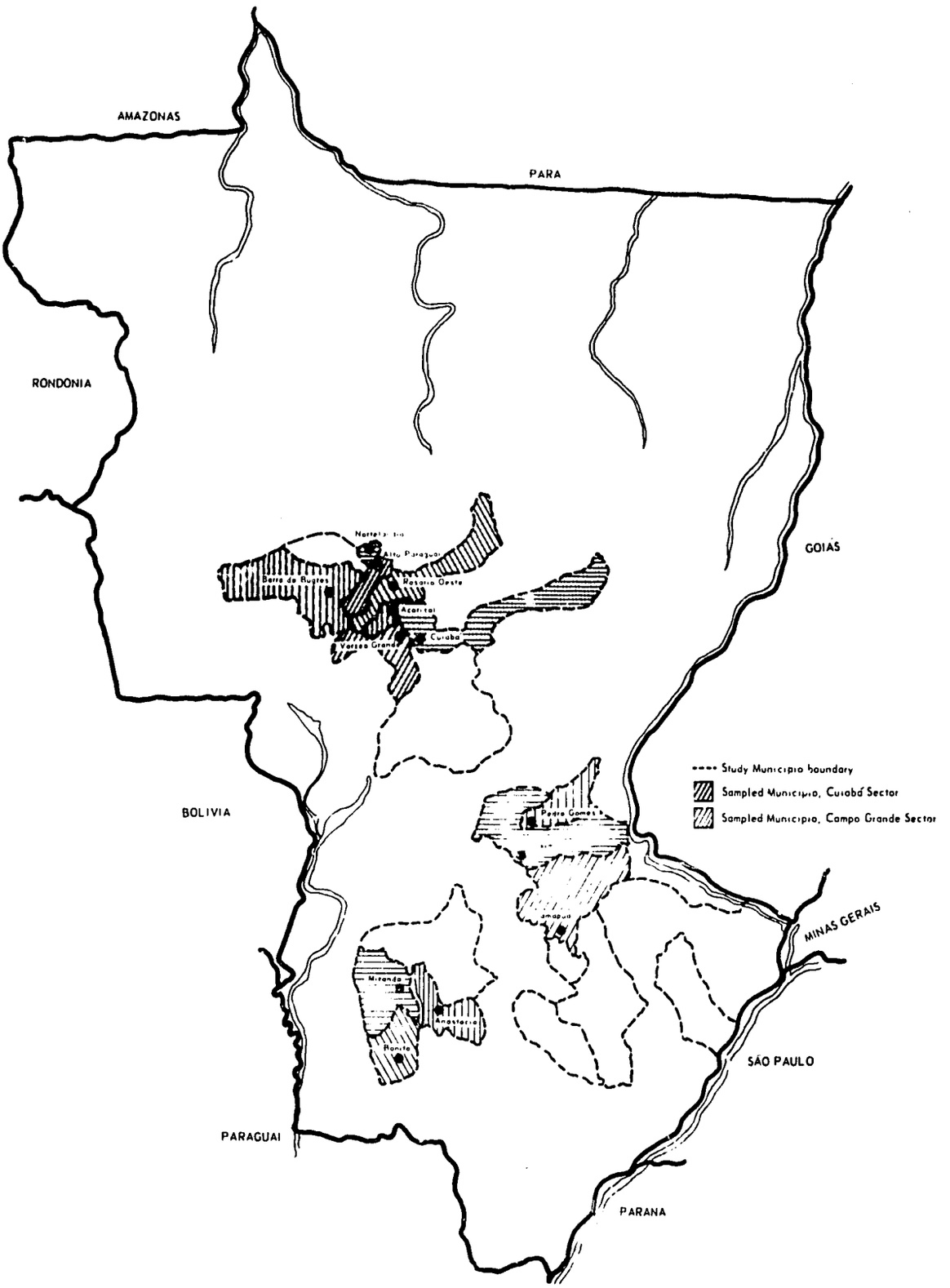
*Figures not available for Barra do Bugres.

TABLE 13
Malaria History in the Proposed Study Area
Mato Grosso, Brazil

Municipio	Population	1966				1967				1968				1969			
		Slides	Positives Total	Positives P.fal.	SPR*	Slides	Positives Total	Positives P.fal.	SPR*	Slides	Positives Total	Positives P.fal.	SPR*	Slides	Positives Total	Positives P.fal.	SPR*
Cuiabá Sector																	
Acorizal	9,095	377	8	4	2.1	561	10	9	1.8	109	5	4	4.6	55	15	6	27.3
Alto Paraguaí	12,513	833	88	70	10.6	936	63	58	6.7	550	61	52	11.1	139	13	8	9.4
Barra do Bugres	4,332	1,369	60	49	4.4	1,665	179	176	10.8	1,837	292	263	15.9	370	64	36	17.3
Cuiabá	88,666	2,242	628	429	28.0	2,482	708	520	28.5	2,695	966	716	35.8	2,871	1,046	705	36.4
Nortelandia	6,250	1,430	261	228	18.3	898	202	170	22.5	1,216	250	196	20.6	423	146	116	34.5
Rosario Oeste	25,598	568	21	18	3.7	619	10	7	1.6	57	7	5	12.3	87	21	12	24.1
Varzea Grande	16,949	290	0	0	-	442	8	8	1.8	126	1	1	0.8	344	40	29	11.6
Total	163,403	7,109	1,066	798	15.0	7,603	1,180	948	15.5	6,590	1,582	1,237	24.0	4,289	1,345	912	31.4
Campo Grande Sector																	
Anastacio	11,219	605	14	0	2.3	733	8	0	1.1	438	4	2	0.9	379	9	2	2.4
Bonito	6,416	180	0	0	-	173	0	0	-	13	1	0	7.7	184	4	0	2.2
Camapuã	14,205	789	22	0	2.8	950	107	1	11.3	205	40	0	19.5	146	12	0	8.2
Coxim	14,972	192	8	2	4.2	1,221	27	5	2.2	12	2	0	16.7	617	18	12	2.9
Miranda	14,533	580	40	6	6.9	756	53	7	7.0	3,454	383	304	11.1	579	103	63	17.8
Pedro Gomes	7,407	362	44	18	12.2	162	16	0	9.9	202	0	0	-	462	60	44	13.0
Total	68,752	2,708	128	26	4.7	3,995	211	13	5.3	4,324	430	306	9.9	2,367	206	121	8.7

*Slide Positivity Rate.

FIGURE 12. Study Municipios, Mato Grosso



The age groups sampled in both areas were somewhat biased to the ages between 6 and 15 because of the inclusion of school populations (Tables 14 and 15). There seemed to be a higher proportion of males sampled in the younger age groups in the Campo Grande sector, but there is no obvious explanation for this distribution.

Cuiabá

Tables 16 and 17 present the malariometric data from this sector by municipio and by age and sex. Only seven positive blood films were found in 957 examinations (0.71%). A clearer picture of the malaria experience in this population may be presented by the serological results. A total of 189 individuals (19.7%) were sero-positive with IFA, the IHA, or both methods. There is a general, but erratic trend toward an age-related percentage of the population with this experience. One would expect a rather regular increase in the positivity of the population with age, in an area of constant endemicity. The erratic increase with age can probably be correlated with the sporadic nature of malaria in the area. The difference in positivity as related to sex was more clear-cut. The increase in malaria experience in the male appeared first in the 16-20-year age group and is probably related to occupational differences, becoming operative at about this same time. The males of this age begin to spend more time in cattle breeding and other occupations that keep them away from village and hacienda for extended periods, often spending nights in the open on cattle ranges. Additionally, as in many cultures, the male here has a greater freedom and frequency of movement in the area, which undoubtedly leads to greater opportunity for exposure.

Campo Grande

Tables 18 and 19 present the parasitological and serological results for the six municipios in this sector. Only five blood films were found to be positive of the 602 examined (0.83%). In this sector, only 48 people (8.0%) gave serological evidence, with either or both of the methods used, of previous experience with malaria parasites. As in the Cuiabá Sector, there was a higher percent of positive response in the males, although the degree of difference was less marked in Campo Grande. Also, the cumulative effect of age on the number of positive serologies was much less apparent here than in Cuiabá. This suggests that the malaria in Campo Grande may be even more sporadic and unstable than it is in Cuiabá.

In both sectors, it was difficult to detect any obvious correlations between the two serologic techniques, or, for that matter, any correlation between the parasite-positive and sero-positive findings. It seems apparent that the IHA method, with 185 positives is more sensitive than the IFA method with 134 positives. Although a total of 237 of the 1,559 people examined, or 15.2 percent, gave serological evidence of malaria experience, only 82 of these specimens were positive with both techniques. The immune response to such a complicated antigenic stimulus as a malaria infection is undoubtedly complex. It would seem possible that the antibodies responsive to the IFA method could be different from those responding to the less specific hemagglutination technique, even though all of the antibodies arose as a result of the same parasite infection. If the differences, on the other hand, are due to false negatives or positives, the percent of the population with previous malaria experience would probably fall somewhere between 8.6 percent (for IFA) and 15.2 percent (total reactors). It would be impossible to say at this point if these deviations represent in part false positives in a highly sensitive IHA test or false negatives in a less sensitive IFA test. If the two techniques are indeed measuring different antibodies, then there would be no direct way to compare them and the 15.2 percent total reactor figure becomes the most probable indicator of malaria experience. The most important factor in the analysis of these serologic responses to malaria is that the interpretation for the whole population examined is essentially the same regardless of what technique is used, considering (1) the relative amounts of past

TABLE 14
Distribution of Population Sampled by Age and Sex
Cuiabá Sector

Age in Years	Total	Percent	Male	Percent	Female	Percent
<5	159	16.6	91	19.1	68	14.1
6-10	224	23.4	106	22.3	118	24.5
11-15	168	17.6	90	19.0	78	16.2
16-20	75	7.8	26	5.5	49	10.2
21-25	68	7.1	37	7.8	31	6.4
26-30	63	6.6	36	7.6	27	5.6
31-35	29	3.0	11	2.3	18	3.7
36-40	55	5.7	22	4.6	33	6.9
41-45	20	2.1	11	2.3	9	1.9
46-50	26	2.7	13	2.7	13	2.7
51-55	20	2.1	8	1.7	12	2.5
56-60	28	2.9	12	2.5	16	3.3
61-65	15	1.6	8	1.7	7	1.5
66-70	3	0.3	2	0.4	1	0.2
71-75	4	0.4	3	0.6	1	0.2
Total	957	-	476	49.7	481	50.3

