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CENTRAL AMERICA MALARIA RESEARCH STATION

Geoffrey M. Jeffery

Center for Disease Control
Atlanta, Georgia

30 June 1972

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RESEARCH FINAL PROJECT REPORT

JUNE 30, 1972

CENTRAL AMERICA MALARIA RESEARCH STATION

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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Center for Disease Control
Atlanta, Georgia 30333

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16. Abstracts

Central America was selected as the site for a research station because the malaria programs of the region had been facing numerous problems. Among the first work initiated by CAMRS were intensive studies on the ecology of the malaria vectors. The information from these continuing studies on activity patterns, breeding cycles and habitats, and vector associations with man has guided the selection and timing of antimosquito activities including dry-season larviciding of river habitats, water-management practices in coastal estuaries, exterior spraying of houses, ULV spraying, and release of sterile male mosquitoes. Characterization of the malaria parasite strains from the area has led to a better understanding of the transmission pattern and yielded information to guide the selection of antimalarial drug regimens. The results of a pyrimethamine-primaquine mass distribution field trial enabled CAMRS to recommend against an expensive change-over of drugs and, in addition, yielded information on the reasons for the low acceptance in mass drug distribution programs. Sero-epidemiologic studies have been at least partially responsible for the cessation of total coverage with expensive intradomiciliary spray programs in areas where transmission was shown to be at low levels, have proved to be efficient in assessing an experimental insecticide application, and have led to training of personnel from one of the national antimalaria programs. Many of the epidemiology and health education studies have included the identification of mechanisms for improving the efficiency and utilization of the voluntary collaborator networks maintained by the national programs. Tests of candidate insecticides for intradomiciliary application and for larviciding have been carried out by CAMRS to assess the usefulness of "promising" formulations against the malaria vectors in Central America, particularly those in the areas of refractory malaria.

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REPORT SUMMARY

A. 1. *Project Title:* Central America Malaria Research Station

Contract Numbers: PASA RA (HA) 2-67
PASA RA (HM) 2-67

2. *Principal Investigator:* Geoffrey M. Jeffery, Ph.D.

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

3. *Contract Period:* July 1966 to June 1972

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

5. *Total A.I.D. Funding of Contract to 30 June 1972:* $ 2,096,152.50

6. *Expenditures and Obligations through 30 June 1971:* $ 1,665,652.50

7. *Expenditures and Obligations for FY 1972:* $ 430,500.00

8. Estimated Expenditures for Next Contract Year: NA

B. Narrative Summary of Accomplishments and Utilization

Central America was selected as the site for a research station because the malaria programs of the region had been facing numerous problems. Among the first work initiated by CAMRS were intensive studies on the ecology of the malaria vectors. The information from these continuing studies on activity patterns, breeding cycles and habitats, and vector associations with man has guided the selection and timing of antimosquito activities including dry-season larviciding of river habitats, water-management practices in coastal estuaries, exterior spraying of houses, ULV spraying, and release of sterile male mosquitoes. Characterization of the malaria parasite strains from the area has led to a better understanding of the transmission pattern and yielded information to guide the selection of antimalarial drug regimens. The results of a pyrimethamine-primaquine mass distribution field trial enabled CAMRS to recommend against an expensive change-over of drugs and, in addition, yielded information on the reasons for the low acceptance in mass drug distribution programs. Sero-epidemiologic studies have been at least partially responsible for the cessation of total coverage with expensive intradomiciliary spray programs in areas where transmission was shown to be at low levels, have proved to be efficient in assessing an experimental insecticide application, and have led to training of personnel from one of the national antimalaria programs. Many of the epidemiology and health education studies have included the identification of mechanisms for improving the efficiency and utilization of the voluntary collaborator networks maintained by the national programs. Tests of candidate insecticides for intradomiciliary application and for larviciding have been carried out by CAMRS to assess the usefulness of "promising" formulations against the malaria vectors in Central America, particularly those in the areas of refractory malaria.

A. General Background

Programs for the eradication of malaria in the six countries of Central America and Panama had been adopted by 1958. Within a very few years resistance of the vector to insecticides emerged and by 1964 the impact of this and many other technical problems facing the malaria programs was being felt. A Public Health Service review team deployed by AID to determine feasibility of malaria eradication in the region expressed strong reservations on the probable success of this endeavor if only residual DDT spraying was to be used. In 1966 one of the conclusions of a joint PAHO, PHS and SNEM Evaluation Group reviewing the programs in the same countries stated: "The Group has grave reservations about the adequacy of presently available attack measures. The achievement of malaria eradication in Central America may well hinge on the development of new and better attack methods. For that reason, the Group feels that it is essential that operational research programs be developed and implemented as soon as possible."

Because of these urgent needs, CDC proposed to AID in 1965 "that a program be established in a tropical area of Central America where various malaria eradication techniques may be evaluated under field conditions." The scope of the studies was to include biological-ecological studies, residual insecticides, larvicides, water management, biological control measures, antimalarial drugs, combinations of methods and epidemiologic investigations. The proposal was implemented in 1967 by the establishment of the Central America Malaria Research Station at a site selected in San Salvador, through a PASA between AID and PHS/CDC.

B. Statement of Project Objectives as Stated in the Contract

The PIO/T accompanying the original PASA stated that the nature of the recognized causes of program failures in the Central American Area indicated that the operational research activities would involve work in the broad investigational areas listed below, and in perhaps still others not identified at that time or in new ones that may be dictated by future laboratory or field discoveries or developments. The investigational areas listed were as follows:

1. Biological-ecological studies pertinent to the development and effective use of control measures.

2. Evaluation of new residual insecticides in field trials.

3. Larviciding techniques.

4. Area water management possibilities.

5. Adulticiding techniques.

6. Investigation of biological control procedures.

7. Use of antimalarial drugs.

8. Combined methods.

9. Epidemiologic investigations.

10. Health education investigations.

The "General Requirements" for the scope of technical services noted that there would be a permanent staff of full-time professional personnel (which was ultimately reduced to a ceiling of nine), that this would be supplemented by temporary professional assignees for specific purposes from CDC and other agencies, and that the required buildings would be provided by the Government of El Salvador. It also noted that, since specific phases of the investigations would have to be determined after field appraisal of the problems, the project would be of a long-term, permissive nature. It was recognized that support would be needed on an undiminished basis through the duration of field requirements, i.e., "until the needs of the malaria eradication program in this area have been met and eradication achieved."

C. Continued Relevance of Objectives

Work plans developed for each fiscal year (1968-1972) have reflected closely the above general aims of the project, with modifications only in emphasis or as a result of completed investigations. The problems confronting malaria eradication programs in the area have proven to be greater than originally anticipated. Based on the recommendation of the World Health Assembly in 1970 a new strategy for eradication has been put forth, recognizing that while eradication may remain the goal, much more time and much more knowledge may be required to achieve this end. In the meantime it is urgent that effective control measures be developed in areas refractory to malaria eradication.

Research remains the key to the future success of malaria control or eradication programs, and the CAMRS program has responded to some of the needs with expanded efforts in studies oriented to a better understanding of the dynamics of malaria as it relates to the current operational problems.

D. Accomplishments to Date

1. Biological-ecological studies pertinent to the development and effective use of control measures

Since its inception in 1967, CAMRS has accumulated entomologic data from more than 2000 field collections and associated observations. Anopheline habitats and populations have been observed through four alternating dry and rainy seasons and the associated transition periods in an area which includes habitats and stations characteristic of much of the malarious area along the Pacific Coast of Central America. Emphasis has been on relating vector studies to malaria and utilizing biological information in the intelligent planning and evaluation of eradication and control measures.

Basic to any studies on a mosquito vector is knowledge of its activity cycle and resting sites. CAMRS has found that, in general, almost any shaded and protective crevice or shelter may attract *A. albimanus*, but bridges and clusters of large rocks in vegetated areas near breeding or feeding sites are the most frequented. The identification of resting sites and observation that

A. albimanus is found consistently in daytime niches in the same vicinity as it is found in night captures, has provided a good and convenient tool for monitoring populations in connection with bionomic and control studies. The 24-hour activity pattern of female *A. albimanus* shows a mass migration from resting places at dusk, followed by a few hours of activity and then by stabilization in a given place after full daylight. This information is essential to application of measures which require direct contact with the adult mosquito, e.g., ULV.

Six basic methods have been used by CAMRS to measure adult densities. The New Jersey light trap collected more *A. albimanus* than other collecting methods and was particularly useful in measuring seasonal and activity patterns. In addition, a major advantage is that the physical presence of collectors is not required. The New Jersey light trap, diurnal collections from natural resting places, and diurnal collections from beneath concrete bridges had not been used extensively in Central America and CAMRS demonstrated the specific utility of these three as well as the long-employed collections from humans, cattle corrals, and inside houses.

Host preference studies, using microprecipitin tests, have shown that, in general, only five kinds of animals (man, cattle, dog, pig, and horse) were involved. A Salvadoran employee was trained by CDC/Fort Collins and has developed the capability for microprecipitin testing at CAMRS. Additional measures of vector potential, *A. albimanus* female dissections and physiological age grading, have shown an apparently young population (but further laboratory work with *A. albimanus* is necessary).

Both *A. albimanus* and *A. pseudopunctipennis* survive the dry season quite efficiently and year round breeding is accomplished through a succession of various larval habitats resulting from changing water levels.

An example of how knowledge of where and when vector breeding occurs has led to sound economical planning of vector control activities is seen in the Estero San Diego. Mosquito breeding there produces peak adult populations for only 4-6 weeks. Therefore, antimalaria measures can be concentrated during this period. Sound information about the vector is equally important to planning control activities for any area.

2. Evaluation of new residual insecticides in field trials

During the four years of CAMRS operation, promising candidate insecticides have been tested for their possible usefulness in the malaria programs of Central America. Entomologic methods for evaluation are standard for most of the studies and included early morning resting densities, early evening density captures, exit densities and mortalities, bioassays of sprayed surfaces, and fumigant tests.

A small study of Dursban at 2 g/m^2 on the outside of houses did not give promising results.

A village-scale trial of fenitrothion indicated a loss of effectiveness in four weeks 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 spraying may have led to a decrease in vector susceptibility.

Evaluation of a carbaryl test (85% wettable powder applied at a dosage of $2g/m^2$) indicated that the insecticide might have some effectiveness for three or four weeks after application.

Propoxur sprayed on the exterior surface of dwellings in a small village trial suggested that such an exterior application effectively decreased inside biting for at least five to six weeks. Houses were sprayed, with coverage of all exterior walls and any overhanging surfaces protected from rainfall. This operation proceeded more rapidly than conventional inside spraying, since the time required to prepare a house for outside spraying is minimal. An average of 9.5 houses were completed per sprayman-day, compared with 5.9 houses for conventional inside spraying in this area, and this technique used 40% less insecticide than the conventional inside spraying.

The effects of a partial (2-swath) application of propoxur once every 35 days diminished by three weeks post-treatment.

Landrin was reported to be similar to propoxur for residual indoor application and somewhat lower in cost and toxicity. Laboratory tests proved encouraging and a village-scale trial was initiated. No difficulties were encountered and no toxic symptoms were noted among the spraymen or householders during the course of the spraying. The period of activity after a second application was increased to at least ten weeks. In general, the studies suggest that Landrin is approximately equal to propoxur in its effectiveness, persistence, and ease of application when used as an indoor spray.

Thus, well supervised field tests have yielded information which obviated the expensive wide-scale adoption of inadequate insecticides and which identified the limitations and potentials of others.

3. Larviciding techniques

CAMRS larviciding trials using Flit MLO, Abate, fenthion, and Dursban have been carried out along river margins, irrigation systems, estuaries, and lakes. Applications have employed both hand sprayers and aircraft to deliver varying dosages. In general, larviciding seems to have greater utility during the dry season and in combination with other attack measures.

4. Area water management possibilities

Coastal estuaries in Central America provide significant anopheline breeding sites. CAMRS studies have yielded detailed information on the problem. During the rainy season sufficient water is carried in the rivers to maintain a constant flow into the ocean; therefore the estuaries are flushed by the tides every twelve hours, precluding the possibility of anopheline breeding. In the dry season the estuary closes, the salinity decreases, and the water-air-plant interface increases. The resulting situation is ideal for *A. albimanus* breeding. Maintenance of an opening to the ocean during the dry season would eliminate this breeding. Trials in one of the larger estuaries have shown that both dynamite and hand labor are inadequate. Studies are continuing to find an economical solution.

5. Adulticiding techniques

Field trials of Ultra Low Volume (ULV) aerial application of technical malathion demonstrated that the system can deliver insecticide in a manner that permits maximum distribution and penetration with a minimum amount of material over wide areas of difficult terrain. The trials did not produce results comparable to those previously observed with this type of treatment in other situations against other vectors. However, much insight was gained into determining the optimum time and conditions for utilizing this technique: (1) Knowledge of the activity patterns is essential to selecting a treatment time when the adult mosquitoes are out of their well-protected resting places. (With the anophelines in coastal El Salvador, this would mean nighttime treatments--possible only in areas of level terrain.) (2) The vector sensitivity to the insecticide in the particular target population is a controlling factor. (In one of the El Salvador tests the vector population manifested resistance while the same species in a nearby area remained sensitive.) (3) Climatic conditions must be monitored to avoid application when unfavorable influences would nullify any potential effect. (Fog and haze during one application resulted in less than desirable droplet size and distribution.)

Both thermal aerosol fogging and cold aerosol fogging have been employed in limited trials in coastal El Salvador using malathion, naled, and fenthion. The coverage obtained, especially with the cold aerosol, is encouraging and this technique may prove to be useful in densely populated areas experiencing a serious malaria outbreak.

6. Investigation of biological control procedures

Most of the CAMRS work in biological control has been through collaborative projects with institutions which have worked extensively in each area.

a. Mosquito pathogens. CAMRS is one of several institutions involved in a screening program to determine the identity and extent of naturally occurring diseases of malaria vectors in Central America. A Salvadoran employee has been trained at the USDA Gainesville, Florida Laboratory and the CDC Technical Development Laboratory in the identification of suspect larvae from routine CAMRS collections.

b. Juvenile hormones. CAMRS has assisted the Technical Development Laboratories in field plot testing of several synthetic juvenile hormone compounds by making density studies and in evaluating results.

c. Sterile male release.* CAMRS vector population studies provided the basic information needed to undertake (in collaboration with USDA Gainesville, Florida) a sterile male release project to control the *A. albimanus* population around Lake Apastepeque, El Salvador. In especially established facilities at CAMRS a colony of the species from the lake

* Includes preliminary data collected after the end of the reporting period.

area was established and up to 50,000 male pupae were produced and sterilized per day. Daily releases of these sterilized males began in April and continued until mid-September 1972. During October, a time when the population in the lake area is generally increasing, no fertile females could be found, indicating that the natural population had essentially been reduced to zero.

7. Use of antimalarial drugs

Based on favorable therapeutic/toxic ratios of the antimalarials pyrimethamine-primaquine found in studies by other workers, the combination was being considered as a replacement for amodiaquine-primaquine throughout the coastal area of El Salvador. In 1969, CAMRS instituted a study to compare the acceptance and effectiveness of the two drug combinations, and also to explore the reasons why drug programs have, in the past, failed as effective eradication methods. The drugs utilized in the malaria eradication programs of the region have been resisted by the people: complaints of side effects have been high, and drug acceptance has been low.

During the first year of the study pyrimethamine-primaquine was given in one district of coastal El Salvador and amodiaquine-primaquine in another and, during the second year pyrimethamine-primaquine was switched to the latter district. The malaria rates were reduced in both districts, but there was no difference between the districts. The complaint patterns did not vary significantly between the two combinations or between the years of pyrimethamine-primaquine use and the acceptance rates averaged 60 to 66% in all cases. On the whole, it was not possible to conclude that either combination had any notable advantage which would have justified an expensive changeover for country programs with large stocks of amodiaquine-primaquine available.

Using the information gleaned from the El Salvador studies that acceptance is high during one or two initial cycles and then drops, CAMRS has employed a single cycle of drug distribution to reduce the parasite reservoir before instituting other attack measures.

8. Combined methods

In one district of El Salvador where experience showed that several attack methods used singly failed to interrupt transmission, the various conditions affecting malaria transmission were studied to design a combination of measures which might be effective. Aerial larviciding of the rivers and major breeding areas with Abate for 20 weeks prior to the transmission season to prevent a buildup in the vector population was followed by one cycle of mass drug distribution offered to the total population to decrease the parasite reservoir before the expected increase in transmission. A single cycle of exteriorly applied propoxur followed to provide some residual effect against the adult mosquitoes and then when cases began to increase another cycle of mass drug distribution was completed.

Other combinations of attack measures being considered include a one-cycle application to reduce the vector population to levels amenable to the sterile

male release technique and larvicides applied within a drainage system at times when the water level and flow may permit breeding.

9. Epidemiologic investigations

In addition to specific studies on the characteristics of the Central American strains of malaria and on the disease in the human population, epidemiologic inquiries have been part of many of the CAMRS studies--especially those involving a combination of measures based on the changing levels of transmission. In addition, assistance has been given to country programs in the investigation of epidemics and reported outbreaks in Honduras, Costa Rica, and Panama.

Plasmodium vivax strains from three Central American countries have been studied in cooperation with the National Institutes of Health (NIAID Laboratory, Chamblee/Atlanta, Georgia). Characterization of the strains has included studies of prepatent periods, relapse activity, and drug response. An early attack was observed in all infected volunteers, followed by a long latent period between the primary attack and first relapse. This pattern, similar in all the Central American strains, is typical of the "temperate zone" malarias described by other workers. Field investigations in El Salvador during the past year have shown patterns of malaria prevalence consistent with the experimental data. Responses of the vivax strains to the usually recommended dosage regimens of chloroquine, pyrimethamine, chlorguanide, or quinine were all within normal limits. Knowledge of the relapse characteristics of the local parasite strain is essential to interpretation of malariometric data from an area; and, obviously, information on expected drug response must be available for the development of antimalarial regimens.

Through sero-epidemiologic studies on malaria in Panama, Costa Rica, and El Salvador, efforts have been made to explore as many different epidemiologic situations as possible. In Panama the impact, extent, and to some degree, the progress of an epidemic of *P. falciparum* malaria were studied through serologic surveys. Preliminary results from the areas of the epidemic indicate that serologic techniques may be useful in evaluating drug distribution programs--the individuals who were "chronic" refusers tended to have higher IFA titers to *P. falciparum* than those who took the drug consistently. In Costa Rica, with a successful antimalaria program, serologic surveys were used to locate areas of possible transmission and areas where expensive attack measures could be withdrawn. The technique was considered useful by the country program and an individual sent to the NIH/NIAID Laboratory in Chamblee/Atlanta, Georgia for training is developing the capability for local IFA testing. In an area of El Salvador under residual propoxur spraying, a group of persons were selected for monitoring of IFA titers. All persons were radically treated at the beginning of the study to eliminate possible relapses. The results of an initial survey and a six-month follow-up were compared to locate individuals who showed evidence of infection (sero-converters) and therefore to demonstrate failure of the attack measure to interrupt transmission.

10. Health education investigations

In most CAMRS studies of antimalaria methods an effort has been made to

evaluate factors which may influence behavioral response to the measures. These have included aerial larviciding, limited cycles of antimalaria drugs, the exterior use of residual insecticides, source reduction by water management, and utilization of surveillance systems as a control method.

One example of the value of inclusion of health education in planning a new activity was seen when PAHO and CNAP initiated a large-scale test of propoxur. Use of the CAMRS health educator's knowledge accumulated in other studies led to the training of spraymen as "educators". The result was no refusals to allow house spraying, in contrast to the high refusal rates in another area of propoxur spraying where population preparation had been inadequate.

Another area in which population attitudes must be understood is in the selection of a surveillance mechanism which is most efficient (high in case finding capability and low in cost). Studies of the passive detection posts manned by lay volunteers revealed an impressive accuracy of self-diagnosis among the general population--in interviews to determine why persons with fever failed to go to the posts, more than one-third said they did not go because they did not think they had malaria or were waiting to see if their fever was caused by malaria. The cost of this highly productive system is a fraction of that of the active case detection system of house-to-house canvassing.

E. Plans for Dissemination and Application of the Expected Results of the Research

The dissemination and application of the findings of research done at CAMRS has been assured through four main avenues of communication: (1) reports, (2) continuing direct contacts with directors and staff at national and regional malaria programs, and with other individuals and organizations in the region and elsewhere with similar interests, (3) regional and other organized meetings of malaria program personnel and other malaria oriented professional groups, and (4) publication in scientific journals.

1. Reports

Regular reports of the activities of the Station are made quarterly and annually to AID/Washington through Malaria Program CDC. Quarterly reports are distributed to allied research groups at CDC, to both technical and line organizational components of AID/Washington, to international organization headquarters groups concerned with malaria eradication (WHO/Geneva and PAHO/Washington), and to groups in various parts of the world considering similar research problems. Annual reports are provided in both English and Spanish and are widely distributed, not only to those recipients listed above but also to national malaria eradication programs in the Western Hemisphere (and elsewhere if deemed appropriate), to laboratories and institutions throughout the world with interests in malaria research, and to any others who may request the annual reports.

2. Continuing Direct Contacts

CAMRS staff members frequently provide consultative services on particular

problems at the request of USAID missions and national programs. Also, there are numerous opportunities in the course of studies in the several countries in the region to visit the country malaria eradication program headquarters and to talk with program directors and members of their staffs. All studies which require field work of any kind are first discussed and cleared with national program directors and are frequently reviewed with them during the course of the study. This provides frequent opportunities, not only to discuss the current study, but to review with them the objectives, progress and results of other CAMRS projects which may be applicable to the individual country programs, and to provide recommendations as to operational methodology based on CAMRS studies and demonstrations.

CAMRS has received approximately 500 visitors during the five years of operation. These have included researchers, management personnel, students, field workers, and trainees. The exchange of ideas has been of benefit to the CAMRS staff and to the visitors representing some 30 countries. The visits have ranged from a few hours to discuss the research activities in general to a period of two months to work with CAMRS staff on a specific problem of mutual interest.

CAMRS staff also maintains direct contact with many other individuals and organizations in the region and elsewhere through the medium of visits to and from these sources of contact. These include the Gorgas Memorial Laboratory (Panama), the Middle America Research Unit (NIH/Panama), the University of Costa Rica ICMRT group (San José), the Canal Zone and U.S. Army Malaria and Vector Control Units (Panama), the Pan American Health Organization Malaria Eradication Division Headquarters and Zone Office (Washington and Guatemala), the El Salvador College of Agriculture, the ROCAP Malaria Advisor (Guatemala), the USAID Public Health Advisors in the several countries, the World Health Organization Malaria Eradication Division (Geneva), the INCAP group (Guatemala), the ODECA Public Health Division (San Salvador), National Institutes of Health groups (Bethesda, Maryland and Chamblee-Atlanta, Georgia), Walter Reed Army Institute of Research (Washington), the U.S. Department of Agriculture Entomology Research Laboratories (Gainesville, Florida and Lake Charles, Louisiana), the Ross Institute of Tropical Hygiene (London), and many others.

3. Regional and Other Organized Meetings

A Working Group of the Malaria Eradication Program Directors of Central America and Panama meets twice yearly to discuss the methods, problems, and progress of the respective national programs. CAMRS participates in these meetings, presenting a report on the problems under investigation, the results of studies, and any recommendations which can be based on the findings. At these meetings the problems considered to be of a high order of importance by the program directors are made known to the CAMRS representatives and are considered for inclusion in the CAMRS work plans.

Annual meetings of the Malaria Eradication Program Directors of the region which includes Central America, Panama, Mexico and the Greater Antilles countries are also attended by CAMRS representatives and a report of activities presented.

CAMRS served as host for the CDC, WHO/PAHO, Government of El Salvador sponsored Inter-American Malaria Research Symposium held in November 1971. Some 210 participants represented 29 countries. Researchers and Latin American malaria program personnel were brought together to discuss the most recent findings from malaria research.

Staff members attend annual meetings of scientific societies to present papers on significant findings of the research to obtain earlier exposure of the research results, making them more quickly available for use. These societies include the Entomological Society of America, the American Society of Tropical Medicine and Hygiene, the American Mosquito Control Association, the American Chemical Society, the American Society of Parasitologists, the American Public Health Association, and others.

A CAMRS representative attends the annual "Tri-Lab" meeting for discussion of vector control technology with representatives from the USDA Entomology Research groups, the TVA Environmental Research and Development Laboratory, and the CDC Technical Development Laboratory.

The CAMRS staff includes a member of the WHO Expert Panel on Malaria and of the Malaria Commission of the Armed Forces Epidemiological Board, and meetings are attended as appropriate.

4. Publication in Scientific Journals

Significant findings are submitted for publication in appropriate scientific journals as information becomes available. (See Appendix H.3.)

F. Statement of Expenditures and Obligations and Contractor Resources

CAMRS Funding Summary

	Fiscal Year						
	1967	1968	1969	1970	1971	1972	Total
Personnel Costs		73,005.51	228,528.62	259,543.13	331,610.29	321,647.87	1,214,335.42
Travel		15,534.07	19,933.60	32,312.77	23,458.59	21,639.40	112,878.43
Transportation of Things		15,557.12	18,291.64	18,918.01	16,045.34	18,737.04	87,549.15
Contractual Services, Rents, Printing, etc.		24,575.93	9,939.48	39,112.71	23,427.89	26,596.13	123,652.14
Supplies & Materials		35,509.23	93,024.55	50,613.13	13,053.66	23,753.76	215,954.33
Equipment		54,894.99	21,309.24	15,016.08	7,400.23	5,564.80	104,185.34
Overhead		4,919.07	9,177.00	10,279.00	10,654.00	12,561.00	47,590.07
Uncoded							
Billed AID/W directly	139,975.89	42,058.50					182,034.39
Billed by CDC	7,973.23						7,973.23
Total	$147,949.12	$266,054.42	$400,204.13	$425,794.83	$425,650.00	$430,500.00	$2,096,152.50

G. Work Plan and Budget Forecast for Coming Year

Not Applicable.

H. Appendices

1. Activities Report, July 1, 1971-June 30, 1972

2. Activities for the Year Ending June 30, 1971
   Activities for the Year Ending June 30, 1970
   Activities for the Year Ending June 30, 1969
   Activities for the Year Ending June 30, 1968
   (available on request)

3. Publications and Presentations

APPENDIX 1

Activities Report, July 1, 1971-June 30, 1972

ACTIVITIES REPORT

JULY 1,1971 thru JUNE 30,1972

CENTRAL AMERICA MALARIA RESEARCH STATION

under Participating Agency Service Agreement Number RA(HM) 2-67
PIO/T Number 931-17-511-474-72-3127701 with U.S. Agency for International Development

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

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ACTIVITIES REPORT

JULY 1,1971 thru JUNE 30,1972

CENTRAL AMERICA MALARIA RESEARCH STATION

under Participating Agency Service Agreement Number RA(HM) 2-67
PIO/T Number 931-17-511-474-72-3127701 with U.S. Agency for International Development

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

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VECTOR ECOLOGY STUDIES

Anopheles Breeding Habitats and Adult Densities

While field studies were concentrated in the Apastepeque area for most of the year, opportunistic collections were made to augment continuing seasonal studies of coastal situations.