TABLE 15
Distribution of Population Sampled by Age and Sex
Campo Grande Sector

Age in Years	Total	Percent	Male	Percent	Female	Percent
<5	63	10.5	38	11.4	25	9.3
6-10	245	40.7	139	41.7	106	39.4
11-15	155	25.7	82	24.6	73	27.1
16-20	39	6.5	22	6.6	17	6.3
21-25	16	2.7	7	2.1	9	3.3
26-30	27	4.5	12	3.6	15	5.6
31-35	8	1.3	5	1.5	3	1.1
36-40	15	2.5	7	2.1	8	3.0
41-45	8	1.3	3	0.9	5	1.9
46-50	7	1.2	5	1.5	2	0.7
51-55	5	0.8	2	0.6	3	1.1
56-60	8	1.3	6	1.8	2	0.7
61-65	1	0.2	1	0.3	0	-
66-70	3	0.5	2	0.6	1	0.4
71-75	0	-	0	-	0	-
76-80	2	0.3	2	0.6	0	-
Total	602	-	333	55.3	269	44.7

TABLE 16
Parasitological and Serological Responses for Malaria in Seven Municipios
Cuiabá Sector, Mato Grosso, Brazil

Municipio	Specimens Examined	Slide Positives		Serologic Positives				
		No.	%	IFA	IHA	IFA and IHA	IFA and/or IHA No.	IFA and/or IHA %
Acorizal	22	0	-	0	0	0	0	-
Alto Paraguaí	53	0	-	13	19	6	26	49.1
Barra do Bugres	275	5	1.8	48	56	29	75	27.3
Cuiabá	298	0	-	7	22	5	24	8.1
Nortelandia	195	1	0.5	37	41	24	54	27.7
Rosario Oeste	62	1	1.6	3	4	1	6	9.7
Varzea Grande	52	0	-	2	5	3	4	7.7
Total	957	7	0.7	110	147	68	189	19.7

TABLE 17
Parasitological and Serological Responses for Malaria by Age and Sex
Cuiabá Sector, Mato Grosso, Brazil

Age in Years	Total	Sex	Total	Slide Pos.	IFA Pos.	IHA Pos.	IFA and IHA Pos.	IFA and/or IHA Pos. No.	IFA and/or IHA Pos. %
<5	159	M	91	0	2	5	2	5	5.5
		F	68	1	0	3	0	3	4.4
6-10	224	M	106	0	2	4	0	6	5.7
		F	118	1	7	11	4	14	11.9
11-15	168	M	90	0	2	9	1	10	11.1
		F	78	0	6	8	5	9	11.5
16-20	75	M	26	2	6	5	2	9	34.6
		F	49	0	5	7	4	8	16.3
21-25	68	M	37	1	8	13	7	14	37.8
		F	31	0	3	5	1	7	22.6
26-30	63	M	36	0	9	10	5	14	38.9
		F	27	0	0	2	0	2	7.4
31-40	84	M	33	0	7	11	5	13	39.4
		F	51	1	8	11	6	13	25.5
41-50	46	M	24	1	9	14	8	15	62.5
		F	22	0	8	4	1	11	50.0
50-75	70	M	33	0	18	17	13	22	66.7
		F	37	0	10	8	4	14	37.8
Total	957	M	476	4	63	88	43	108	22.7
		F	481	3	47	59	25	81	16.8

TABLE 18
Parasitological and Serological Responses for Malaria in Six Municipios
Campo Grande Sector, Mato Grosso, Brazil

Municipio	Specimens Examined	Slide Positives		Serologic Positives				
		No.	%	IFA	IHA	IFA and IHA	IFA and/or IHA No.	IFA and/or IHA %
Anastacio	18	0	-	1	3	1	3	16.7
Bonito	49	0	-	2	5	2	5	10.2
Camapuã	92	3	3.3	9	12	6	15	16.3
Coxim	106	1	0.9	2	5	1	6	5.7
Miranda	202	0	-	5	6	1	10	5.0
Pedro Gomes	135	1	0.7	5	7	3	9	6.7
Total	602	5	0.8	24	38	14	48	8.0

TABLE 19
Parasitological and Serological Responses for Malaria by Age and Sex
Campo Grande Sector, Mato Grosso, Brazil

Age in Years	Total	Sex	Total	Slide Pos.	IFA Pos.	IHA Pos.	IFA and IHA Pos.	IFA and/or IHA Pos. No.	IFA and/or IHA Pos. %
<5	63	M	38	0	1	1	0	2	5.3
		F	25	0	0	0	0	0	-
6-10	245	M	139	1	1	5	1	5	3.6
		F	106	1	2	5	0	7	6.6
11-15	155	M	82	1	3	4	1	6	7.3
		F	73	1	1	3	1	3	4.1
16-20	39	M	22	0	4	5	3	6	27.3
		F	17	0	0	1	0	1	5.9
21-25	16	M	7	0	0	0	0	0	-
		F	9	1	1	1	1	1	11.1
26-30	27	M	12	0	0	0	0	0	-
		F	15	0	1	1	1	1	6.7
31-40	23	M	12	0	3	4	2	5	41.7
		F	11	0	1	0	0	1	9.1
41-50	15	M	8	0	2	3	2	3	37.5
		F	7	0	0	0	0	0	-
50-80	19	M	13	0	2	3	1	4	30.8
		F	6	0	2	2	1	3	50.0
Total	602	M	333	2	16	25	10	31	9.3
		F	269	3	8	13	4	17	6.3

malaria compared to current slide positivity rate, (2) the influence of age and sex on exposure to malaria, and (3) the relative levels of endemicity in the areas examined.

Table 20 summarizes the parasitological findings in relation to the associated serology. Parasite densities were relatively high in a large proportion of the P. vivax cases. Negative serologies in such cases are not entirely unexpected. Possible explanations for this phenomenon include a lack of detectable antibody during the early stages of an infection and the possible antibody absorption in the presence of large amounts of antigen, i.e., during periods of high parasitemia.

TABLE 20
Analysis of Parasitologically Positive Specimens
Cuiabá and Campo Grande Sectors

Sector	Sex	Age	Species Diagnosis	Intensity Parasites/cmm.*	Max. IFA Response (Species)	IHA Response
Cuiabá	F	32	P. vivax	8,520	0	0
	F	6	P. vivax	4,210	0	1:16
	F	2	P. vivax P. falciparum**	24,540	0	0
	M	23	P. vivax	1,400	1:80 (f)	1:32
	M	19	P. vivax	60	1:20 (v)	1:1024
	M	50	P. vivax	240	1:640(v)	1:2048
	M	18	P. vivax	11,640	1:80 (v)	0
Campo Grande	F	24	P. vivax	4,320	1:20 (f)	1:128
	F	10	P. vivax	360	1:40 (v)	0
	F	13	P. vivax	60	1:20 (v)	1:256
	F	11	P. vivax	60	0	0
	M	8	P. vivax	60	0	0

*Estimated on the basis of a standard 6,000 white cells/cmm.
**Gametocytes of P. falciparum seen--count reflects both species.

The IFA responses for specific malaria antigens in the two sectors are summarized in Table 21. It can readily be seen that the largest percentage of maximum endpoints was for P. falciparum in the Cuiabá sector, but that this was not the case in Campo Grande, where P. vivax predominated in maximum responses. It should be noted that the greater than 100 percent total for maximum responses reflects a certain amount of sharing of the maximum response between two antigens. This distribution of maximum titers in the area seems to be confirmed and supported by the blood film results reported from these two sectors during the past four years (Table 13). In the Cuiabá sector, 75.5 percent of the blood film positives were diagnosed as P. falciparum; 80.9 percent of the present samples responded highest to the P. falciparum antigen. In the Campo Grande sector, 52.2 percent of the positive blood films reported from 1966 to 1969 were diagnosed as P. vivax; in this sector, 66.7 percent of the IFA tests showed highest titers to this antigen. In the latter case, the percentage of slides positive for P. vivax would have been somewhat higher had not an obvious P. falciparum epidemic occurred in 1968. However, in any case, the slide positivity during this period correlated well with the fluorescent antibody titers from the same sectors.

TABLE 21
Summary of IFA Responses for Three Human Malaria Antigens
Cuiabá and Campo Grande Sectors

Sector	Antigen	Number of IFA Endpoints ≥1:20	Maximum IFA Endpoints (≥1:20)	
			No.	Percent
Cuiabá	P. falciparum	90	89	80.9
	P. vivax	51	20	18.2
	P. malariae	15	2	1.8
Campo Grande	P. falciparum	12	10	41.7
	P. vivax	15	16	66.7
	P. malariae	4	2	8.3

Summary

The study in Mato Grosso yielded some valuable findings, not only in development of epidemiologic information on that area, but also on the methodology of undertaking such a study. Active cases of malaria were limited in the study areas and P. vivax was the dominant parasite found. When the serologic and parasitologic results in the Cuiabá Sector are evaluated, certain conclusions relative to the epidemiology of malaria in the area become reasonable. Endemicity and immune levels are low, as evidenced by the few positive serologic responses and the fact that most of the active cases were relatively intense. Transmission is sporadic but occurs with sufficient frequency to produce an erratic but gradual increase in positive responses according to age. The dominance of P. falciparum maximum IFA endpoint titers is explained by the constant movement into this area of individuals from highly malarious sectors to the north where this parasite is common. Many of the relatively large numbers of P. falciparum cases from Cuiabá Municipio are undoubtedly imported in this fashion (Table 13). This situation is further augmented by the movement of the people within the study area, which would serve to involve several localities once a carrier had initiated an outbreak in one family group, hacienda, or pueblo. Vectors are probably present, at least in limited numbers, through much of the year for new cases have been reported in every month. However, the rainy season undoubtedly produces an increase in the level of transmission.

The situation is somewhat different in the Campo Grande Sector. The dominant parasite species here is P. vivax, serologically as well as parasitologically. The serological responses indicate that the malaria in this area is even more sporadic and localized than in the Cuiabá Sector. Much of the Campo Grande Sector is further removed, both geographically and in terms of transportation, from highly endemic areas with heavy P. falciparum concentrations. Therefore, it has less malaria but a higher proportion of the more persistent P. vivax. Outbreaks of P. falciparum do occur, as can be seen in Miranda in 1968 (Table 13). However, this outbreak was apparently localized since the present survey failed to sample the appropriate villages and there was no serological evidence of the documented surge in P. falciparum cases in 1968.