The several rivers in the La Libertad area between the Tunco and Huiza rivers were virtually void of breeding by mid-June 1971 with only one positive collection (three *A. pseudopunctipennis* larvae in 100 dips from an isolated pool in Rio Chilama, July 7) being found after that time until favorable habitats again developed in late December. This was followed by consistent and increasing breeding from early January. A total of 1180 dips from 11 rivers during January, February, and March, normally the months when river breeding is at a peak, yielded 961 larvae, for an average of 0.8 larvae per dip, and an *A. albimanus* to *A. pseudopunctipennis* ratio of 9:11. For the most part, adult anophelines were absent from the associated bridges by late June, but two female *A. albimanus* were found under the Rio Aquisquillo bridge on July 27; and both *A. albimanus* (one female) and *A. pseudopunctipennis* (two males, one female) were found under the Rio Huiza bridge on the same date. An unusual occurrence of *A. pseudopunctipennis* resting under the Comasagua bridge in late August and early September was observed when a total of 60 specimens (20 males, 40 females) were collected on August 30 and 22 specimens (21 males, one female) on September 7.

The seasonal nature of *Anopheles* breeding in Estero San Diego was again reflected in 1971 by results of larval sampling in the estuary (estero) and associated adult captures in a New Jersey light trap at San Diego Beach. Larval sampling yielded an average of 10.0 *A. albimanus* larvae per dip on May 4; 8.0 per dip on May 20; 4.0 per dip on June 15; and 1.8 per dip on July 20. No larvae were collected after that date until the dry season of 1972 when breeding was detected on March 13. Meanwhile, the San Diego light trap averaged 180 *A. albimanus* per trap night in May; 203 in June; and 208 in July 1971. The average then dropped to 20 per trap night in August and to nine in September, after breeding had been eliminated due to the natural opening of the estuary in late July. The trap reflected the general absence of breeding between July 1971 and March 1972 with negligible captures (average of one per trap-night). The number increased to 11 on March 27 and to 46 by April 4 with the return of favorable conditions resulting from the natural closing of the estuary.

Breeding was moderate in Estero Ticuiziapa during both the rainy season of 1971 and the dry season of 1972. During 1971 there was an average of only 0.2 larvae per dip in July; 0.4 in August; and 0.3 in September. During the dry season of 1972 the average was 0.8 in January; 1.6 in February; and 0.2 in March. The associated light trap generally reflected the dry season breeding showing an average of 6.0 *A. albimanus* per trap-night in January, 1.0 in February and 1.0 in March.

Lowland wet weather habitats followed the seasonal pattern of rather heavy breeding in early rainy season (June-July) with reduced breeding during mid-rainy season (August-October) when waters tend to stagnate, and with breeding

rebounding somewhat in late rainy season (November-December), following the gradual receding of basin water, when fresh intermittent rains created new plant-air-water intersection line. This important point may be illustrated by results of light trap catches at Ticuiziapa Beach compared with light trap captures from San Diego Beach. The Ticuiziana light trap reflects pasture and marsh breeding which is subject to habitat conditions described above, whereas the San Diego trap reflects estuary breeding uncomplicated by significant outside breeding. Results from the Ticuiziapa trap show an average of 64 *A. albimanus* per trap-night in June and 17 in July. The mid rainy season lull followed with counts of 2.6, 1.1 and 1.2 for August, September, and October, respectively; followed by an increase to 1.8 in November, 7.8 in December, and 14.0 for the first week of January with an average of 6.0 for the month. By contrast, the San Diego trap averaged 203 *A. albimanus* per trap-night in June, 208 in July, and dropped to 20 in August after the estuary opened in mid-July, and reduced to nine in September, 2.5 in October, 0.4 in November, 0.5 in December, and zero for the first week of January with no late rainy season rebound. These data help validate the annual recurrence of a late season population increase in coastal pasture and marsh situations. The described buildup might be of significance in "seeding" the same pasture during dry season when irrigation is commenced. For example, anopheline breeding in irrigated portions of pastures was unusually high in January 1972 in both large pastures of the area, previously discussed Ticuiziapa, east of La Libertad, and Santa Emilia, west of La Libertad. Sampling in the Ticuiziapa pasture in January yielded 869 larvae in 180 dips (4.8 per dip) 77% of which were *A. albimanus*, while the Santa Emilia pasture sampling yielded 639 larvae in 165 dips (3.9 per dip), 89% being *A. albimanus*. The same pattern has been observed in other seasons in both of these pastures, i.e., the early rainy season peak, the mid-season lull, and the late season peak followed by large populations during the early part of the subsequent dry season.

The coastal area comprises a complex of habitats available to anophelines and these habitats are utilized as they become suitable. In combination they support a rather constant level of breeding, although the components might contribute different levels of habitat availability at different times, depending upon prevailing conditions. For the period January-March, river breeding dominated the study area in 1970, estuary breeding dominated in 1971, and pasture breeding dominated in 1972 (Table 1). In spite of this population "shift", the total larval sampling showed a rather constant density for the period ranging from 1.8 to 2.5 larvae per dip.

TABLE 1
Anopheline Breeding from Major Sources
La Libertad Area of Coastal El Salvador
January-March, 1970-1972

	Larvae per dip		
Source	1970	1971	1972
Rivers	3.2	1.8	0.8
Estuaries	0.2	3.7	0.5
Pastures	1.0	2.8	4.4
Collective	2.5	2.4	1.8

Collections from miscellaneous rainy season breeding sites have been sparse during the year due to special attention being given to population studies at Lake Apastepeque, discussed separately. However, even in the small sample, densities seem to be similar to those of other years in that 655 dips from such sites yielded 997 anopheline larvae, or 1.52 per dip (4 year average - 1.76 per dip).

Studies of the anopheline populations in the Lake Apastepeque area are included in this report in Vector Control Studies: Lake Apastepeque Sterile Male Release Project.

Host Preference Studies

The results of microprecipitin tests to determine vertebrate host preference during 1970-1971 were fully reported in the 1971 CAMRS Annual Report. However, some additional results from specimens taken during 1971 were received after that report was prepared, and these have been added to previous data to show complete results from all tests conducted between March 1970 and June 1971. The data added during the year were similar to our overall experience except for four double feedings by *A. albimanus* on chicken-human and one double feeding by *A. pseudopunctipennis* on pig-horse. Neither of these double feeding combinations had previously occurred in the study and only one other collection of four specimens has been positive for chicken.

A review of the data of Table 2 shows that 48.3% of 1,132 specimens of *A. albimanus* collected from inside houses had fed on humans and 26.1% on cattle. In contrast, collections from outdoor resting sites included only 1.7% human feedings and 88.3% cattle. Double feedings have occurred in seven combinations from intradomiciliary collections, five involving man, and in four combinations from outdoor natural resting sites, only one involving man. Five kinds of animals (humans, cattle, dogs, pigs, and horses) furnished the vast majority of feedings whether specimens were collected indoors or out-of-doors.

In both *A. albimanus* and *A. pseudopunctipennis* from natural resting sites the same five animals (cattle, horses, pigs, dogs, and man) accounted for over 97% of all feeding (Table 3). Cattle was the major host and man a very minor one in both cases--an expected finding in outdoor collections.

The highlight of host preference studies during the fiscal year centers around the development of a precipitin testing competency at CAMRS for blood meal identity in field collected anophelines. During the first quarter of CY-1972 capability had been developed for identifying blood meals from humans, cows, and horses. In a sampling of 60 specimens of known blood meal sources, no errors were made by the technician in identifying a lot of specimens including the above animals plus guinea pigs (listed as unknown mammals) from *A. albimanus* in filter paper preparations. Pig and dog antisera were prepared during the subsequent quarter so that by the end of the fiscal year, CAMRS had the competency to determine blood meal sources from the five animals which have made up about 95% of approximately 2,700 specimens representing collections of all types from eight departments of El Salvador. Thus, it is not anticipated that preparation of antisera from additional kinds of animals is indicated at this time. This competency at CAMRS will allow essentially on-the-spot blood meal identifications which will be extremely useful in epidemiologic investigations.

TABLE 2
Host Preference of *Anopheles albimanus*
El Salvador, March 1970-June 1971

Host	Intradomiciliary Collections		Collections From Natural Resting Sites		All Collections	
	No.	%	No.	%	No.	%
Man	547	48.3	19	1.7	566	24.9
Cow	295	26.1	1,007	88.3	1,302	57.3
Dog	164	14.5	15	1.3	179	7.9
Pig	49	4.3	47	4.1	96	4.2
Horse	5	0.4	17	1.5	22	1.0
Unknown Mammal	33	2.9	29	2.5	62	2.7
Other	10	0.9	0	-	10	0.4
Man/Cow	15	1.3	1	0.1	16	0.7
Man/Dog	3	0.3	0	-	3	0.1
Man/Pig	2	0.2	0	-	2	0.1
Man/Chicken	4	0.4	0	-	4	0.2
Man/Racoon	1	0.1	0	-	1	0.1
Cow/Horse	2	0.2	1	0.1	3	0.1
Cow/Dog	2	0.2	0	-	2	0.1
Cow/Pig	0	-	3	0.3	3	0.1
Pig/Horse	0	-	1	0.1	1	0.1
Total	1,132		1,140		2,272	

TABLE 3
Host Preferences of *A. albimanus* and *A. pseudopunctipennis*
in Natural Resting Sites
El Salvador, March 1970-June 1971

Host	*A. albimanus*		*A. pseudopunctipennis*	
	No.	%	No.	%
Cow	1007	88.3	340	82.1
Pig	47	4.1	3	0.7
Man	19	1.7	4	1.0
Horse	17	1.5	46	11.1
Dog	15	1.3	7	1.7
Unknown Mammal	29	2.5	10	2.4
Man/Cow	1	0.1	1	0.2
Cow/Pig	3	0.3	1	0.2
Cow/Dog	0	-	1	0.2
Cow/Horse	1	0.1	1	0.2
Pig/Horse	1	0.1	0	-
Total	1140		414	

Natural Plasmodium Infections in *Anopheles*

Since March 1970, a total of 5,066 *A. albimanus* females from 535 collections in nine departments of El Salvador have been dissected for malaria parasites (Table 4). Through June 1972, the dissections showed four salivary glands positive for sporozoites and 4,273 negative glands. All of 789 mid-guts have been negative for oocysts. Additionally, a total of 827 *A. pseudopunctipennis* yielded 754 negative glands and 73 negative mid-guts. A single gland dissection from *A. punctimacula* was negative.

TABLE 4
Anopheline Dissections for Presence of Malaria Parasites
El Salvador, March 1970-June 1972*

Species	Salivary Glands		Mid-Guts		Dissected
	Pos.	Neg.	Pos.	Neg.	
A. albimanus	4	4273	0	789	5066
A. pseudopunctipennis	0	754	0	73	827
A. punctimacula	0	1	0	0	1
Total	4	5028	0	862	5894

* Data from 535 collections, 9 Departments

Longevity Studies (Physiological Age-Grading)

For reasons explained in the CAMRS Annual Report for 1971, ovariole dissections to determine gonotrophic cycles in *Anopheles albimanus* were temporarily suspended at CAMRS. Meanwhile, dissections to determine only the parous or nulliparous state of population samples by the tracheole method were conducted during January-March 1972. Results show 49 (13.2%) parous among 370 *A. albimanus* dissected from collections representing five departments (Table 5). In earlier studies using ovary dissections (April-September 1970) 49 of 423 specimens (11.6%) were parous. The agreement of these data by the two methods lends added importance to resolving the question of gonotrophic concordance or dissociation in *A. albimanus*, for future studies on longevity will almost certainly require techniques providing more accurate age-grading than is afforded by the tracheole method.

TABLE 5
Tracheole Dissections of *A. albimanus* for Age-Grading
El Salvador, January-March 1972

Department	Number		%
	Nulliparous	Parous	Parous
Ahuachapán	20	4	16.7
La Libertad	4	4	50.0
La Paz	90	11	10.9
San Miguel	28	4	12.5
San Vicente	179	26	12.7
Total	321	49	13.2

Anopheles albimanus Strain Variants

Circumstantial evidence has accumulated which suggests the possible existence of subgroups in the *Anopheles albimanus* population in Central America. These groups seem to exhibit behavioral variations in feeding preferences, tendency to enter houses, breeding sites, affinities for different types of traps, etc. Investigations are now underway at CAMRS which are designed to search for morphologic or physiologic markers that may assist in identifying these behavior groups. Colonies of *A. albimanus* are being established from a variety of backgrounds. Populations from interior as well as coastal areas, from coastal estuaries and flooded pastures, from the dry as well as wet seasons, from houses and stables, from areas with localized epidemics, and isolates from different countries are either now colonized or will be studied in the future. Each population is evaluated morphologically and, in cooperation with Dr. James B. Kitzmiller of the University of Illinois, variations in chromosome patterns are also studied. Possible variation in susceptibility to malaria, potentially a highly important factor, is investigated through the use of monkeys infected with human malaria parasites.

At present colonies under study include, Melara (collected from a coastal cattle stable), San Diego Sur (collected from a coastal estuary), San Diego Norte (collected from a permanent interior lagoon), Canoñcito (collected from a house near the beach at Cangrejera where an unusual number of *P. falciparum* cases had occurred), and Apastepeque (collected from an interior lake where a sterile male mosquito release project is underway). It is hoped to add isolates from Haiti, the Atlantic coast of Nicaragua, and the Lake Gatun area of Panama in the near future.

One morphologic variant has been recorded which is currently under study. The Salvadoran *A. albimanus* produce two varieties of pupae, one that is plain and one that has a distinct white stripe. Ratios of striped to non-striped pupae vary geographically in El Salvador. The San Diego Norte colony has been selected so that there are now two sub-colonies, one producing only plain pupae and one only striped forms. Genetic studies are now underway to define the basic aspects of this character. It is interesting that initial observations on a population from Panama reveal only non-striped pupae while all those from Haiti seem to have stripes.

These studies are directed toward the identification of a possible group or strain within the *A. albimanus* complex that is more involved in human malaria than the population as a whole. Some workers believe that several species will eventually be recognized from what is now called *Anopheles albimanus*. The advantages, in terms of malaria control, would be obvious if a specific, identifiable part of the very large mosquito populations in this area could be labeled as the malaria vector, possibly providing a "vector population" more amenable to control.

VECTOR CONTROL STUDIES

Residual Insecticides

During the year, studies were continued on two carbamate compounds: propoxur (OMS-33) and Landrin (OMS-597). These studies included village scale and large scale field trials, with associated chemical and entomologic evaluations, as well as laboratory studies on the characteristics of the insecticides and their effect on the vector.

Studies on Landrin (OMS-597)

Village scale trials: In July, 197 houses in the locality of La Zunganera, near the Pacific coast, were sprayed with Landrin (70% W.W.P.) using a target dose of 2 grams/square meter. All inside wall surfaces to a height of 3 meters from the floor were sprayed. No difficulties were experienced in preparing the suspension, nor did the material clog nozzles or settle unduely in the pump tank during the operations. No toxic symptoms were noted among the spraymen or householders during the course of the spraying. Spraymen were instructed to wash hands and face after completing a house, bathe at the end of the day and change to a clean uniform daily. Householders were instructed to remove all foodstuffs and cooking utensils from the house and to remain outside themselves until one hour after the spraying.

The entomologic evaluation was by weekly bioassay testing of sprayed surfaces, following techniques described in the Seventeenth Report of the WHO Expert Committee on Insecticides (1968). Mosquitoes were exposed under plastic cones to wall surfaces of palm thatch, brick, wood and mud. Approximately 180 specimens were exposed in each individual test, and the mortalities recorded 24 hours after exposure. Considering 70% or greater mortality to represent effective control of exposed anophelines, it appears that the period of activity was five to seven weeks, depending on the surface tested (Table 6).

TABLE 6
Bioassay of Landrin Applied as an Interior Spray:
Percentage Mortality in Mosquitoes

	Week after Treatment							
Surface	1	2	3	4	5	6	7	8
Palm	100	100	100	92	79	73	76	48
Wood	92	92	87	89	76	65	53	69
Brick	97	90	86	73	71	44	55	42
Mud	97	98	89	74	78	64	59	68
Control	21	0	5	1	2	7	1	2

Studies on the fumigant activity of Landrin were conducted in this field trial. Air samples were collected from the interior of treated houses of

differing construction materials and analyzed for insecticide content. The findings showed a similar trend as was found for propoxur previously, in that the insecticide was no longer detectable in the air 17 to 20 days following spraying. The 2, 3, 5 trimethylphenyl methylcarbamate isomer, which is present at a concentration of 1/4 that of the 3, 4, 5-isomer, was absent from the air samples collected after ten days.

Because of a hypothesized increase in persistence of effectivity after multiple applications of this compound, 12 selected houses were resprayed in October, approximately 12 weeks after the first treatment. The residues were evaluated in a similar fashion in the same houses utilized during the first cycle. Figure 1 details the mortality seen during the ten week periods following both spraying cycles. After the first application the average mortality decreased below the 70% level after the fifth posttreatment week, although, as detailed above (Table 6) the wood surface retained this level of effectivity through the seventh week. After the second spraying of these premises the average mortality remained well above the 80% level for the entire ten-week period, and the differences between the surface types seemed to be minimized. Effective levels (i.e., >70%) persisted for two additional weeks.

The analysis of air samples collected after the second spraying showed a similar pattern. While after the first cycle the insecticide persisted for only a relatively short period as a fumigant (17-20 days), the compound could be detected after the second treatment through 63 days, with the 2, 3, 5-isomer being present in some samples as long as 35 days.

Table 7 summarizes the air sampling results in palm, wood, and mud wall houses.

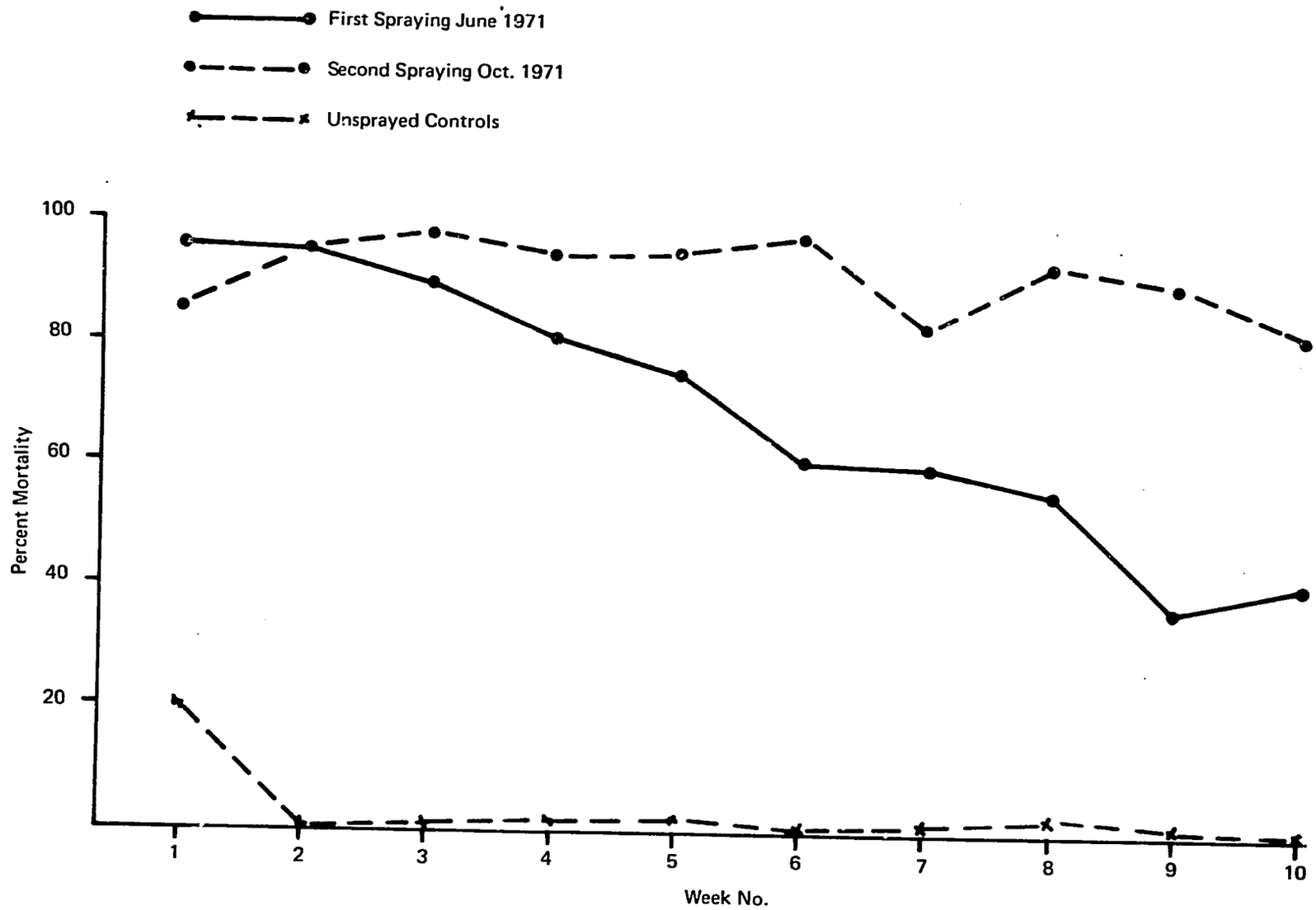
TABLE 7
Air Concentrations of Landrin in Houses Following a Second Interior Application at a Rate of 2 grams per Square Meter*

Days After Application	House Construction			Temp °C	RH %
	Palm	Wood	Mud		
1	16.06	27.10	56.71	29	77
7	11.99	14.99	18.27	30	70
14	7.78	12.71	8.34	29	84
21	8.28	11.64	8.20	30	70
35	3.42	3.78	2.78	32	45
63	3.72	2.80	1.17	33	41

* Results are expressed as nanograms per liter and are the average of two replicate samples for each house type. All results have been corrected for recovery.

A possible explanation for the enhanced persistence following the second application can be suggested: The first application of the compound occupied various sorption sites on the treated surfaces, particularly those of a more porous type, and because of this deeper sorption the chemical was less available for surface activity or vaporization; on the second application, many of the sorption sites were still occupied, allowing a greater percentage of

FIGURE 1
Bio-assays After First and Second Applications of Landrin
Average of Palm, Wood, Brick and Mud Interior Surfaces of Houses



the compound to remain at the surface and to be available for contact or for vaporization. Whether the enhancement in bioassay mortality after the second treatment was due to persistence of surface activity, persistence of fumigant activity, or a combination of the two, remains to be clarified.

The studies completed to date suggest that Landrin is approximately equal to propoxur in its effectiveness, persistence and ease of application when used as an interior residual house spray.

Exterior Application of Propoxur

Small scale tests on the application of propoxur to the outside walls and eaves of houses were done by CAMRS in 1970. These tests indicated that exterior application of the insecticide significantly reduced inside resting and biting of *Anopheles albimanus* for a period of approximately six weeks after spraying. Since outside spraying has obvious operational advantages compared with traditional inside spraying, it was decided to test this method of application on a wider scale, treating all houses in District 13 in the Department of La Libertad.

In late June 1971 outside spraying was completed in District 13, covering 1,260 houses and using 420 kilograms of propoxur 70% WWP. The spraying was done by locally recruited spraymen trained by CNAP supervisors. In the spraying team of six men, two showed mild symptoms of nausea and one man vomited during the first day of spraying. These symptoms disappeared after a rest period, and did not reappear in any spraymen during the two weeks of spraying. No householders reported any ill effects from the insecticide application.

The entomologic evaluation of this trial was carried out during July-September. Weekly captures of resting mosquitoes were made in 40 reference houses, chosen to represent all of the house construction types found in the area. In addition, weekly human bait captures were made inside and outside of eight houses, simultaneously, from 6:00 to 8:00 p.m. As a measure of the total resting population of *A. albimanus* in the area, weekly captures were made also in eight unsprayed stables. The entomologic findings indicated that outside spraying with propoxur significantly reduced inside anopheline resting and inside biting compared with outside biting for a period of approximately six weeks following the insecticide application.

In the operational analysis, a sprayman could exteriorly treat an average of 9.5 houses per day, compared with 5.9 houses per man-day in conventional total inside spraying. This spraying technique used 40% less insecticide than the amount used in total inside house spraying.

Chemical analyses of fumigant and surface activity were done to supplement the entomologic findings, with periodic sampling of treated houses of several types of construction. In air sampling, the findings closely paralleled those of previous investigations on this insecticide when applied for the first time. No detectable amounts of the chemical could be found in the air 21 days after application. (Table 8).

TABLE 8
Concentration of Propoxur* in Air Surrounding
Houses Receiving Total Exterior Coverage
Santo Tomás, El Salvador

Days After Application	Type House Construction			Temp. °C	RH %
	Mud	Pole	Palm		
1	5.7	40.7	12.5	31.6	72
2	4.8	13.2	5.5	31.1	60
7	1.2	4.3	3.7	32.2	57
14	0.0	0.0	0.6	32.1	58
21	0.0	0.0	0.0	31.6	50
28	0.0	0.0	0.0	32.2	58

* Results are expressed as nanograms per liter of air, and as the average of four replicated samples.

The sampling of mud wall and palm thatch surfaces consisted of 5 cm. x 5 cm. areas scraped or cut from the surface and analysis of extracts by the usual methods for such materials. For the mud surfaces, areas protected by overhanging eaves from direct sunlight, rainfall or other adverse environmental factors were compared with areas of the wall not so protected. The results (Table 9) showed that concentrations of propoxur were consistently higher for the protected surface for the first 30 days. However, with increasing time the concentrations became equal, probably reflecting that portion of the insecticide which is tightly sorbed by the surface and only slowly lost by volatilization or degradation.

TABLE 9
Propoxur Residue* on/in Treated Mud Surface
Santo Tomás, El Salvador

Days After Application	Mud Surface		Temp. °C	RH %
	Protected	Unprotected		
1	3.47	2.00	31.6	72
2	3.04	1.33	31.1	60
9	2.09	0.90	32.2	57
16	1.69	1.00	32.1	58
23	0.85	0.22	31.6	50
30	1.77	0.50	32.2	58
37	0.28	0.38	30.0	60
65	0.15	0.15	31.0	77

* Results are expressed as milligrams per 5 cm x 5 cm surface scrapings, and as the average of two replicated samples.