The results of the present studies indicate that malaria in the sampled sections of the Mato Grosso is sporadic and confined to localized outbreaks. The frequency of the outbreaks is probably dependent upon a number of things; undoubtedly, the introduction of carriers at appropriate times is tremendously important. The Cuiabá Sector is closer to reservoir areas, and the few roads available tend to funnel in potential parasite infective sources. This factor combined with lesser seasonal extremes in temperature and apparently higher rainfall causes the outbreaks to be more frequent in Cuiabá than in Campo Grande.

In the Campo Grande Sector, the situation is essentially similar in most respects, with the malaria being somewhat more sporadic and more unstable. Here, the annual rainfall is less, the winter temperatures drop to near frost levels, and the rate of introduction of malaria carriers is lower. However, adequate vectors are present and outbreaks can be quite intense even if localized.

The techniques used in this study have tremendous potential in assessing the endemic level of malaria in an unknown area, in augmenting malaria information in an area where accumulated data is incomplete, and in helping to evaluate on-going antimalaria programs. Some means by which the malaria situation in an area can be understood without having to examine tens of thousands of blood films over a period of two to three years is essential. The application of a combination of serological and parasitological techniques such as described here, when administered by individuals with field malaria experience, may answer this need.

Drug Studies

Studies on the collective use of antimalarials as a method for malaria eradication or control were continued through the reporting year. Most of these studies represented a continuation or a modification of those initiated during the previous year. The background and preliminary results are contained in last year's report. By the end of FY 1970, several of the studies had been completed and a single study was continuing. Because the theoretical effectiveness of the compounds under study is well documented, our primary interest has been in the acceptance of the drugs by the population, obviously the controlling factor in the success or failure of such a program. Discussion of population attitudes as controlling factors in the acceptance of chemotherapeutic and other antimalaria measures are discussed further under Health Education.

Pyrimethamine-Primaquine Combination

During the calendar year 1969, a study was undertaken to evaluate the combination of pyrimethamine-primaquine. This study was planned with a two-fold purpose:

1. To evaluate the complaint patterns in the population receiving this combination.

2. To evaluate the effectiveness of this combination, especially in regard to *Plasmodium vivax*.

In both aspects of the study, a comparison was made with the routinely used amodiaquine-primaquine combination.

Two areas approximately 15 kilometers apart were selected with the cooperation of the National Anti-Malaria Campaign of El Salvador (CNAP). These areas, CNAP Zone 2, Districts 8 and 13 (Figure 13), had a long history of endemic malaria. Both districts are located on the coastal plain with the elevation ranging from sea level to approximately 100 meters. District 13 extends to the Pacific coast while District 8 stops approximately one to two kilometers short of the coastline. Each area has a registered population of approximately 5,500 persons. The primary industry is cotton farming and ranching.

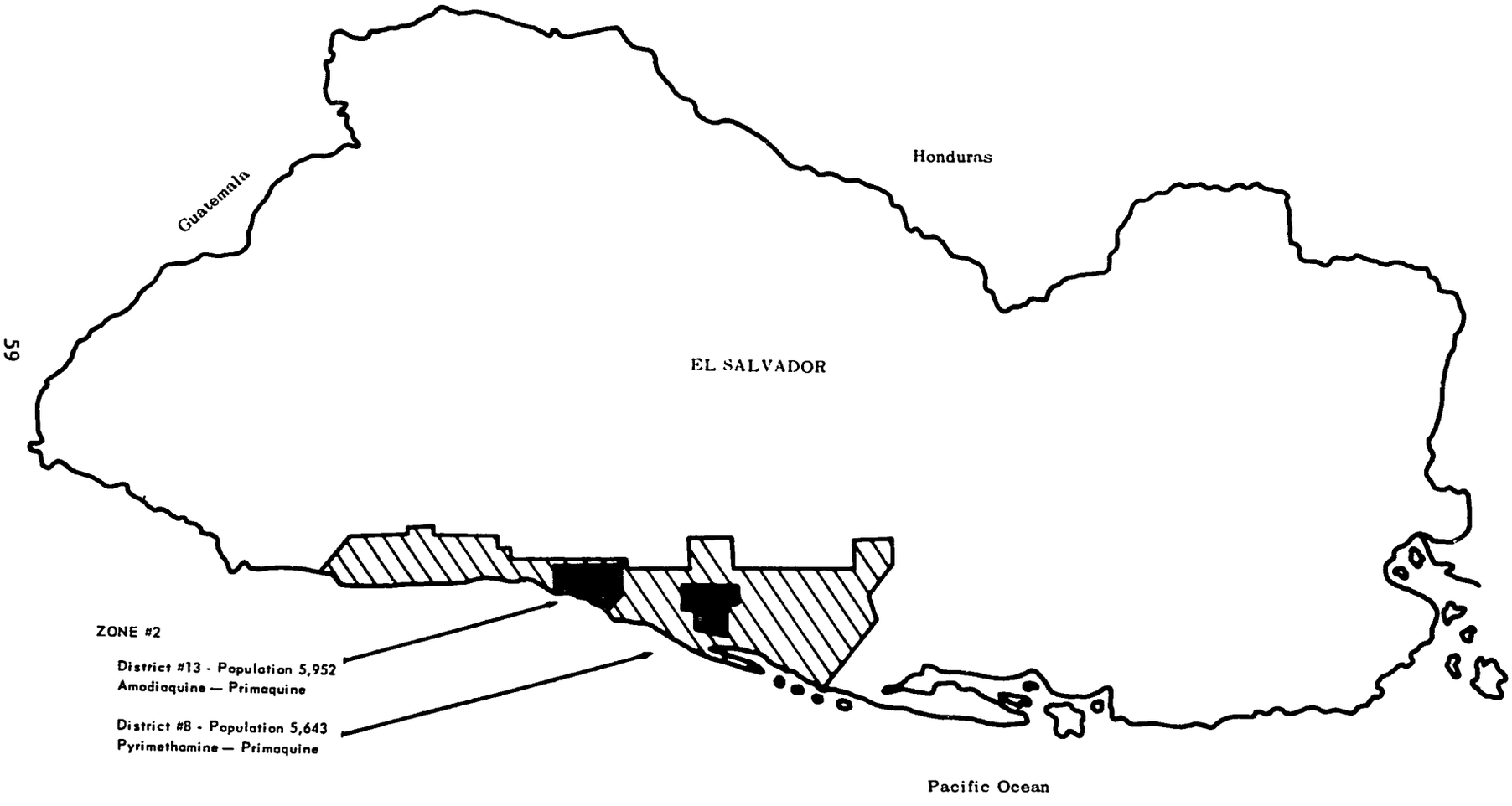
Meteorologic data for this general area were obtained from the Servicio Meteorologico Nacional. The average annual rainfall for the past 41 years is 1,745 mm., with a maximum average of 351 mm. in September and a minimum average of 1 mm. in January and February. There is a distinct wet and dry season with more than 95 percent of the precipitation falling during the months of April through October. For the past 19 years, the average temperature was 26.7° C with a high monthly average of 28.1° C for April and a low of 25.9° for December.

DDT has been used in these areas for many years. A review of CNAP records from 1966 confirms that all houses have been sprayed twice yearly with two grams of DDT per square meter of surface area. In addition, collective drug treatment has been used intermittently, and an established passive case detection system has been operational for many years.

In September 1967, a complete census was conducted in both districts; then collective drug distribution (amodiaquine-primaquine) was initiated and continued, until the beginning of this study, at fortnightly intervals except for an interruption from January until the third week of March 1968.

In the present study, the population of District 8 was treated with pyrimethamine-primaquine. Adults' tablets contained 37.5 mg. pyrimethamine base and 25.0 mg.

FIGURE 13. Drug Study Areas, El Salvador



primaquine base and children's tablets contained 18.75 and 12.5 mg., respectively. The dosage schedule was as follows:

Pyrimethamine-Primaquine Dosages in Mg. Base

Drug	Weight in Pounds			
	20*	20-49	50-99	100
Pyrimethamine	9.375	18.75	37.5	75.0
Primaquine	6.25	12.5	25.0	50.0

*Children under 6 months not treated.

The standard CNAP combination, amodiaquine-primaquine, used in District 13 was supplied in adult tablets containing 150 mg. amodiaquine base and 15 mg. primaquine base and in children's tablets with 75 mg. chloroquine base and 7.5 mg. primaquine base. The dosage schedule for this drug was the following:

Amodiaquine-Primaquine Dosages in Mg. Base

Drug	Age in Years				
	1*	1-2	3-6	7-12	13
Amodiaquine	75**	150	225	300	450
Primaquine	7.5	15	22.5	30	45

*Children under 6 months not treated.
**Chloroquine.

Infants younger than six months of age were not administered drugs unless febrile; then they received one-half of the lowest dose indicated in the preceding tables.

Initially, a complete registration of the population was made in the two study areas. Beginning on January 6, 1969, the drugs were administered fortnightly to all the people who could be contacted for a period of twenty-four distribution cycles. The drugs were distributed by personnel employed by the National Anti-Malaria Campaign (CNAP). In addition, all voluntary collaborators involved in passive case detection and presumptive treatment of fever cases were supplied with the type of drug used in their respective districts.

Surveys were begun in District 8 in February 1969 and in District 13 the following month. These were conducted on every fourth cycle of drug distribution for a total of five surveys. Each survey required approximately two weeks to complete; the last one was completed in November. Each survey was made by personnel from CAMRS, who accompanied the drug distributors on their normal rounds. Thick and thin blood films were made on all people encountered who would submit to a blood examination. Survey films were examined for malaria parasites by CAMRS personnel, while all those taken by voluntary collaborat 's and drug distributors were read by CNAP personnel. On the first survey, all persons, and subsequently all new residents, were interviewed and the following information was recorded: age, sex, educational level, past history of malaria, and the number of times they had ever visited a voluntary collaborator. In addition on each survey visit, spontaneous complaints were recorded from each person accepting drugs. Those refusing drugs were questioned only a

single time as to their reasons for refusal. Recorded complaints were categorized into five major groups: gastrointestinal, neurological, psychological, allergic, and others. In the study period, acceptance of drugs varied from 48 to 67 percent in District 8 and from 56 to 74 percent in District 13 (Figure 14). Based on the average population for the year, the overall acceptance was 60.1 percent for District 8 and 63.6 percent for District 13. Percent refusal was 35.9 for District 8 and 33.6 for District 13. Percent absent was 4.0 and 2.8, respectively. In both districts, gastrointestinal complaints were the most prevalent (Tables 22 and 23). The number of complaints by survey and category varied considerably during the five surveys; but in general, the largest number of complaints was reported in the first survey. The only exception to this was in the category "psychological" in District 8; this category increased from 70 for the first survey to 221 for the last one. The total complaining for all categories represented 20.5 percent of the population interviewed for District 8 and 23.1 percent for District 13. Multiple complaints were not uncommon in the population contacted, with 3.0 percent of those contacted in District 8 and 4.5 percent of those in District 13 having two, three, or four complaints.