For the palm thatch surfaces (Table 10) a loss of approximately 80% of the insecticide could be seen by the end of the first week after application. Since the entomologic evaluations during this test showed satisfactory control for a period of six weeks after treatment, and the residual concentration of propoxur on the mud and palm surfaces after five weeks averaged 0.30 mg. per 5 cm. x 5 cm. sample, it would appear that this concentration closely approximates the lower limit at which effectiveness of the insecticide may terminate, at least in this type of exterior application.

TABLE 10
Propoxur Residues* on/in Palm Roofing Material
From Houses Treated Exteriorly
Santo Tomás, El Salvador

Days After Application							
1	2	9	10	23	30	37	65
4.22	3.67	1.01	0.98	0.70	0.35	0.30	0.07

* Results are expressed as milligrams per 5 cm x 5 cm sample and as the average of three replicated samples.

Interior Application of Propoxur

As part of the investigations being undertaken in District 13 during the calendar year 1972, all houses in the area were sprayed with a partial (2-swath) application of propoxur once every 35 days, in the manner utilized by the WHO AMRO-0216 project previously underway. Spraying was initiated March 1972. All houses in the study were of the pole-wall, thatched-roof construction. Chemical analyses showed a greater degree of persistence of the chemical in the air when applied interiorly than with the exterior applications reported above (Table 11). This can undoubtedly be attributed to the reduced effect of weathering and the semi-confinement of the vapors which almost any type of house construction would provide. Fumigant bioassay tests, using caged, propoxur-susceptible colony mosquitoes, were done in sprayed premises in three localities of the area treated (Table 12). In general, while a full range of interior periods was not explored, the high mortality seen during the first week after spraying diminished somewhat by three weeks posttreatment. In some cases, mosquitoes exposed near sprayed walls showed a greater mortality than those exposed in more central areas.

As an additional check on the effectiveness of the insecticide, weekly searches for daytime resting mosquitoes were made in 16 houses in each of six principal localities in the area. Resting mosquitoes were found in houses in two of the localities as early as one week after spraying. The presence of resting adults during the day in a sprayed house is considered indicative of a failure of insecticide effectiveness in that particular house.

The epidemiologic aspects of this study are detailed elsewhere in this report.

TABLE 11
Air Concentration of Propoxur, Expressed as Nanograms per Liter in Houses Receiving 2-Swath Interior Applications Once Every 35 Days

House #	Days After Application					
	3	10	17	24	32	3
56	34.68	14.20	20.00	12.85	4.40	24.28
57	7.14	0.0	-	-	-	-
57A	24.28	7.14	5.71	6.42	3.57	8.57
57C	21.43	4.29	4.28	2.84	5.71	21.43
57E	-	-	-	-	6.43	14.29

TABLE 12
Fumigant Bioassays, District 13, 1972
(Each Test: 20 Mosquitoes Exposed 90 Minutes)

Locality	Date Sprayed	Date Tested	House No.	24-hour Mortality	
				Wall	Center
Melara	6 June	14 June	63	100	100
			64	100	100
			64a	100	100
			66	100	100
Control 1				0	
Control 2				30	
Toluca	24 May	15 June	14	55	5
			15	100	85
			13	70	5
			12	0	0
Control 1				0	
Control 2				0	
Chaguiton	30 May	22 June	177-S	100	100
			173-A	45	50
			177-K	35	50
			177-F	100	95
Control 1				0	
Control 2				0	

Laboratory Studies on Propoxur

A study was made on the air concentration of propoxur necessary to produce 100% mortality in *Anopheles albimanus*. A solution of the insecticide in acetone was pipetted into a 500 ml flask and the acetone removed by gentle heat and vacuum on a rotary evaporator, the insecticide residue being retained and distributed about the lower portion of the flask. Air from this treated flask was then drawn through glass tubing packed with glass wool into a second clean flask containing five blooded *A. albimanus* mosquitoes. The air from this second flask was in turn drawn into an impinger charged with a trapping solution of aqueous sodium hydroxide. The entire system was made with glass to glass connections where possible and teflon-lined rubber tubing was employed elsewhere. The process was a continual flow operation with air entering the insecticide flask, passing into the flask with the mosquitoes and then into the impinger at a rate of 2.8 liter/minute. The time for 100% knockdown was recorded and the concentration of insecticide present in the trapping medium determined in the manner previously described for air samples.

The average time to knockdown in 14 tests was 12.9 minutes (range: 8.5-18.3) and the average air concentration determined as 16.06 nanograms per liter (range: 14.34-18.09). Following knockdown, the mosquitoes were transferred to clean holding flasks covered with nylon screening and recovery recorded after 24 hours. In all instances, mortality was 100%.

In a second series of tests, after knockdown was complete, the flasks in which knockdown occurred were left unstoppered in the laboratory for two and one-half days. Fresh blooded *A. albimanus* were then introduced into the flasks. The flasks were unstoppered but covered with nylon screening to prevent mosquito escape and to support cotton saturated with sugar-water. Mortality was 80% after 24 hours in three such tests.

The entire test procedure was repeated and, in addition to analysis of the impingers, the walls of the flasks were washed with acetone, the acetone added to aqueous sodium hydroxide and analyzed separately. The air concentration was approximately the same as in the first tests. The residue from the walls of the flasks averaged 2.22 micrograms (range: 1.92-2.64).

Observation of the mosquito mortality in flasks into which insecticide containing air had been drawn and left unstoppered for two and one-half days suggested a residual on the walls of the flask which was later confirmed by chemical analysis. It is evident therefore that the insecticide can be readily sorbed by various surfaces and makes it difficult to ascertain the route of incorporation into the mosquito body. Further controlled experiments are necessary to establish the mechanism involved.

Studies on Propoxur Resistance in *A. albimanus*

In 1971 propoxur resistance was reported in a strain of *A. albimanus* which originated in Melara, a locality in District 13. The absence of mortality among locally captured mosquitoes used in bioassays and the presence of resting mosquitoes in presumably sprayed houses, provided suggestive evidence of resistance in widely distributed populations of the area. Since

propoxur is the only control measure being attempted in the area, it is important to determine the distribution and degree of resistance in wild populations. To date, all tests of mosquitoes from three localities, using the WHO test method with one-hour exposure to 0.1% propoxur impregnated papers, have shown propoxur resistance (Table 13). In contrast, control tests conducted with colony reared mosquitoes (Metapán and Apastepeque colonies) showed complete sensitivity to propoxur. CAMRS continued to collaborate with the University of California at Riverside in a study of *A. albimanus* resistance to organophosphorus and carbamate insecticides. Data have been gathered on ten compounds since June 1970 including propoxur, carbaryl, malathion, fenitrothion, Dursban, fenthion, and Abate. Progressive increase in resistance has been shown to some of the key compounds (see 1971 CAMRS Report) and all are being evaluated for patterns of resistance development. With a collection supplied by CAMRS in June 1972, two full cycles of testing for resistance will have been completed for wild populations from the same geographic area of El Salvador where early detection of *A. albimanus* resistance to organophosphorus and carbamate compounds was detected and demonstrated. Cumulative data will be analyzed and results published in the near future.

TABLE 13
Susceptibility Tests of *A. albimanus*, District 13, 1972
One-Hour Exposure to Propoxur 0.1%

Locality	Type of Capture	Date of Capture	Mosquitoes Exposed (#)	24-Hour Mortality (%)
Cangrejera Sur	Intradom.	3 Mar.	75	13
Control	"	"	25	4
Melara	Stable	16 May	17	35
Control	"	"	20	0
Santa Lucía	Stable	23 May	40	30
Control	"	"	20	0
Melara	Stable	23 May	20	50
Control	"	"	20	0
Melara	Stable	30 May	20	40
Control	"	"	20	5
Santa Lucía	Stable	30 May	18	50
Control	"	"	18	5
Santa Lucía	Stable	6 June	73	44
Control	"	"	25	8
Cangrejera	Stable	6 June	20	45
Control	"	"	20	0
Apastepeque Col.	Colony	13 June	102	100
Control	"	"	25	0
Metapán Col.	Colony	15 May	100	100
Control	"	"	25	0
Metapán Col.	Colony	15 May	100	100
Control	"	"	25	4

Lake Apastepeque Sterile Male Release Project

The Gainesville, Florida, USDA Laboratory, in cooperation with CAMRS, in May 1971 initiated a project to determine the feasibility of sterile male releases for controlling *A. albimanus* and to study population dynamics of the species under isolated field conditions. Based on data gathered by CAMRS and the El Salvador malaria program since 1968, the vicinity of Lake Apastepeque, Department of San Vicente was selected as the study site.

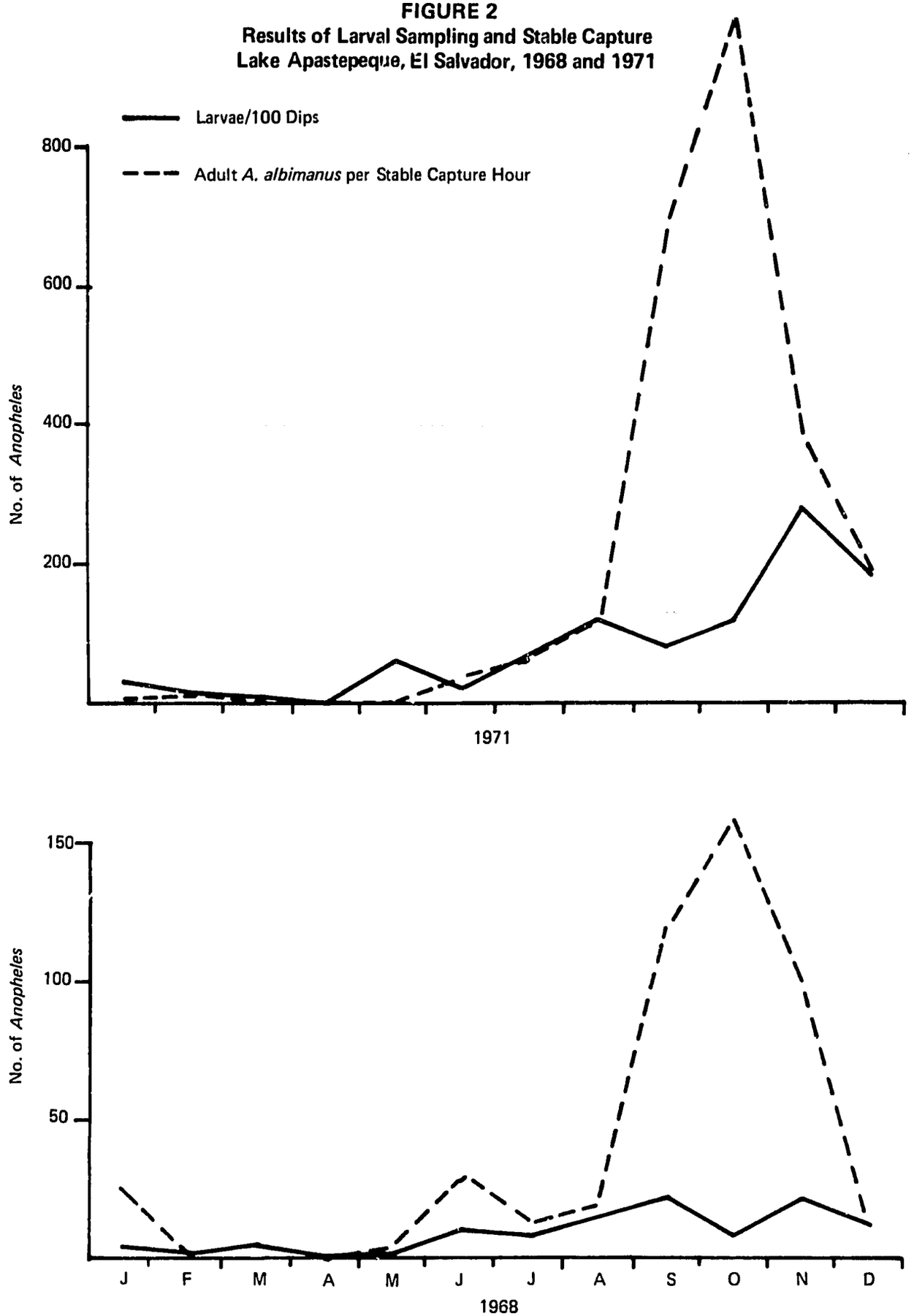
Population Studies

Population studies were actively pursued throughout the year to establish the seasonal pattern (Figure 2), but were intensified in May 1971 when systematic pre-release surveys were begun. Regular releases began in mid-April 1972, according to plan, and are to continue through August in an effort to begin pressuring the natural population while still low, just prior to the beginning of the expected seasonal buildup, and to continue the pressure sufficiently long to prevent the annual peak expected during the last quarter of the year.

The success of the project depends upon rather accurate estimates of population densities and movements of mosquitoes in the area. Larval collections were made at least once weekly throughout the year to determine anopheline production, by species, in both Lake Apastepeque and the nearby pasture east of the lake. Also, a variety of adult-capture techniques were used at frequent intervals to measure existing densities, to determine if released males were integrating with the natural population, and to identify the most useful method(s) for evaluating the cumulative effect of sterile male releases on the *A. albimanus* population. Anopheline breeding was minimal between January and March 1971 (3.3 to 1.1 larvae per dip), increased (to 6.5 per dip) in May with fresh inundations of the lake margins, and generally continued to increase to high densities in the ensuing months producing the predicted late season peaks (28.5 larvae per dip in November). Larval densities, though still high in early 1972, did decline prior to releases which began in mid-April 1972. The nearby pasture did not become a factor in 1971 until July. It was hoped that the same situation would prevail in the release year of 1972, providing additional isolation of the lake population, but a series of rains in early May produced breeding in the pasture; additional release stations were established to overwhelm the newly emerging population with sterile males. A prolonged dry period followed this initial flooding and the pasture was virtually dry again by late July. The effect of the early flooding will not be known until results are tabulated.

Rather high *A. albimanus* densities were measured by all routinely used collecting methods, again reflecting the predicted pattern of annual buildup during the last quarter of the calendar year followed by a decline during the early months of the ensuing year (Table 14). Steady declines were recorded between October 1971 and March 1972: cattle corral counts from 1,337 to 27, New Jersey light trap counts from 145 per trap-night to 7, and natural resting counts from 190 to 7. Human bait counts, on the other hand, have remained consistently high because, in contrast to the other methods with fixed-point capture stations, the human bait captures have been used to obtain specimens for sterility and fecundity studies; therefore, quantity, rather

FIGURE 2
Results of Larval Sampling and Stable Capture
Lake Apastepeque, El Salvador, 1968 and 1971



than population sampling, has been the basic objective. Thus, captures have been made in situations with high biting activity. In spite of an apparently larger population during the first three months of calendar 1972 than in recent years, the population was at a relatively low point when releases were begun in April. In many ways the larger population is desirable for the pre-release period since associated studies require a representative population. The relatively good correlation between larval counts and adult captures lends a degree of confidence to the collecting methods being employed in the project.

TABLE 14
Larval and Adult *Anopheles albimanus* Collections
Lake Apastepeque, 1971-1972

	Larvae/Dip		Adults			
Month	Lake	Pasture	Cattle Corral[1]	N.J. Light Traps[2]	Natural Diurnal Resting Corral[3]	Human Bait[4]
1971-January	3.3	0	1	-	-	-
February	1.4	0	9	3.0	0	7
March	1.1	0	-	0.7	7	2
April	-	0	-	-	-	-
May	6.5	0	-	-	26	3
June	2.1	0	42	7.0	13	3
July	7.4	0.03	67	13.0	31	4
August	12.3	0.3	122	15.0	24	4
September	8.2	1.0	688	55.0	132	7
October	11.8	0.9	1337	145.0	190	36
November	28.5	2.5	386	59.0	35	103
December	18.3	1.1	191	40.0	27	167
1972-January	24.5	0.08	171	15.0	55	260
February	19.1	0	93	12.0	23	77
March	12.8	0	27	7.0	7	108
April	10.6	0	67	5.6	10	135
May	9.4	0.6	95	13.6	60	147
June	3.8	0.4	52	6.8	43	45

[1] Females per nighttime collection.

[2] Females per trap-night, 4 trap locations.

[3] Females per daytime collection.

[4] Bites per man-hour, peridomiciliary.

Insectary Colonies

Basic to the entire project was the establishment of insectary colonies to produce 50,000 male pupae per day. By the end of December 1971, the Apastepeque colony was maintained at a level of 8,000 new larvae per day and Metapan colony at a level of 4,000 per day. The Apastepeque colony was increased during the first quarter of 1972 to a level of 120,000 new larvae daily (60 trays, 2,000 larvae each). The average development time is 7-8 days after the trays are set with first stage larvae.

Field Releases

The Gainesville group made preliminary sterile male releases November 8-20, 1971. Pupae were harvested, separated by sex, using a mechanical separator, and exposed to the sterilant in the CAMRS laboratory, essentially daily for two weeks. The sterilized pupae were then transported to release sites around the edge of the lake (60 km. from the CAMRS laboratory) and allowed to emerge from sheltered structures and escape into the environment.

Having developed insectary and release procedures, preparations were made in early 1972 for systematic sterile male releases to begin in mid-April. To estimate the natural population at Lake Apastepeque for determining the number of sterilized males necessary, two releases of dye-marked females were made in early and mid-April, two weeks apart. The first release of about 3,100 females on April 3 yielded a recapture of 1-1.5% and a second release of about 4,000 females on April 17 yielded a similar return.

All preliminary work was completed by mid-April, and the first of the regular releases was made on April 19, 1972 when a total of approximately 7,700 males were released from four stations. The remainder of the daily releases during April averaged between 7,000 and 8,000. The daily average reached approximately 25,000 in May and exceeded 30,000 in June. Based on estimates of the natural population, higher numbers are not considered necessary. As previously mentioned, breeding during May in the pasture required the establishment of two additional release stations, but by mid-July this source of production was essentially absent because of drought conditions.

Before the releases were begun, approximately 50% of all female *A. albimanus* captured at Lake Apastepeque deposited fertile eggs. By July 1972, less than 15% of captured females were depositing eggs and of these, the sterility level, i.e., the percentage of these depositing non-viable eggs, exceeds 60%. The releases will be continued at least through August after which time results will be jointly evaluated by personnel representing CAMRS and the Gainesville Laboratory.

Mosquito Pathogen Studies

A protocol for participation of CAMRS, the CDC Technical Development Laboratories, and the USDA Laboratories of Gainesville, Florida and Lake Charles, Louisiana, in a program for screening pathogens of anophelines in El Salvador and other areas of Central America has been agreed upon. A member of the CAMRS Staff was provided training in the three U.S. laboratories for collection, isolation, preliminary identification, and shipping procedures for isolates collected by CAMRS during field studies.

One fortuitous isolation of a potential pathogen has been accomplished. Adult *A. albimanus* collected at Hacienda Santa Emilia, La Libertad, El Salvador, on January 25, 1971 (CAMRS Coll. #71-1470), were sent to the USDA Laboratories, Gainesville, Florida, for the purpose of establishing a colony. When the adults died, they were examined for pathogens. One of 170 adults was infected with *Nosema stegomyiae*.

Spores from this mosquito were fed to first instar *A. albimanus* larvae from the Gainesville colony. Three of 60 adults reared from these larvae were infected. Enough spores were obtained from these three infected individuals to induce 100% infection in subsequent exposures. The *Nosema* propogated from this isolation has been designated as the El Salvador strain and it is being maintained and studied at the laboratory in Gainesville.

Aedes aegypti were also infected with this *Nosema* in the Gainesville laboratory. Continuous exposure of larvae from the first instar to pupation to 5×10^3 spores/ml. resulted in infection in 83% of the adults. The pathogen does not multiply to any appreciable extent in *A. aegypti*, but it has been possible to maintain this *Nosema* for five generations in this species.

MALARIA CONTROL DEMONSTRATION PROJECT

Multiphasic Control in an Endemic Area

Organization

The year-long study in a limited coastal area of El Salvador (District 13) was completed in December 1971. The organization and operations during the first six months of this project were described in the CAMRS FY-1971 report. Surveillance of malaria in the District continued through the year with routine passive case detection (voluntary collaborators), active case detection with contact of the entire population every two weeks for febriles, and blood film surveys of the entire available population every eight weeks. Entomologic surveillance was continued during the year with routine determinations of larval and adult mosquito densities in selected points in the District.

Control operations during the year were as follows:

1. Dry season application of aerial larviciding with Abate of rivers and other major breeding areas at a target rate of 0.01 lbs. per acre of water surface; areas were treated weekly from January 27 through June 11.

2. One cycle of mass drug distribution of amodiaquine, offered to the total population, July 5 to 10, following the larviciding activities and designed to decrease the parasite reservoir at the beginning of expected high seasonal transmission.

3. A single cycle of house spraying, using exteriorly applied propoxur (described elsewhere in this report) during the period of June 14 to July 6.

4. A second cycle of mass drug distribution (amodiaquine), October 4 to 14, in response to an increase in malaria positives during September.

Epidemiologic Evaluation

During the year a total of 2145 positive blood films were taken in the area by all sampling methods. The malaria positives by months are compared with the same information from the previous two years in Table 15. It is apparent that the malaria prevalence in the area was considerably higher than that seen during the two previous years, when the area was under continuing mass drug distribution programs (60-65% acceptance). The approximate 4-fold increase in the number of positives in the study area probably reflects both the general increase in malaria incidence throughout the coastal area and the lesser efficiency of the control measures applied in 1971.

A comparison of the yield of the three surveillance systems in the three years is presented in Table 16. The increase in the proportion of the slides taken by the passive case detection network can be at least partially accounted for by an increase in the number of voluntary collaborator posts from 8 to 14 during the year 1971. Additionally, for at least part of the year, there was a conscious effort on the part of the active case detection person-

TABLE 15
Malaria Positives by Month,
District 13, El Salvador, 1971

Month	1969*	1970**	1971***
January	5	12	3
February	8	36	113
March	18	60	168
April	15	33	226
May	23	18	120
June	50	17	340
July	111	45	159
August	120	91	319
September	144	187	475
October	14	27	68
November	17	18	106
December	3	7	48
Total Positives	528	551	2,145
Attack Rate/1,000+	89.3	93.2	362.8
Total Slides	22,975	16,636	18,238
Slide Positivity Rate	2.3	3.3	11.8

* Mass biweekly distribution of amodiaquine + primaquine entire year

** Mass biweekly distribution of pyrimethamine + primaquine March through December

*** Single cycle of amodiaquine during periods July 5-10 and October 4-14, 1971.

\+ Based on population estimated at 5,912

TABLE 16
Production of Three Surveillance Systems: Active Case Detection (ACD), Passive Case Detection (PCD) and General Surveys
District 13, El Salvador, 1970 and 1971

		Slides		Positives		
		Total	%	Total	%	SPR
January-December 1969	PCD	889	3.9	211	40.0	23.7
	ACD	6,381	27.8	195	36.9	3.1
	Survey	15,705	68.4	122	23.1	0.8
	Total	22,975	100.0	528	100.0	2.3
January-December 1970	PCD	334	2.0	94	17.1	28.1
	ACD	1,450	8.7	269	48.8	18.6
	Survey	14,852	89.3	188	34.1	1.3
	Total	16,636	100.0	551	100.0	3.3
January-December 1971	PCD	2,356	12.9	1,140	53.1	48.4
	ACD	2,040	11.2	479	22.3	23.5
	Survey	13,842	75.9	526	24.5	3.8
	Total	18,238	100.0	2,145	100.0	11.8

nel in the area to encourage by several methods the public utilization of the voluntary collaborator posts. This phase of the study will be discussed below.

Of interest was a marked shift in the *P. vivax* - *P. falciparum* ratios during the three years of observations in the study area. During 1969, 43.3% of the cases detected were *P. falciparum*; this decreased to about 5.8% in 1970 and to 0.6% in 1971. Results for the first six months of 1972 showed an increase in the proportion of *P. falciparum* cases, to 14.7% of 1214 cases. (For the comparable six-month period in 1971, only 0.8% of 997 cases were *P. falciparum*.) The decrease from 1969 through 1971 may represent the cumulative effect of a well organized drug distribution program in the area, combining mass distribution with the presumptive treatment available at the passive case detection posts. This provided curative treatment for *P. falciparum* infections and could have reduced the reservoir of this species in the study area; the same drug availability would not have reduced materially the reservoir of *P. vivax*.

Entomologic Evaluations

The District 13 study area consists principally of five large cotton farms (haciendas). As in all of coastal El Salvador, the cotton growing areas are sprayed with large quantities of aerially applied insecticides during the entire growing season, through January. A study was made of the possible relationship between this agricultural use of insecticides and the malaria vector populations.

Routine entomologic observations in the district from July through December 1971 included weekly stable captures of *A. albimanus* in four different localities. In addition, information was collected from four of the landing fields in the area which kept records of the number of spraying flights per day. Since all of the aircraft are of the same type and carry approximately the same amount of insecticide, the number of flights per day may be used as a measure of the spray delivered to the fields.

Spraying starts in July and is applied in increasing amounts until a peak is reached during October (Figure 3). Adult *A. albimanus* density reaches a peak in late August, then abruptly decreases in the span of a few weeks. This decrease comes at the peak of the rainy season which continues through October. Adult anopheline density data collected from coastal areas where cotton is not grown also show a rapid buildup in August or September, but a more gradual decline through the remainder of the rainy season. Although there are other considerations in the decrease of anopheline densities in the late rainy season, such as deterioration of breeding sites as flooded pastures become polluted and stagnant, the presence of large quantities of insecticide in the environment during this period may exert an effect on anopheline densities.

Utilization of Voluntary Collaborator Posts

An efficient epidemiologic surveillance system is necessary for a successful multiphasic control program because selection and application of measures are dependent on reliable and timely reporting of disease prevalence. The stan-

FIGURE 3
Intensity of Cotton Spraying and *A. albimanus* Adult Densities
District 13, 1971

dard surveillance system in District 13 is a network of voluntary collaborator (V.C.) posts. For this reason efforts were made in District 13 to study the normal operation of the V.C. posts and to explore methods for improvement of utilization.

As a preliminary step, available data were examined to determine the extent to which persons, on whom blood films were made by CAMRS personnel at the time that the multiphasic project was initiated, utilized voluntary collaborator posts for presumptive treatment. Identifying data were available for only the 86 persons who were found to have malaria. Of these, seven had visited a voluntary collaborator post and 79 had not. In a follow-up survey, to explore reasons why persons with malaria did not utilize V.C. posts for presumptive treatment, six of the persons who did utilize a post and 57 of those who did not were interviewed. Based on responses, failure to go to a post could not be attributed to the absence of symptoms, unusually short duration of symptoms, or to symptoms not characteristic of malaria. Inconvenient location of post and fear of side effects from medication were the reasons most frequently given for not going to a post. None of the respondents indicated that the fact that a second blood film would be taken by the voluntary collaborator influenced their failure to go to a V.C. post for presumptive treatment. Nevertheless it is suspected that this was a factor.