TABLE 22
Frequency of Complaints by Category in 5 Surveys
District 8

Survey No.	1	2	3	4	5	
Survey Dates 1969	Feb 17-28	Apr 21-May 2	June 16-27	Aug 4-15	Sep 29-Oct 10	Total
Persons Contacted	3,201	3,509	3,535	3,498	3,481	17,224
Persons Complaining	986	792	612	618	519	3,527
% Complaining	30.8	22.6	17.3	17.7	14.9	20.5
Complaints:						
Gastrointestinal	634	430	335	254	211	1,864
Neurological	459	278	205	211	119	1,272
Psychological	70	174	144	194	221	803
Allergic	24	24	8	8	8	72
Others	23	10	24	6	5	68
All	1,210	916	716	673	564	4,079

FIGURE 14. Acceptance Levels for District 8 (Pyrimethamine-Primaquine) and District 13 (Amodiaquine-Primaquine) – 1969

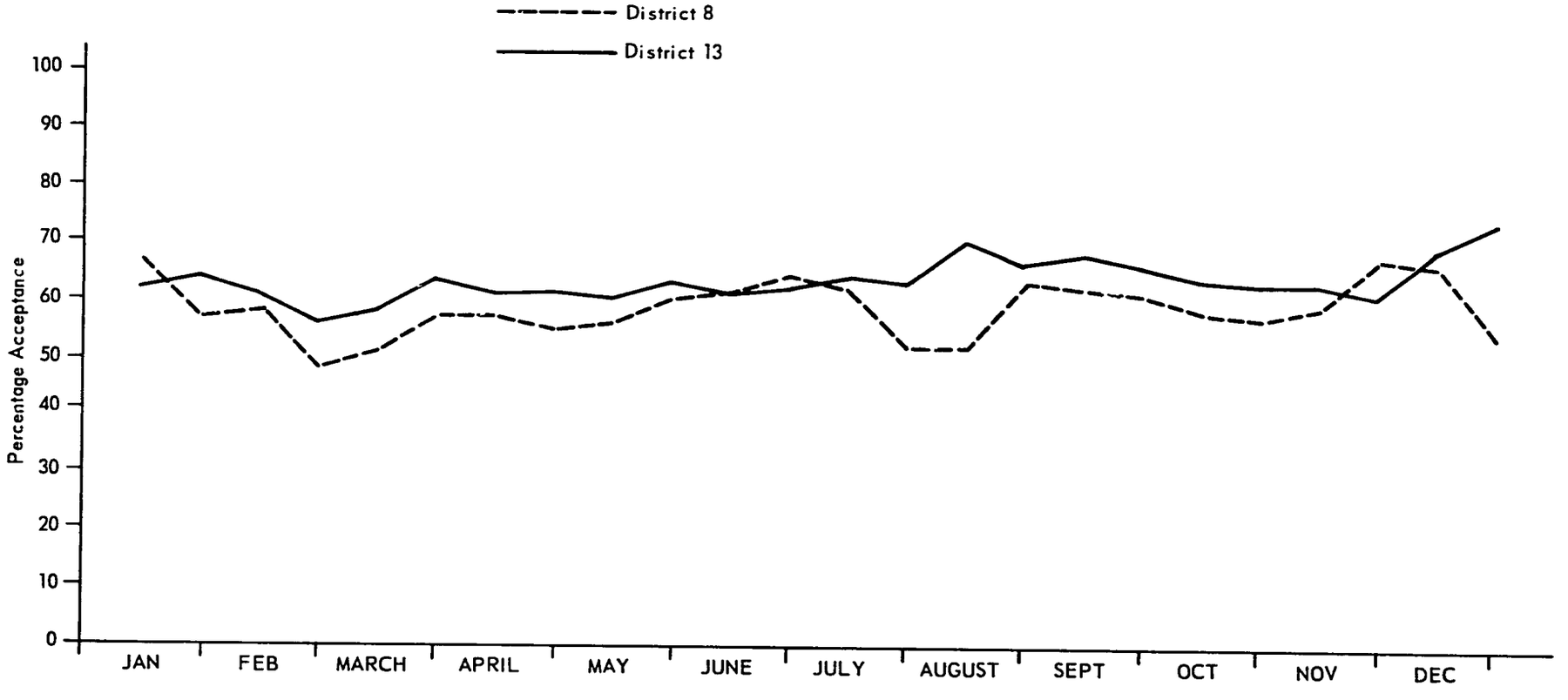


TABLE 23
Frequency of Complaints by Category in 5 Surveys
District 13

Survey No	1	2	3	4	5	
Survey Dates 1969	Mar 17-28	May 19-30	July 14-25	Sep 1-15	Oct 29-Nov 7	Total
Persons Contacted	3,555	3,987	3,511	4,040	3,471	18,564
Persons Complaining	1,323	679	691	912	677	4,282
% Complaining	37.2	17.0	19.7	22.6	19.5	23.1
Complaints:						
Gastrointestinal	738	326	297	431	365	2,157
Neurological	708	219	173	247	240	1,587
Psychological	269	158	229	327	227	1,210
Allergic	61	28	25	26	26	166
Others	34	17	56	29	13	149
All	1,810	748	780	1,060	871	5,269

Complaints were also classified more specifically under subjective headings (vomiting, dizziness, headache, abdominal pain, anorexia, and diarrhea) to determine possible differences in the two drug regimens. However, the complaints by "category" and the "specific complaints" were closely similar in the two districts. In each of the complaint groupings, District 8 had fewer than District 13, but the difference was never large. While the general category of "gastrointestinal complaints" was the most prevalent, this may not represent a true causal relationship with the taking of drugs. In the area under study, the continual high incidence of gastroenteritis, with its multiple etiologies, may reduce the reliability of any such observations.

During the year, a total of 644 cases of malaria were reported from the two study areas. Of 123 cases reported in District 8, 33 were P. falciparum and 90, P. vivax. District 13 reported 521 cases; 228 were P. falciparum and 293, P. vivax. The slide positivity rate for District 8 was 0.8 percent and for District 13, 2.4 percent. All slides--survey, voluntary collaborator, and drug distributor--were included in calculation of the SPR; therefore no definite conclusions can be drawn.

The SPR's for the last two years show a reduced malaria incidence in both districts (District 13: 9.2% in 1968 and 2.4% in 1969; District 8: 9.1% to 0.8%). The many factors which can be responsible for changes in malaria incidence make definite conclusion impossible. The slightly greater drop in incidence in the pyrimethamine-primaquine area may indicate greater efficacy of the drug combination in a mass administration program, or it may be a result of any of several other epidemiologic variables.

For 1970, a continuation of the study on the pyrimethamine-primaquine combination with the exchange of the study drug and the control drug in the two areas was planned, but District 8 was assigned for an insecticide study. District 13 was placed under

pyrimethamine-primaquine in April, after approximately three months with no drug distribution. There was a marked increase in malaria cases during the first three months of the year (Table 24), but within two weeks after the first cycle of drug distribution, there was a reduction in the number of cases. The acceptance level in this district is slightly higher than during the previous year when amodiaquine-primaquine was in use (Table 25); but with the completion of only seven cycles of drug distribution, no conclusions can be reached.

TABLE 24
Number of Cases of Malaria by Species
District 13, January-June 1970

Date (1970)	falciparum	vivax	Total
January	-	12	12
February	2	39	41
March	2	50	52
*April 1-11	-	37	37
April 12-30	-	13	13
May	-	14	14
June	-	7	7
Total	4	172	176

*1st drug distribution.

TABLE 25
Percent Acceptance by Drug Distribution Cycles
District 13, 1970

Cycle	Number Registered	Percentage		
		Acceptance	Refusal	Absent
30 Mar - 11 April	5,230	65.8	32.7	1.5
12 Apr - 25 April	5,228	62.3	34.6	3.1
26 Apr - 9 May	5,241	63.1	36.0	0.9
10 May - 23 May	5,289	68.5	30.6	0.9
24 May - 6 June	5,768	64.4	34.5	1.1
7 June - 20 June	5,752	65.7	33.3	1.0
21 June - 4 July	5,829	67.4	31.1	1.5
Total	-	65.4	32.5	2.1

Amodiaquine (OH)-Primaquine Combination

This project was first discussed in 1968 when it was learned that the substitution in amodiaquine of a hydroxyl group for the chloride radical at seven (7) position on the quinoline ring might produce a reduction in the percentage of complaints about the drug combination as currently administered in the country program. The difference was theoretically because of the possible lesser gastric irritant activity of the OH formulation. Protocols were originally submitted for a double blind study using the new combination tablet amodiaquine (OH), 450 mg. base, + primaquine, 45 mg. base, and the current country medication, amodiaquine (CL), 450 mg. base, + primaquine, 45 mg. base. In addition, placebos were to be inserted every sixth cycle of drug distribution to better evaluate complaints from those receiving drugs. All tablets were to be identical in appearance.

The study area was located some 150 to 170 kilometers from San Salvador at the eastern end of the country. Three districts consisting of 18 sectors were equally and randomly divided between the two drugs. During a complete census of the districts in early May 1969, a total of 3,970 houses were visited and 17,663 persons registered. Treatment was given to 503 persons with a current or recent history of fever. Of 485 slides taken, four were positive for P. vivax.

A delay of some six months in receiving the proper amodiaquine (CL) and placebo tablets resulted in altering the protocol as follows:

1. Amodiaquine (OH)-primaquine was distributed throughout the entire study area for the first six months followed by the amodiaquine (CL)-primaquine combination for the remaining six months.

2. No placebos were distributed. Drugs were distributed at fortnightly intervals beginning on May 19, 1969.

After only two cycles of drug distribution, the acceptance level of the drug fell drastically. At this time, hostilities broke out between El Salvador and Honduras. The study area was near the border where much of the strife occurred. During this period the acceptance level continued to decrease, falling below 50 percent by the middle of August 1969; and, with the exception of one instance the following year, acceptance never again exceeded 50 percent. There appeared to be little difference in acceptance between the two combinations used (Tables 26 and 27). The impossibility of mounting a significant drug study in an area where, for whatever reason, acceptance decreases to this point is obvious.

TABLE 26
Acceptance Levels of Amodiaquine (OH)-Primaquine
1969

Cycle	Percentage		
	Acceptance	Refusal	Absent
19 May - 31 May	74.7	20.3	5.0
2 June - 14 June	70.5	25.3	4.2
16 June - 28 June	59.0	38.9	2.1
30 June - 12 July	55.0	42.6	2.4
14 July - 24 July	53.0	44.8	2.2
Vacation			
4 Aug - 16 Aug	51.7	46.4	1.9
18 Aug - 29 Aug	47.3	50.6	2.1
1 Sept - 13 Sept	43.9	54.3	1.8
16 Sept - 27 Sept	43.7	55.2	1.1
29 Sept - 11 Oct	44.8	54.3	0.9
13 Oct - 25 Oct	46.1	51.9	2.0
27 Oct - 8 Nov	44.6	53.6	1.8
10 Nov - 22 Nov	46.1	52.8	1.1
24 Nov - 6 Dec	46.7	51.6	1.7
8 Dec - 17 Dec	45.9	52.4	1.7

TABLE 27
Acceptance Levels of Amodiaquine (CL)-Primaquine
1970

Cycle	Percentage		
	Acceptance	Refusal	Absent
17 Jan - 24 Jan	44.6	52.2	3.2
26 Jan - 7 Feb	44.1	53.4	2.5
9 Feb - 21 Feb	48.9	49.8	1.3
23 Feb - 5 Mar	50.1	47.2	2.7
9 Mar - 19 Mar	49.2	49.1	1.7
30 Mar - 11 Apr	48.7	50.0	1.3
13 Apr - 25 Apr	49.9	47.7	2.4
27 Apr - 9 May	49.1	48.9	2.0
11 May - 23 May	48.9	49.0	2.1

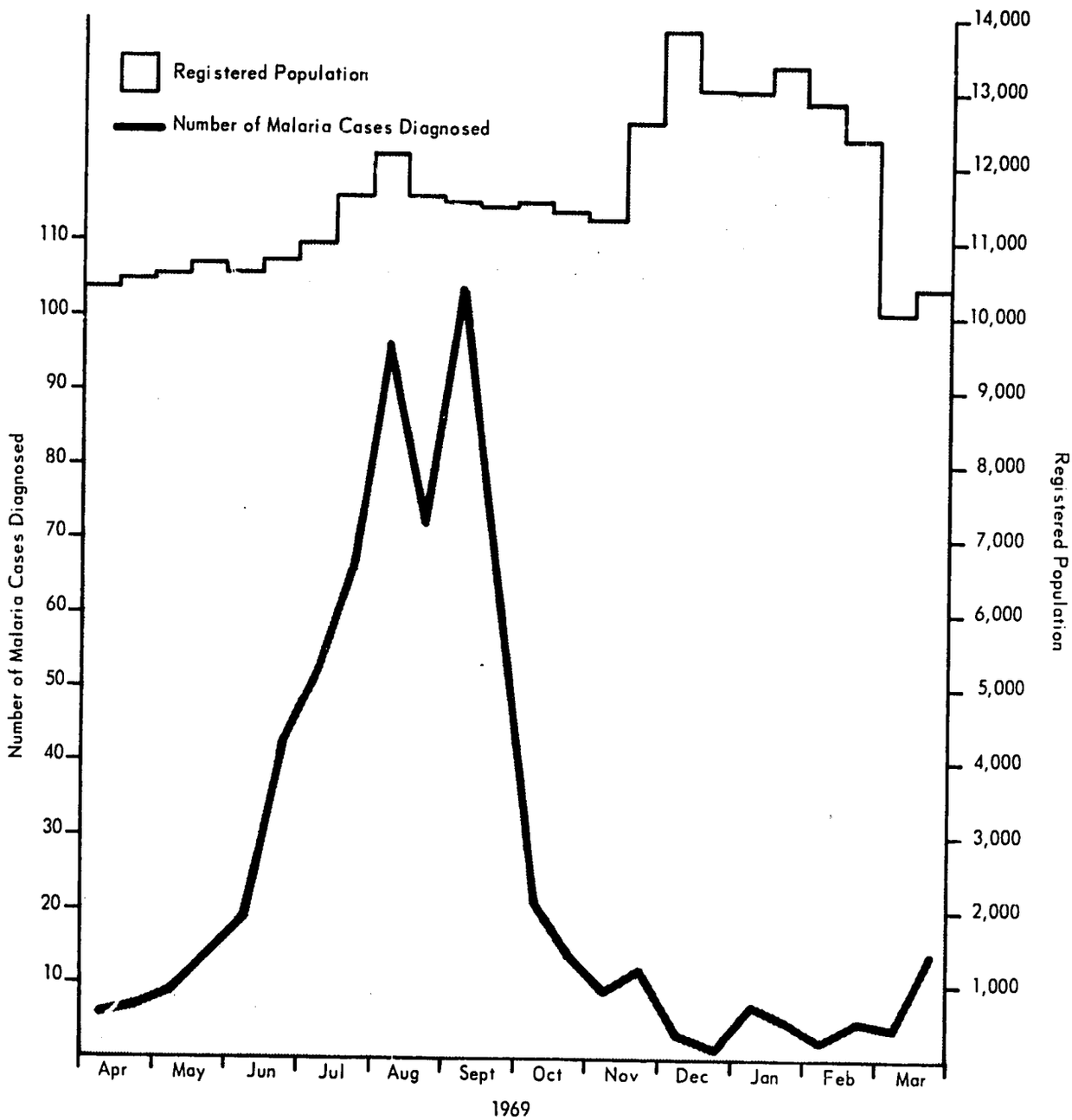
Epidemiologic Factors Affecting Transmission

During the course of drug studies, several factors which may affect the transmission of malaria have been studied. As expected, the occurrence of malaria in coastal areas used for drug studies is related to the rainy season, which extends from April to October. The largest single industry in the region is cotton farming, which is subject to seasonal influx of farm labor. Data suggest that the incidence (Figure 15) of malaria is not closely related to this seasonal influx, as has been postulated. There are two labor migrations, one in the summer months when planting occurs and one of greater magnitude in late fall and winter for harvesting. While there seems to be some relationship between the increase of malaria with the first migration, there is none with the latter, suggesting that the malaria increase is more likely related to seasons than to influx of labor.

Another factor which often affects the transmission of malaria in cotton-growing areas is the widespread application of agricultural insecticide which takes place from July to February. The greatest amounts of insecticides are applied during the months of August, September, and October. It has long been felt that this application of insecticides has a significant effect on the anopheline populations and the malaria transmission in these regions.

Consideration of these multiple factors is a reminder that assessment of the malaria situation is never simple. Any definitive results must always be viewed with the reservations imposed by the many factors not primary to the investigation.

FIGURE 15. Population Registered and Malaria Cases Diagnosed by Fortnightly Cycle Districts 8 and 13, 1969



HEALTH EDUCATION ACTIVITIES

La Libertad Study

Origin and Purpose

The study, which was carried out under the auspices of the national malaria program just before introduction of mass drug distribution early in 1969, originated with a household survey of experience and beliefs associated with malaria. Junior high school students from La Libertad served as interviewers. The national program used survey findings to orient the content of educational materials and activities. Subsequently, completed interview forms were made available to CAMRS for use in the present study, which was designed to test the following hypotheses:

That drug acceptance level would be higher among--

students who served as interviewers and their families than among comparable students not involved in such an activity and their families.

households that were interviewed than among non-survey households.

households in which the head of household consistently accepted medication than in cases where the head of household consistently refused.

households of survey respondents who stated that they believed malaria to be the most dangerous disease than among those in which some other disease was named.

households of survey respondents who stated that they believed prophylactic drugs to be the most important way to eliminate malaria than among households where some other measure was listed as most important.

households in which the respondent reported a personal and/or family history of malaria than among households where no malaria history was reported by the respondent.

Procedures

Originally, the study was to be based on results from the first twelve fortnightly medication cycles beginning March 22, 1969, and ending September 19, 1969. The national program had scheduled a total of nineteen cycles for the calendar year. The study design called for a probability sample of 20 percent (146) of the 731 survey households and a like percentage (20.0%) of the 1,011 non-survey households in the same community. Students who served as interviewers were to visit sample households to copy medication records. A side study to examine the impact of these students' visits on acceptance during the remaining seven cycles was contemplated.

The only source of data on individual and family response to mass drug distribution was the household medication record (TC-1), a standard form used by the national program. This form, placed in homes by those who administer antimalaria drugs on a house-to-house basis, lists occupants by name, sex, and age and provides for recording by cycle the data for individuals on acceptance, refusal, or absence. The form also identifies the head of household and carries the DDT house number. Medicator reports, from which zone and national offices routinely compile statistical summaries, consist of a head count by cycle of individuals present and absent at the time of the medicator's fortnightly visit, with acceptance or refusal of medication

indicated for the former. To the home visit count is added that of groups offered medication at school or place of work. Rarely were the latter data transferred to the household record. Since TC-1's are single copies, their loss or destruction, which can occur at any point in the program, means that the data recorded is gone forever except as an unidentified contribution to a statistical summary. If such loss occurs, a new form is placed in the household to pick up where the previous one left off. Even if forms survive until all blanks are filled and are replaced by new forms, generally they are not collected or preserved. For the operating program, the system appears to be reasonably adequate since it is supplemented by special records on case detection and treatment.

The same form is used for recording household visits when an area is under "strict observation" which entails fortnightly visits to take blood smears on persons who report a recent history of fever. Although strict observation is not carried on simultaneously with mass drug distribution, the same copy of the form may be used to record data for both activities during different periods. Consequently, TC-1's may contain data for a series of strict observation cycles followed and/or preceded by medication records. This was the case, in many instances, in La Libertad.

Since the only way to get medication data on sample households was to visit these and copy the information from TC-1 forms, arranging for students to perform this task had a practical aspect as well as a possible value in terms of influencing drug acceptance.