Consequently, a field test of simple procedure was initiated to encourage not only greater utilization but also immediate visits to a detection post when symptoms appear. Objectives of the study were to examine the immediate and longer term effect on V.C. post utilization of a time-limited referral form; and to determine the possible impact of an educational message delivered during active case detection to persons reporting symptoms.

A referral slip was developed for distribution by evaluators to persons on whom blood films were made during bi-weekly rounds of active case detection in District 13. The referral slip was issued during the second week of the initial round and the following seven rounds to all persons on whom slides were made (those reporting current or recent malaria-like symptoms). Evaluators were instructed to explain to those receiving the slip about malaria transmission and the importance of going immediately to a voluntary collaborator post for presumptive treatment. Slips were to be left with the V.C. and subsequently collected by CAMRS personnel. Voluntary collaborators were instructed not to take another blood film from those who visited the post within 24 hours after the slip was issued, and the blood film made.

A 30% sample of the entire first cycle was taken to compare the referral slip group with those not receiving referral slips. Of the 18 who received slips, 13 (70.2%) went to a V.C. post and of the 27 not receiving slips, 15 (55.5%) went to a post. In the referral slip group all who went to a post did so within 24 hours and in the non-referral group 75% of those going to a post went within 24 hours.

During the eight rounds some 524 persons had a blood slide taken and received a referral slip; among these laboratory examination resulted in 136 positives. The utilization of the V.C. posts was higher among those with positive slides (Table 17). The V.C. post utilization averaged about 70% on all rounds (Table 18) and in all age groups, except the less than one year age group where utilization was lower (Table 19).

TABLE 17
Utilization of Voluntary Collaborator Post for Presumptive Treatment by Persons Issued a Referral Form

Diagnosis	No. of persons referred	Went to V.C.	
		No.	%
Positive	136	107	78.7
Negative	388	263	67.8
Total	524	370	70.6

TABLE 18
Distribution by Cycle of Visits to Voluntary Collaborator Posts for Presumptive Treatment

Cycle	No. of persons referred	Went to V.C.	
		No.	%
1	56	37	66.1
2	112	84	75.0
3	72	57	79.2
4	59	41	69.5
5	45	28	62.2
6	72	53	73.6
7	58	35	60.3
8	50	35	70.0
Total	524	370	70.6

TABLE 19
Distribution by Age Group of Visits to Voluntary Collaborator Post for Presumptive Treatment

Age in Years	No. of persons referred	Went to V.C.	
		No.	%
< 1	15	7	46.7
1-5	124	87	70.2
6-14	154	114	74.0
15-19	51	35	68.6
20-34	88	61	69.3
35-49	50	37	74.0
50-59	23	16	69.6
≥ 60	19	13	68.4
Total	524	370	70.6

PARASITE BIOLOGY

Characterization of Malaria Parasites of Central America

Additional information has been added to that included in the FY-1971 CAMRS report on the strains of *P. vivax* isolated in Central America and under study in volunteers and *Aotus* monkeys in cooperation with the NIH/NIAID Laboratory at Chamblee, Georgia. A summary of the activity patterns seen in volunteers inoculated with the strains provided through CAMRS is presented in Table 20. Included are two strains from coastal El Salvador (Sal-I and Sal-II), one from Panama, and one from the Atlantic side of Nicaragua (NI[illegible]). All cases not experiencing relapses had either received curative therapy with chloroquine-primaquine, or, in the case of the Nicaragua strain, not enough time had elapsed at the time of writing for occurrence of the expected relapses. It is apparent that in all strains a prolonged latent period, averaging from 166 to 219 days, was characteristic preceding the first relapse, and that subsequent relapses appeared after shorter latent periods ranging from 20 to 79 days. Field investigations in El Salvador, detailed elsewhere in this report, have shown patterns of malaria prevalence which are supported by this experimental data, and which can now be more precisely understood as to the origin of cases, i.e., relapses or new infections.

Isolation of Malaria Parasites in Monkeys

Continuing efforts are being made to isolate parasite strains from Central America in owl monkeys (*Aotus trivirgatus*), the only monkey species now being maintained at CAMRS. These isolates will be used in studies with the NIH Laboratory in Chamblee, Georgia. Isolates are desired from a number of geographic and ecologic situations in the region where differences may be seen in parasite biology or in the transmissibility of the parasites by various isolates of vectors.

One strain of *Plasmodium vivax* is now well established in owl monkeys at CAMRS. This parasite was isolated from a CAMRS employee who worked several nights each week at Lake Apastepeque where a sterile male (*Anopheles albimanus*) project is underway. After several blood passages of this parasite in monkeys, parasitemias of more than 10,000 parasites per mm^3 of blood are obtained during the initial period of patency. However, splenectomies are required if extended periods of active parasitemias are to be achieved.

Two samples of human blood infected with *P. vivax* from Costa Rica have been inoculated into owl monkeys but to date no parasites have been seen in these animals. Three inoculations of human blood infected with *P. falciparum* from coastal El Salvador have also failed to produce a parasitemia in recipient monkeys.

TABLE 20
Activity Patterns of Four Central American Strains of *P. vivax**

Strain	No. of Inoc.	Prepatent Period (Days)		No. of 1st Relapses**	Latent Period (Days)		No. of 2nd Relapses**	Latent Period (Days)		No. of 3rd Relapses**	Latent Period (Days)	
		Range	Mean		Range	Mean		Range	Mean		Range	Mean
Panama	10	12-17	13.5	8	94-226	166	5	20-60	42	3	52-57	54
Sal-I	11	12-16	14.3	5	192-229	206	1	30	30	-	-	-
Sal-II	11	10-16	12.8	4	172-262	219	3	31-79	57	2	28-58	43
NICA	6	10-12	10.8	1	194	194	-	-	-	-	-	-

* Information Provided by Unit on Primate Malaria, NIAID, NIH, Atlanta-Chamblee, Georgia

** All relapses were after treatment of previous attack with chloroquine, pyrimethamine, chloroguanide or quinine in normal suppressive doses.

EPIDEMIOLOGIC STUDIES

District 13 Study - 1972

Organization

The use of District 13 (El Salvador) as a CAMRS study area is being continued for the fourth year. The area is being used currently to evaluate the effectiveness of propoxur as a residual insecticide when applied every five weeks as a partial coverage spray in the interior of houses. This measure is being employed by the national antimalaria program in approximately half of the coastal area of El Salvador, while in the remainder of the coastal area propoxur is being used as a full coverage spray on an every-three-month schedule. Since a rather elaborate surveillance program is required for adequate evaluation of the propoxur spraying, the information being collected is being used, along with additional more specific studies, to elucidate the basic epidemiology of malaria in the area. In addition, an effort is being made to determine the utility of a basic resident field worker (case detector, evaluator) to carry out broader responsibilities as a local "malaria agent" and, possibly eventually, as a multipurpose health visitor.

The project was initiated with a complete geographical reconnaissance of the area, with mapping of vector breeding sites and existing houses on 1:5000 cadastral topographic maps obtained from the National Institute of Geography. Working copies of the maps were prepared for field use. Rainfall, temperature and humidity data from an official National Meteorological Service Weather Station at nearby Hacienda Melara has been made available for CAMRS use, and daily rainfall measurements are provided from a gauge located at Hacienda Santa Lucía, approximately in the center of the District.

During early January field staff were recruited and trained, and routine epidemiologic and entomologic observations were initiated in late January. Four "evaluators" were assigned to specific geographic divisions of the District, where they were to participate in case detection, case investigation, entomologic, and control activities.

Routine entomologic observations included weekly adult mosquito captures in stables at three different locations and human bait captures at another point where no suitable stable could be found. Weekly visits were made to some 18 different vector breeding sites to measure larval densities.

Additional studies included periodic insecticide sensitivity tests, search for resting mosquitoes in houses during the day in various locations, and limited sporozoite surveys of *A. albimanus* mosquitoes collected in areas where malaria transmission was suspected.

Epidemiologic surveillance was conducted through the voluntary collaborator posts, through active case detection by contacting the entire population approximately every two weeks for symptomatics, and by mass surveys of selected populations. Blood films and filter paper blood spot samples are collected from each person contacted for sampling. All blood films collected are processed and examined by CAMRS staff and filter paper samples preserved frozen for possible future serologic evaluation.

Malaria cases detected through the regular surveillance activities are investigated by the field staff to provide further epidemiologic information. At the time of these investigations, household or neighborhood contact blood film surveys are made. Persons with positive slides are followed-up every month with additional blood films. Filter paper samples are collected from all persons contacted for blood films during case investigation and follow-up.

Among additional information monitored in the District is the usage of agricultural insecticides, the movement of populations into and out of the District, daily activity patterns of the populations, pasturing and stabling practices for livestock herds, irrigation practices in the area which would relate to vector breeding, and any other apparent factor which might have a relationship to the transmission of malaria.

Insecticide effectivity is monitored by periodic evaluation by chemical and chromatographic assay of fumigant and surface activity and by bioassay with caged *A. albimanus* at intervals after spraying. These results are recorded elsewhere in this report.

Control Operations

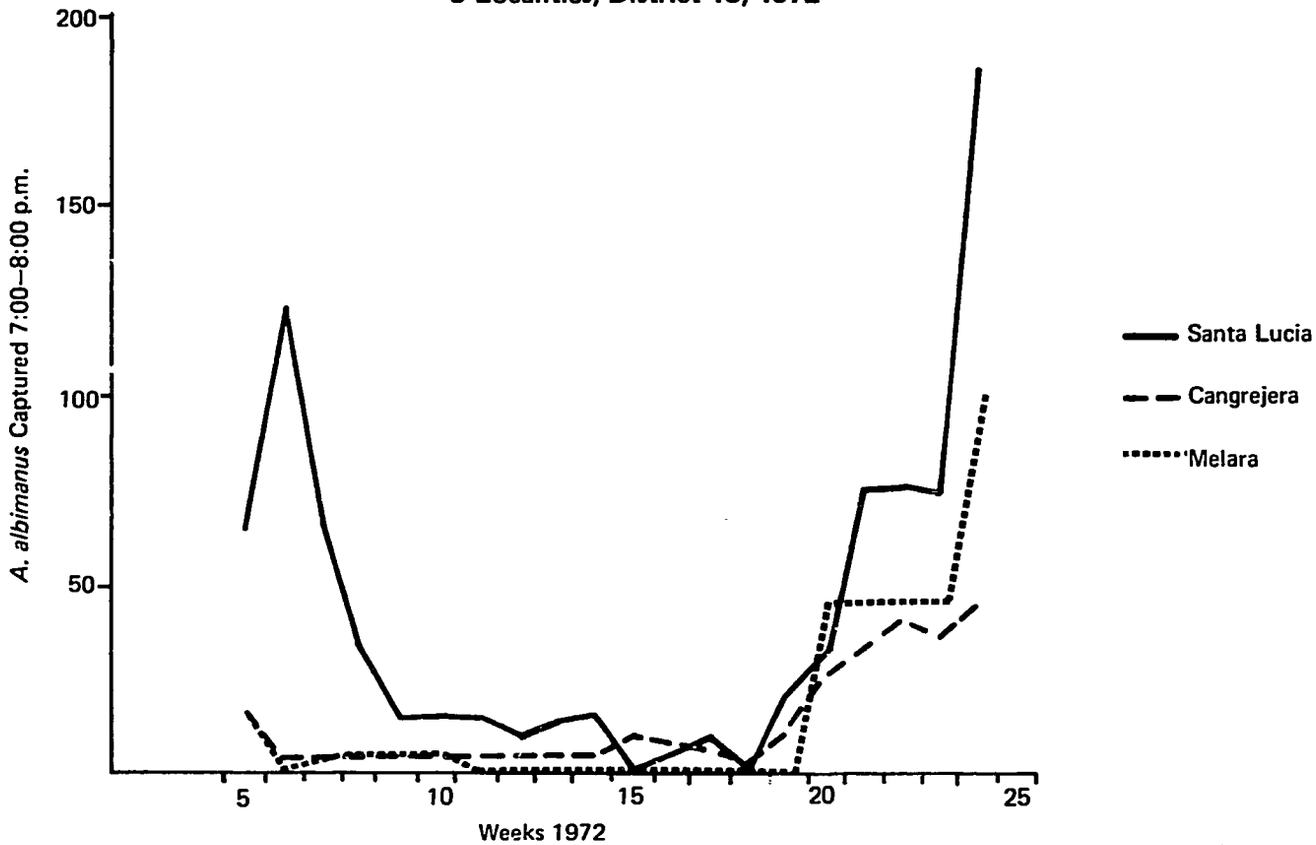
Spraying with propoxur was initiated in March on a 35-day schedule, and three cycles had been completed and the fourth initiated by the end of the first 6-month period (June 30, 1972). The technique of application has been the spraying of two 1-meter-wide swaths around the interior walls of the houses, delivering an average of 80 grams of propoxur 70% W.W.P. per house in each cycle. The total of sprayable houses in the area is 1,396. The spraying is done by two spraymen, employed by the national program, but supervised by one of the four sub-area evaluators, who accompanies the spraymen on their rounds in his own assigned area, accomplishing surveillance duties simultaneously. Each sprayman completes about 31 houses per day. The acceptance of the spraying by the householders has been good; coverage during the three completed cycles averaged 98%. No significant problems have been encountered.

The only additional control measure in the District has been the presumptive treatment of all fever cases identified through active and passive case detection activities, using a single dose of amodiaquine (Basoquin) scaled from an adult dose of 600 mg. of base.