Because of problems which arose with respect to the identification of households, however, the original plan for selecting the sample and for student participation in data collection had to be abandoned. Another unexpected development was the sudden suspension, after the fifteenth cycle, of drug distribution to be replaced by strict observation.

Those TC-1 forms which still had blanks for several cycles were used for recording strict observation data. The only course open for recovering household data was for medicators to collect those household records which were to be replaced by new forms. Ultimately, 571 household records were recovered, as were medication rosters for elementary schools and for the junior high school. A roster for only one employee group was recovered--that of workers at Customs facilities. When positive identification of individuals listed in these rosters could be made, CAMRS staff transferred their medication record to the appropriate household form. The household records netted data for 3,200 individuals (Table 28).

TABLE 28
Study Sample

	Households			Individuals	
		Sampled			
	Total	No.	%	Total	Sampled
Survey	731	221	30.2		1,415
Non-Survey	1,011	350	34.6		1,785
Total	1,742	571	32.8	7,000	3,200

The sample represented 32.8 percent of the households in La Libertad and 45.7 percent of the estimated total population of 7,000. The ratio of total individual records in the sample to total population is higher than the ratio of dwellings in the sample to the community total. This may be accounted for, at least in part, by population turnover. Twenty-four percent of the individuals in the study sample were found to have lived in the community for less than 12 cycles (24 weeks). The population figure of 7,000, given by CNAP, was probably an accurate estimate for any given point in time. Study data indicated that shifts in population were more likely to be the result of some members moving in and out of households rather than the movement of an entire family in or out of the community.

Of the 3,200 individual records, 1,374 or 19.6 percent of the total population group (7,000) had data on acceptance and refusal recorded for at least 12 of 15 cycles. The remaining 1,826 were classified as incomplete (Table 29). Reasons for incomplete data were:

Absence from the household at the time of the medicator's visit.	49%
Residence in the community for less than 12 cycles.	24%
Babies too young to be given medication for 12 cycles during the course of the program (the drugs were not administered to babies under 6 months of age).	2%
Data covering 12 or more cycles either not recovered or not recorded on household records (TC-1 forms).	25%

The households from which data were recovered were spotted on sector sketch maps to examine geographic and population density distribution. The sample followed a pattern comparable to that of the total population. This, together with the fact that the population of La Libertad is relatively homogeneous with regard to education level and socioeconomic characteristics, resulted in what appeared to be a representative sample. Sample size also favored reduction of bias.

Forty-six percent of the individuals in the sample were males, and 54 percent females. With minor exceptions, age-sex distribution was well balanced. As one might expect, there appeared to be relatively fewer males with complete records in the age group ranging from 20 to 49 years. On the other hand, males in younger age groups were better represented by complete data than were females of corresponding age.

The unprinted side of a McBee keysort card was used for listing the variables to be examined. Hand punching and sorting were used for transferring and tabulating data. Tests for statistical significance of differences between variables were done when indicated.

Findings

Data from the 3,200 individual medication records were examined in two ways: first, on the basis of the total potential* for taking prophylactic drugs and secondly, on the basis of recorded offers of medication. The first gave some indication of the level of community protection as well as behavior; the second reflected only behavior.

---

*"Potential" is defined as 15 cycles x number of persons included in a given group.

TABLE 29

Distribution of Complete and Incomplete Individual Medication Records* in Study Sample by Age and Sex

Age in Years	All Persons in Study (3,200)						Persons with Complete Data** (1,374)						Persons with Incomplete Data*** (1,826)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<1	33	2.2	35	2.0	68	2.1	8	1.6	9	1.0	17	1.2	25	2.6	26	3.1	51	2.8
1-5	263	17.7	303	17.7	566	17.7	154	30.3	181	20.9	335	24.4	109	11.2	122	14.4	231	12.7
6-14	446	30.0	441	25.7	887	27.7	210	41.3	229	26.5	439	32.0	236	24.2	212	24.9	448	24.5
15-19	153	10.3	196	11.4	349	10.9	45	8.8	79	9.1	124	9.0	108	11.1	117	13.8	225	12.3
20-34	268	18.0	364	21.2	632	19.8	36	7.1	158	18.3	194	14.1	232	23.8	206	24.2	438	24.0
35-49	180	12.1	203	11.8	383	12.0	23	4.5	109	12.6	132	9.6	157	16.1	94	11.1	251	13.7
50-59	68	4.6	83	4.8	151	4.7	13	2.6	49	5.7	62	4.5	55	5.6	34	4.0	89	4.9
≥60	74	5.0	90	5.2	164	5.1	20	3.9	51	5.9	71	5.2	54	5.5	39	4.6	93	5.1
Total	1,485	100.0	1,715	100.0	3,200	100.0	509	100.0	865	100.0	1,374	100.0	976	100.0	850	100.0	1,826	100.0

*Absences as well as no data were counted as incomplete data for classification of records.

**Persons whose record showed acceptance or refusal for 12 or more of 15 cycles.

***Persons whose record showed acceptance or refusal for less than 12 of 15 cycles.

Acceptance Levels Based on Total Potential for Taking Prophylactic Drugs

Based on the potential for taking medication for 15 cycles, the acceptance level for the total study group was 32.4 percent. Refusal accounted for 30.6 percent and absences for 21.6 percent of the total. Acceptance levels for both survey and non-survey households were essentially the same as for the total group. When only recorded acceptance, refusals, and absences were considered without taking lack of data into account, the acceptance level was a little higher, 38.4 percent. On this basis, the refusal level was 37.6 percent and absences accounted for 24 percent of the total. Acceptance levels were higher but followed a downward trend for the first three cycles, then leveled off to little change for the remaining 12 cycles. Initially, absences and refusals increased at about the same rate. However, after six treatment cycles, absences decreased and refusals increased, reaching a jointly stable relationship which persisted through the last six cycles (Figure 16).

Of the various subgroups studied, junior high students, who did not serve as interviewers, had the highest acceptance level (65.1%). Student interviewers were second highest with 53.3 percent. When the two student groups were compared across the board, however, taking into consideration acceptance, refusal, absence, and cases of no data, the data presented a confusing picture. Student interviewers refused half as often but were absent twice as often (Table 30).

Records for households with both a survey respondent and student interviewer showed a considerably higher acceptance level (51.8%) than did survey households with junior high students who were not interviewers (38.7%). Non-survey homes with student interviewers were about the same as the latter (37.6%), but non-survey households with students who were not interviewers had an acceptance level of 45.9%. The acceptance level (45.2%) was a little higher for student interviewers and their families than for students who were not interviewers and their families (40.7%).

The poorest acceptance level for any one group was that for survey respondents (25.6%). This group also had the highest refusal level (45.7%) and correspondingly, less occurrence of absence and lack of data.

Acceptance Levels Based on Recorded Opportunities to Take Medication

The most accurate picture of behavior alone with regard to taking medication was that based on recorded opportunities to take or refuse drugs, i.e., on outright acceptance or refusal. Using this approach, the acceptance level for survey households (50.5%) was a little lower than that for non-survey households (52.3%). Although the acceptance level of student interviewers (94.7%) was a little higher than that for students who were not interviewers, (89.5%) the difference was not significant. Student interviewers and their families did, however, show a significantly higher acceptance level (70.7%) than students who did not serve as interviewers and their families (57.7%) (Table 31).

FIGURE 16. Acceptance, Refusals, and Absences by Cycle, La Libertad, March–October 1969

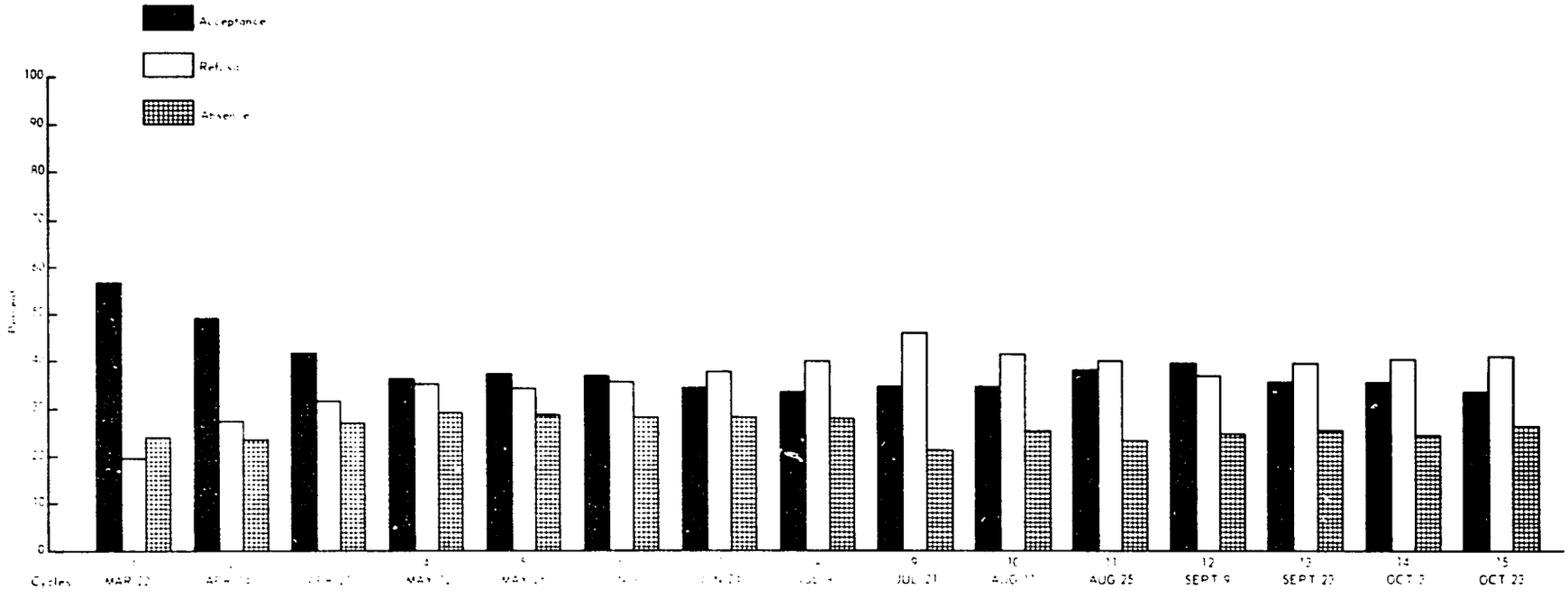


TABLE 30
Medication Record for Selected Groups for 15 Cycles

Group	Number Persons in Group	Total Potential for Medication*	Acceptances		Refusals		Absences		No Data	
			No.	%	No.	%	No.	%	No.	%
Respondents (Initial Survey)	218	3,270	838	25.6	1,494	45.7	583	17.8	355	10.9
Respondents + Families	1,415	21,225	6,912	32.6	6,766	31.9	4,316	20.3	3,231	15.2
Non-Respondent Families	1,785	26,775	8,656	32.3	7,915	29.6	6,087	22.7	4,117	15.4
Student Interviewers	20	300	160	53.3	9	3.0	128	42.7	3	1.0
Students not Interviewers	21	315	205	65.1	24	7.6	73	23.2	13	4.1
Student Interviewers + Respondent Families**	68	1,020	528	51.8	154	15.1	226	22.2	112	11.0
Students not Interviewers + Respondent Families***	111	1,665	645	38.7	486	29.2	371	22.3	163	9.8
Student Interviewers + Non-Respondent Families	58	870	327	37.6	200	23.0	321	36.9	22	2.5
Students not Interviewers + Non-Respondent Families	43	645	296	45.9	204	31.6	109	16.9	36	5.6

*No. of persons in group x 15 cycles.
**10 student interviewers were members of survey households.
***12 students, who were not interviewers, were members of survey households.

TABLE 31
Comparison between Acceptance by Student Interviewers and Families and Students Who Were Not Interviewers and Families

	Student Interviewers and Families (126)		Student Not Interviewers and Families (154)		Total
Acceptance	855	(70.7%)	941	(57.7%)	1,796
Refusal	354	(29.3%)	690	(42.3%)	1,044
Total	1,209		1,631		2,840
		$\chi^2 = 50.7$		$P < .01$	

Relation between Responses Given on Survey and Drug Acceptance

Reported Malaria History: Survey respondents were asked what disease they themselves had had most frequently. Of the 218 respondents in the sample, 39 reported having had malaria more frequently than any other disease. The acceptance level (48.7%) for those who reported having malaria most frequently was significantly higher than that (32.9%) for the other group (Table 32). Though to a lesser degree, respondent reports of a family history of malaria were also positively and significantly related to respondent acceptance. The acceptance level was 40.3 percent for the former and 33.9 percent for the latter (Table 33). The acceptance level (55.8%) for households in which respondents reported a history of malaria was not significantly different from that (51.1%) in households where respondents stated that nobody had had malaria.

TABLE 32
Drug Acceptance in Relation to Diseases Respondents Reported Having Most Frequently

	Reported Most Frequently				
	Malaria (39)		Other Diseases (179)		Total
Acceptance	217	(48.7%)	621	(32.9%)	838
Refusal	229	(51.3%)	1,265	(67.1%)	1,494
Total	446		1,886		2,332
		$\chi^2 = 38.8$		$P < .01$	

Table 33
Relationship of Respondents' Acceptance
to Their Reporting a Household History of Malaria

	Family History of Malaria				
	Yes (68)		No (150)		Total
Acceptance	301	(40.3%)	537	(33.9%)	838
Refusal	445	(59.7%)	1,049	(66.1%)	1,494
Total	746		1,586		2,332
		$\chi^2 = 9.3$		$P < .01$	

Relationship of Acceptance to Stated Belief that Malaria Was Most Dangerous Disease: Forty-one respondents stated that they believed malaria to be the most dangerous disease, and 177 named some other disease. The difference between acceptance for those respondents who said malaria was the most dangerous disease (35.3%) and that for those who named some other disease (36.1%) was not significant.

Relationship between Acceptance and Statements on Best Way to Get Rid of Malaria: When asked what they believed to be the best way to get rid of malaria in the community, 89 respondents (39%) replied "take medicine" and 108 (50%) said "kill mosquitoes." The remaining 11 percent gave one of several other answers. The data did not support the hypothesis that drug acceptance would be higher among those who said taking medicine was the best way to eradicate malaria. The acceptance level of this group was 30.5 percent and for those who gave priority to killing mosquitoes, 39.3 percent.

Distribution by Drug Acceptance Category of Persons for Whom Data Were Recovered for 15 Cycles

As a side study and preliminary to examining the relationship between drug-taking behavior of heads of household and that of other household members, the 1,374 individuals for whom complete data on acceptance and refusal were recovered were classified as "acceptor," "refuser," or "intermittent." Criteria applied were acceptance of medication for 12 of 15 cycles for acceptors, for three or less cycles for refusers, and for no less than 4 or more than 11 cycles for intermittents. The classifiable group was divided almost equally among the three categories (Table 34). A much higher proportion (64.7%) of the 6-14 year-olds were acceptors than of any other age group. Both males and females had a large percentage of acceptors. This was accounted for by the fact that elementary school facilities are available and well attended and that children generally accepted drugs administered in the school setting. In general, a larger proportion of males (43.0% overall) than females (29.7%) were in the acceptor group.

TABLE 34
Classifiable Individuals by Drug Acceptance Category, Age, and Sex

Age in Years	Sex	Total Number	Acceptors		Intermittent		Refusers	
			No.	%	No.	%	No.	%
<1	M	8	0	-	0	-	8	100.0
	F	9	1	11.1	1	11.1	7	77.8
	T	17	1	5.9	1	5.9	15	88.2
1-5	M	154	26	16.9	50	32.5	78	50.6
	F	181	27	14.9	67	37.0	87	48.1
	T	335	53	15.8	117	34.9	165	49.3
6-14	M	210	146	69.5	49	23.3	15	7.1
	F	229	138	60.3	64	27.9	27	11.8
	T	439	284	64.7	113	25.7	42	9.6
15-19	M	45	28	62.2	11	24.4	6	13.3
	F	79	30	38.0	29	36.7	20	25.3
	T	124	58	46.8	40	32.3	26	21.0
20-34	M	36	9	25.0	12	33.3	15	41.7
	F	158	23	14.6	64	40.5	71	44.9
	T	194	32	16.5	76	39.2	86	44.3
35-49	M	23	3	13.0	10	43.5	10	43.5
	F	109	23	21.1	36	33.0	50	45.9
	T	132	26	19.7	46	34.8	60	45.5
50-59	M	13	2	15.4	4	30.8	7	53.8
	F	49	5	10.2	22	44.9	22	44.9
	T	62	7	11.3	26	41.9	29	46.8
≥60	M	20	5	25.0	5	25.0	10	50.0
	F	51	10	19.6	13	25.5	28	54.9
	T	71	15	21.1	18	25.4	38	53.5
Total	M	509	219	43.0	141	27.7	149	29.3
	F	865	257	29.7	296	34.2	312	36.1
	T	1,374	476	34.6	437	31.8	461	33.6

Influence of Drug-Taking Behavior by Heads of Household on Other Family Members

Records for the classifiable group included those for 130 heads of household; 62 males and 68 females. Age and sex distribution in this group is shown in Table 35. Males predominated in the acceptor category and females in the refuser; there was little difference in those classified as "intermittent." The sex-related differences occurred largely in the 20-34-year age groups.

TABLE 35
Distribution of Heads of Household for Whom Complete Data Were Available by Drug Acceptance Category, Age, and Sex

Age in Years	Acceptors			Intermittent			Refusers			All Categories		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total
15-19	0	0	0	0	0	0	1	0	1	1	0	1
20-34	6	0	6	6	6	12	3	10	13	15	16	31
35-49	5	4	9	6	7	13	9	7	16	20	18	38
50-59	1	2	3	7	6	13	3	6	9	11	14	25
≥60	4	2	6	7	9	16	4	9	13	15	20	35
Total	16	8	24	26	28	54	20	32	52	62	68	130

In all three categories a positive relationship existed between the drug-taking behavior of heads of household and that of other household members (Table 36).

TABLE 36
Behavior of Household Members in Relation to That of Heads of Household with Regard to Taking Prophylactic Drugs

Classification of Head of Household		Total*	Acceptance		Refusal	
			No.	%	No.	%
Acceptor						
Male	(16)	1,097	868	79.1	229	20.9
Female	(8)	411	302	73.5	109	26.5
Total	(24)	1,508	1,170	77.6	338	22.4
Intermittent						
Male	(20)	1,250	610	48.8	640	51.2
Female	(32)	1,429	810	56.7	619	43.3
Total	(52)	2,679	1,420	53.0	1,259	47.0
Refuser						
Male	(26)	1,775	522	29.4	1,253	70.6
Female	(28)	1,474	634	43.0	840	57.0
Total	(54)	3,249	1,156	35.6	2,093	64.4
All Groups	(130)	7,436	3,746	50.4	3,690	49.6

*Total opportunities to accept or refuse medication by all household members including head of household.

Acceptor households headed by males had a slightly higher acceptance level than those with female heads. The acceptance level (50.4%) for the 130 households did not vary significantly from that of the total classifiable group nor from that of the total study group.

Discussion

Although the study was designed as a hypothesis-testing investigation and yielded information of practical value on the variables examined, its greater value may have been the identification of some of the problems involved in conducting a field study in the type of setting in which it occurred. Among these problems were the impossibility of selecting a probability sample and the use of records maintained by operational personnel to carry out procedures associated with data collection and hypothesis-testing. Among program-operating procedures which contributed to problems were lack of duplicate household records, the practice of not transferring school and worker group records to household records, loss of household records, and frequent turnover and changes in assignment among medicators (which contributed to lapses in maintaining current lists of households and inability of medicators to identify persons and households).

Such problems are inherent in field studies and perhaps more likely to occur when administrative direction for the research entity and operational program stems from different sources. Even though program objectives and research objectives are compatible in a global sense, pressures and emergencies at the field level frequently dictate that field personnel subordinate research objectives to pressing problems at hand. Only through the sustained and dedicated cooperation of national program staff could problems be resolved to the point that the study could be completed, although not strictly in accord with the original design.

Although records were incomplete with respect to total scope of the population and individual household forms recovered, the size and distribution of the study population and the results from cross checking acceptance levels for major subdivisions lend some support to the sample having been representative.

The procedures for recording and analyzing data constituted a pilot effort for CAMRS in the use of Keysort cards on a fairly large scale. Though time consuming, tedious, and exacting, the method proved feasible. (Operating programs would be more likely to have adequate manpower for the relatively easy and inexpensive use of Keysort cards than funds and skills for using automatic data processing facilities even when such are available. Thus, personnel in malaria programs could, with short term training, use this method to carry out or participate in health education studies.)

Results from hypotheses tested which may hold potential for practical application were:

1. The favorable relationship between student participation and acceptance levels of students and families.

2. Positive association between head of household drug-taking behavior and that of other household members.

3. The finding that personal or household malaria history was a better predictor of subsequent drug acceptance than stated beliefs about danger from malaria or efficacy of mass drug distribution.

All of these findings need further testing.

In the case of student participation, it was not possible to establish a true control group since there was no other community in which a similar student body could be matched under similar circumstances. Characteristics of staff in the La Libertad school favored student impact whether students were interviewers or not. The principal was noted for his interest in promoting community betterment and he, as well as other staff, gave enthusiastic support to the program. This may have been an intervening variable which favored higher drug acceptance by both interviewer students and those who were not interviewers and their families. Also, although student families did not represent a socioeconomic strata different from that of other families in the community the fact that they, sometimes at some sacrifice, keep children in school beyond the elementary grades may indicate that they have a different value system which favored receptivity to the mass drug distribution program. One incontestable fact does stand out as having merit for repetition in operating programs; junior high school students can, when given an appropriate interview schedule and well-planned, short training, function remarkably well as interviewers. Probably, La Libertad is not the only community in this or other Central American countries which would manifest pride in the enterprising effort of its youth and give full cooperation in the interviews. CAMRS hopes that the impact of additional student involvement, such as that originally planned for this study, can be tested.

The La Libertad study showed that drug-taking behavior of household members is likely to follow a pattern similar to that of the head of household. Therefore, a deliberate, well-planned effort should be directed toward heads of households. From documented research and experience, this should likely be done on an interpersonal level.

The positive association between malaria history and a higher level of drug acceptance resulting from this study seemed a logical outcome. However, the El Jocotal study described elsewhere cast doubt on its reliability in all situations. Other variables, such as behavioral resistance and a lower case rate resulting from previous experience with mass drug distribution or a high case rate but absence of acute illness, may have a bearing on the relationship between personal experience with the disease and acceptance of prophylactic medication.

Visual observation and questions about personal experience and behavior are more likely to result in accurate predictions of future behavior than statements in answer to questions about what people believe. The lower acceptance of drugs by persons purporting to believe malaria to be a dangerous disease and mass drug distribution to be the most effective eradication measure supports this contention.

Summary and Conclusions

The measurement of behavioral and educational aspects of a malaria prevention effort was complicated by difficulties in collecting data and in isolating and controlling variables in order to establish causal relationships. Nevertheless, a field study such as this one, in which actual program operation and its outcomes serve as the "laboratory," offers opportunity for identifying variables, testing hypotheses, and emerging with findings that may serve as useful guidelines for improved health education programs.

A valuable by-product is the possibility of actually using findings in ongoing programs because both program personnel and investigator are a part of the operational scene.

This study showed that there was a positive relationship between student participation and drug acceptance and also produced other useful information. Replication or adaptation of such involvement in ongoing malaria control efforts as both an educational and practical operational measure merits consideration.

Study findings pointed to the need for an effective, frontal educational approach to heads of household.

Verbalized beliefs about danger from malaria and the efficacy of mass drug distribution as a preventive measure did not favorably influence drug acceptance.

Surveys Conducted in Association with a Test of Outside Spraying with OMS-33 (Baygon) in El Jocotal

Two surveys were conducted in the Jocotal area. The first of these included 20 of the 57 households in Borbollón which comprised the cluster of dwellings used as the experimental site for spraying outside surfaces with OMS-33 (Baygon). Interviews in this survey, conducted before the test spraying, were focused primarily on knowledge and opinions about spraying as a malaria control measure. The second survey, conducted two-and-a-half weeks after the spraying, covered a 25 percent probability sample of households in both La Curruncha, the untreated control group for the OMS-33 test, and Borbollón. In both localities, this survey concerned opinions about malaria and prophylactic use of antimalarials; in Borbollón, impressions about the effectiveness of the OMS-33 test were also sought.

First Survey

The Borbollón population was stable. Only one of the 20 families interviewed had lived in the locality less than five years. They had, therefore, been through many cycles of DDT spraying as well as several previous insecticide studies.

Thirty percent of the households had at least one functionally literate member (completion of four years elementary schooling). None of the respondents reported that anyone had finished more than five years. Interest in education of children was indicated by the community's having recently built its own school.

Sixteen respondents recalled that program personnel had given instructions on precautions in association with earlier spraying programs. Only two respondents mentioned explanations about the purpose of spraying. One of these said that CNAP personnel told him the spraying was to kill mosquitoes and one stated that PAHO personnel said the spraying was to test the insecticide (Borbollón was included in a previous OMS-33 inside spraying test). Responses on duration of the effect of spraying appeared to be based on the time during which post-spray effect was highly visible for arthropods other than mosquitoes (such as roaches or bedbugs). Responses on duration were:

10 days	7
1 month	6
3-4 months	3
6 months	1
Don't know	3
	20

Only one of the 20 respondents, a 16 year old girl who attends school in San Salvador, knew that three agencies (CNAP, PAHO, AID) had been involved in spraying activities. Three identified the national program and the rest seemed vaguely aware that the Salvadorean government was involved.

None of the respondents manifested any opposition to spraying. Several expressed the hope that the spraymen would use a "stronger" insecticide to get rid of "bugs" and mosquitoes because of discomfort from biting and crawling.

Because Jocotal had been used as a test area several times, an attempt was made to assess reaction to the rather frequent visits by program personnel. When respondents were asked about visitors to the locality, they said they had many visitors. However, in relating why visitors came, they usually spoke only of those who came to hunt and fish. Apparently, persons associated with malaria programs had become such an accepted part of their lives that they did not count them as visitors--rather, like the postman or meter reader in the U.S. There was no indication that their feeling

was anything but cordial about "visitors" ("we like new faces," "they are good for business," "usually nice friendly people"). No indication of adverse feeling about malaria workers from any source was noted.

Second Survey

With regard to the test spraying in Borbollón, responses to open end questions were as follows:

Question	Response	No.
Benefits	Very good	10
	Helped a little	6
Nature of benefits	Killed all arthropods	13
	Killed or repelled mosquitoes	3
Duration of effect	3-10 days	11
	Still helping some (only for mosquitoes)	5
Reports of illness or discomfort from insecticide	None	16
Preference between interior and exterior spraying	Exterior	7
	Interior	4
	Both at some time	3
	No preference	2
Why spraying was done	To kill bugs and mosquitoes	13
	Prevent malaria	2
	To test spray	1
Explanations given by program personnel before spraying	Precautions to take	11
	No recall	4
	Precautions and that spraying was for malaria	1
Comparison of results with previous spraying	Better	13
	Less	2
	Same	1
Spray still showing results	None	9
	Some ("still helps to keep mosquitoes away")	7

Among respondents in both Borbollón and La Curruncha, assessment of the period of greatest incidence of malaria during the year was consistent with epidemiologic and entomologic data. They also knew that the malaria rate was high in the locality. Responses also revealed, however, that malaria was not considered a very serious matter. In fact, several respondents went into great detail about when malaria was really malaria in bygone years. Some of them expressed doubt about or total disbelief in present day diagnoses because symptoms did not conform to their criteria of the "real thing"--severe chills, high fever accompanied by excruciating aches and pains and violent nausea, followed by turning yellow and culminating in death or long-term debilitation. Apparently, there is a semantic "gap" between the health worker and this populace when discussing malaria. Even in households with current or recent cases (approximately 25%), great concern about the disease was not expressed.

When respondents were asked specifically what malaria programs do to prevent malaria, responses were:

Give tablets to cure malaria	12
Spray	10
Give green tablets to prevent malaria	9
Take blood smears	7
Catch mosquitoes	3
Don't know	2
Maintain voluntary collaborator posts	1

These responses indicate that even those persons who recognize spraying as a malaria control measure evaluate an insecticide's effectiveness on the basis of ridding them of biting and crawling arthropods.

Respondents were asked if they planned to take the "green tablets" (prophylactic medication) if distribution were resumed.* Twenty-seven (73%) answered affirmatively. On the basis of 16 medication records for 1969 found in survey homes, the acceptance level, based on recorded opportunities to accept or refuse drugs, was 38.4 percent.

Summary

The effectiveness of spraying and of the insecticide used was evaluated by the study group on the basis of its killing power for all visible arthropods which may cause discomfort from their biting and crawling. No strong feeling about the kind of insecticide used or how it was applied was detected; visible killing power was the major criterion. Whether the content of prior educational effort has fostered or contributed to this criterion was not clear but seemed a possibility.

There appeared to be a semantic gap between the study group and malaria program personnel on what malaria and a malaria problem are. Current or recent cases did not seem to be regarded as particularly serious.

Since the study population described the transmission pattern accurately, this might serve as a starting point for program personnel in future educational efforts.

*Mass drug distribution was suspended in 1969 and had not been resumed at the time of this study in 1970. However, a medicator in the locality reported that prophylactic drug distribution was to start again early in April.

General Observations

Progress has been made in identifying some variables which seem to have potential as predictors of receptivity and others which appear to indicate educational approaches largely on a family basis. Findings have been shared with staff of the national program, and they plan to use this information in an OMS-33 spray project which is being planned.

Further study is needed on variables already examined as well as effort to identify additional variables which may influence behavior in relation to malaria prevention measures and/or have operational implications. Certain impressions in the course of conducting the studies may merit investigation:

1. The cognitive structure of acceptor and refuser respondents seemed tighter on factors related to drug taking behavior than that of the intermittent group. If this impression were confirmed, one might speculate that the intermittent group may be more susceptible to educational effort than the refuser group, therefore a more productive target for educational action.

2. There was considerable confusion about the transmission of malaria and the nature of the disease as well as actual doubt about a diagnosis of malaria in some cases.

3. Behavioral resistance to taking drugs is apparently at a lower ebb during the early cycles of mass drug distribution, following a change in drugs or resumption of collective treatment after suspension of distribution for several months. The possibility that a more propitious climate for educational effort may exist during this period has not been exploited or studied in El Salvador or in other Central American countries, with the possible exception of Costa Rica.

4. In general, the population does not seem "future-oriented." Time-limited programs of relatively short duration, strategically scheduled in relation to peak transmission periods and promoted as such, might, therefore, result in better response than longer, indefinite periods of drug distribution.

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