Entomologic Observations

Adult *A. albimanus* densities have been monitored since February 1, 1972 through weekly captures of resting adults in stables at Melara, Congrejera, and Santa Lucía. The densities were generally very low during the dry season except at Santa Lucía, where the stable is located near a pasture which is irrigated periodically during the dry season. With the onset of rains, densities began to increase in all three stables in mid-May and will probably reach a peak in mid-August (Figure 4). A light trap was operated one night weekly in the village of Valle Nuevo and the results of these captures also reflected the *A. albimanus* population buildup in mid-May (from 0 in week 14 to 35 per trap-night in week 24). Human bait captures were carried out weekly in the village of Toluca for measuring adult densities as well as man-

FIGURE 4
Weekly Stable Captures of Resting Adult *A. albimanus*
3 Localities, District 13, 1972



vector contact. Starting the first of June weekly human bait captures inside and outside houses have been carried out in the six localities of the District. While the captures were variable it was apparent in all localities that man-vector contact, both intra- and extra-domiciliary, were adequate to account for continuing transmission of malaria, in spite of the propoxur house-spraying program. The bioassay of propoxur fumigant activity is detailed in Vector Control Studies; Residual Insecticides.

Larval *A. albimanus* densities have been monitored in District 13. In January a complete survey was done to locate, measure, and map all of the actual and potential anopheline breeding places. Of the 180 breeding places found, 18 were chosen for weekly larval dipping. All types of larval habitats, except permanent lakes, are found within the study area. The 18 sampling stations were chosen to include estuaries, rivers, brackish swamps, fresh-water swamps, drainage ditches of various kinds and irrigated pastures. Attempts are being made to correlate *A. albimanus* production in specific breeding places with malaria transmission in specific houses or groups of houses by season.

Permanent rivers produce few *A. albimanus* during the dry season in this area, but do produce large populations of *A. pseudopunctipennis*. This is well illustrated by the weekly findings for the Huiza River (Figure 5). Irrigated pastures are important sources of *A. albimanus* breeding during the dry season, and probably during the rainy season as well when the low spots fill with rain instead of irrigation water. The potential of this type of breeding place during the dry season is illustrated in Figure 5 with the weekly larval densities in the pasture at Cangrejera.

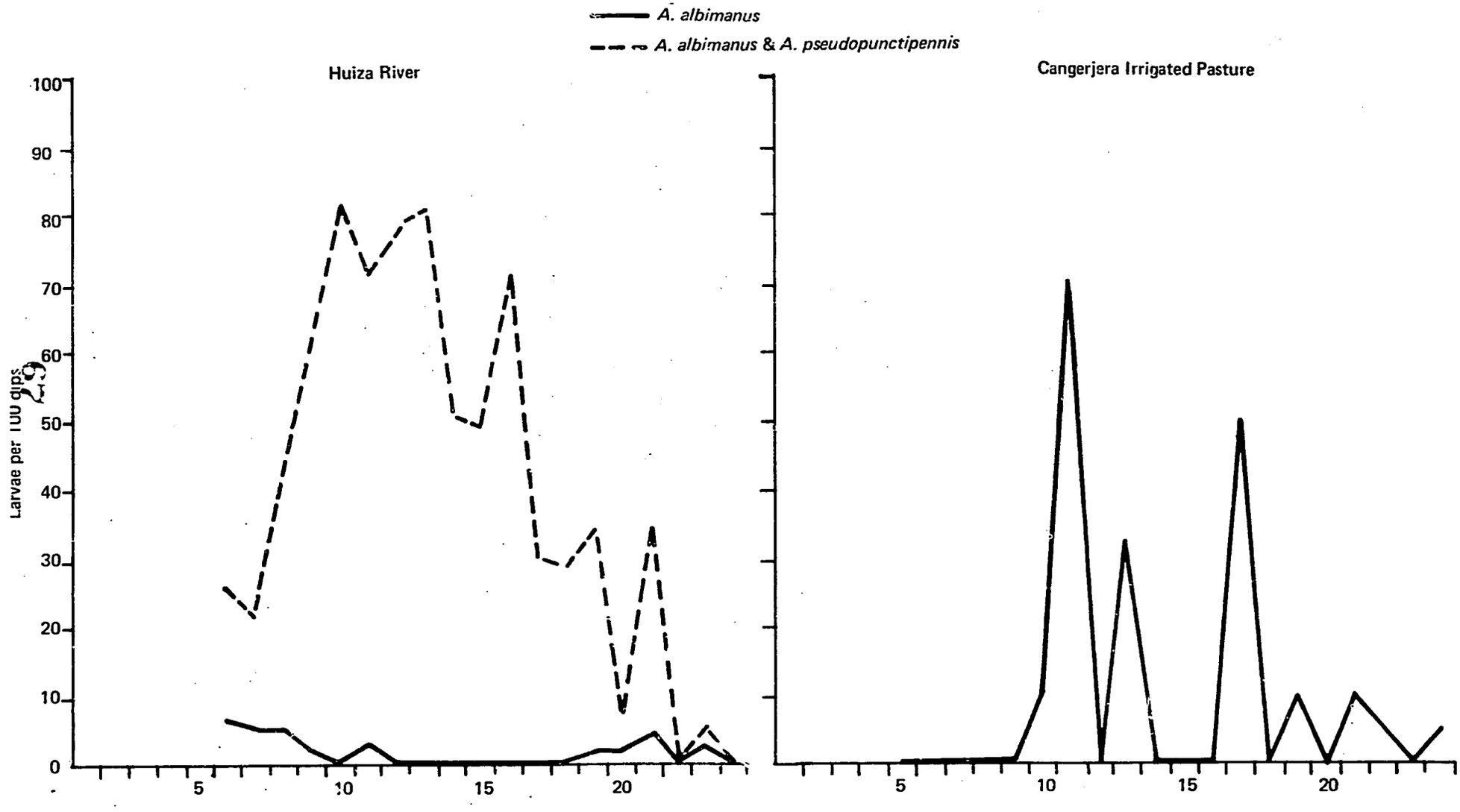
Case Detection

A total of 9,160 blood films were collected and examined during the six months of 1972, and 1,707 (18.6%) were positive for malaria (Table 21). Of the positives, 279 (16.3%) were *P. falciparum* and the remainder *P. vivax*. Mass surveys provided 38% of the total slides taken, voluntary collaborators 17%; and active case detection, contact investigations, and follow-up of cases about 15% each. The greatest yield of positive slides was from the voluntary collaborator (V.C.) posts, providing 47% of the positives, with the other sources yielding 8 to 19% of the positives.

TABLE 21
Case Detection by Source and Sex
District 13, January-June 1972

	Male			Female			Total		
	Slides	Pos.	% Pos.	Slides	Pos.	% Pos.	Slides	Pos.	% Pos.
Vol. Col	879	427	48.6	674	376	55.8	1553	803	51.7
ACD	682	156	22.9	700	164	23.4	1382	320	23.2
Mass Survey	1566	77	4.9	1918	89	4.6	3484	166	4.8
Contact Surveys	605	78	12.9	746	70	9.4	1351	148	11.0
Follow-up	632	118	18.7	758	152	20.1	1390	270	19.4
Totals	4364	856	19.6	4796	851	17.7	9160	1707	18.6

FIGURE 5
Weekly Larval Surveys
Rio Huiza and Pantano Cangrejera, District 13, 1972



From all sources 48% of the total slides were collected from males and 52% from females. Although a greater proportion of the slides collected by the V.C. posts were from males, this difference was more than equalized by collections made during house-to-house visits during regular working hours when a larger proportion of females was encountered.

In regard to age distribution, somewhat fewer slides were collected from young children (0-4 years) at the V.C. posts than would have been expected, but this was equalized to some degree by the greater number included in the mass surveys. More slides were collected from older children (5-14) and fewer from persons 15 years of age and over than would have been expected from the known age distribution of the population. The age-group of 5-14 years had the highest attack rate and also the highest positivity rates.

When the blood films from fever patients only, i.e., from V.C. posts and ACD, are considered, the 5-14 age-group provided 45% of the positives, although they made up only about 28% of the population (Table 22). Persons under 20 years (about 55% of the population) contributed 70% of the positives, and those under 30 years 89%.

TABLE 22
Blood Slides Positive for Malaria by Age-Group
January through June 1972,
District 13 Study Area
(From V.C. Posts and Active Case Detection)

Age Group	Total Slides	Pos. Slides	% Pos.	Est. Pop.	% Pop.	% Total Slides	% Total Pos.	Attack rates per 1000 pop.
≤ 4	417	156	37.4	1140	19	14	14	137
5-9	611	293	48.0	960	16	21	26	305
10-14	432	211	48.8	720	12	15	19	293
≥ 15	1459	463	31.7	3180	53	50	41	145
Totals	2919	1123	38.5	6000	100	100	100	187

Case Investigation

An attempt was made during the first six months of 1972 to investigate all *P. falciparum* cases and as many of the *P. vivax* cases as possible, resulting in a total of 943 cases investigated. Of this number 57 (6.0%) could not be found, and presumably had left the District. Of the remainder, only 40 (4.2%) were classified as imported from outside the study area, and the great majority (846 or 89.7%) were classified as indigenous.

The classification of cases to distinguish the origin of attacks (relapse, recrudescence, or new infection) was difficult because of the possible continuing transmission in the area. However, knowledge of transmission potential in various parts of the District permitted some tentative assumptions and conclusions (Table 23).

TABLE 23
Case Investigations, District 13 Study Area
January-June 1972

		P. vivax	
No. Investigated			777
	New:	Indigenous	140
		Imported	3
	Relapse:	Indigenous	544
		Imported	34
No. Lost			56
		P. falciparum	
No. Investigated			166
	New:	Indigenous	153
		Imported	2
	Recrudescence:		
		Indigenous	9
		Imported	1
No. Lost			1

Practically all of the *P. falciparum* cases could be considered as new infections, since very few cases were detected in the entire District during the previous year. For *P. vivax*, because of the probable limitation of transmission during the dry season period extending through the first four or five months of the year, all infections which had a known history of previous infections were arbitrarily classified as "relapses", and those with no previously documented infection as "new infections". The possible fallacy of these assumptions is obvious, but this classification is useful as a basis for consideration of a number of epidemiologic factors. On this basis, 578 of the 721 *P. vivax* cases actually investigated were classified as "relapses", and 143 as "new infections" (Table 23).

While sporadic low level transmission may have gone on in scattered situations throughout the District during the "non-transmission" season, there is a good evidence that transmission at fairly high levels did occur in at least two locations during this period. In these two foci (Playa Cangrejera and El Chaguiton) concentrations of *P. falciparum* were seen, including cases in young infants and new arrivals. Also, the percentage of "new" *P. vivax* cases was somewhat higher at these sites than in the District as a whole. Perhaps more significantly the attack rate at these two places during this period was about twice as high as the rate for the rest of the District (354.8 against 170.1 per 1000 population). The presumption of transmission at these sites was also supported by entomologic observations of *A. albimanus* breeding and house-resting.

It is of interest that, although *P. falciparum* transmission had started in Playa Cangrejera and El Chaguiton before the initiation of propoxur spraying, it has continued at a significant level in both places in spite of the propoxur spraying program. With the advent of the rainy season in May and a subsequent increase in vector densities, there has been a general increase in malaria incidence throughout the area, both *P. vivax* and *P. falciparum* (Figure 6). The proportion of cases which were *P. falciparum* has increased from about 6% in February and March to about 30% in June.

Relapse Patterns of *P. vivax*

During the investigation of *P. vivax* infections in the District during the first six months of 1972 information became available which may provide confirmatory evidence on the normal life patterns of this species in coastal El Salvador. A general increase in numbers of slides positive for *P. vivax* occurred beginning in mid-February, with a greater increase in late June (Figure 6). The overall incidence during this period was somewhat higher than seen during the comparable period of 1971. The peak of the malaria transmission season in 1971 had occurred at the end of August and through early September, and, according to current information on expected latency prior to the initial relapse in this area which had been developed in studies in volunteers, relapses from the infections would be expected to occur some five to seven months later. Because of the detailed studies during 1971, the past histories of most of the cases occurring in early 1972 could be determined, and, in fact during the first five months of 1972, 244 *P. vivax* infections were detected in persons with a previous history of a *P. vivax* positive in 1971. When the intervals in months between the "initial" attack in 1971 and the subsequent "relapse" in 1972 were tabulated (Figure 7), it could be seen that most of the repeat positives were found after an interval of four to eight months and that the mean length of latency was about seven months.

It was also seen that after the initial recurrence of *P. vivax*, a considerable number of persons continued to show additional recurrences during the first five months of 1972. When the intervals in weeks between these attacks are tabulated, it appears that, when each attack was treated with amodiaquine, the subsequent attack usually occurred after three to nine weeks of latency, with an average of about six weeks (Figure 8).

Thus, the hypothetical life pattern which can be constructed from these observations coincides very closely with that which might be constructed from the information derived from volunteers inoculated with *P. vivax* strains from El Salvador. In volunteer studies of two El Salvador strains the latent periods in nine primary relapses ranged from 172 to 262 days and averaged 212 days, or approximately seven months. Six subsequent relapses occurred 28 to 79 days later, averaging about 47 days. Because these latter periods were calculated using the time period between the first day of parasite occurrence in each attack, the actual time period between treatment and relapse would have been several days shorter. The field studies used the same criteria, but the actual time of onset would be obscured by a probable delay in acquiring the first positive blood film. In any event, the relapse patterns apparent from the field observations, are well within the ranges seen in the controlled studies, and reinforce significantly the applicability of these

FIGURE 6
Positive Slides from Voluntary Collaborator and Active Case Detection by Week of Collection and Rainfall by Week District 13, El Salvador, 1972

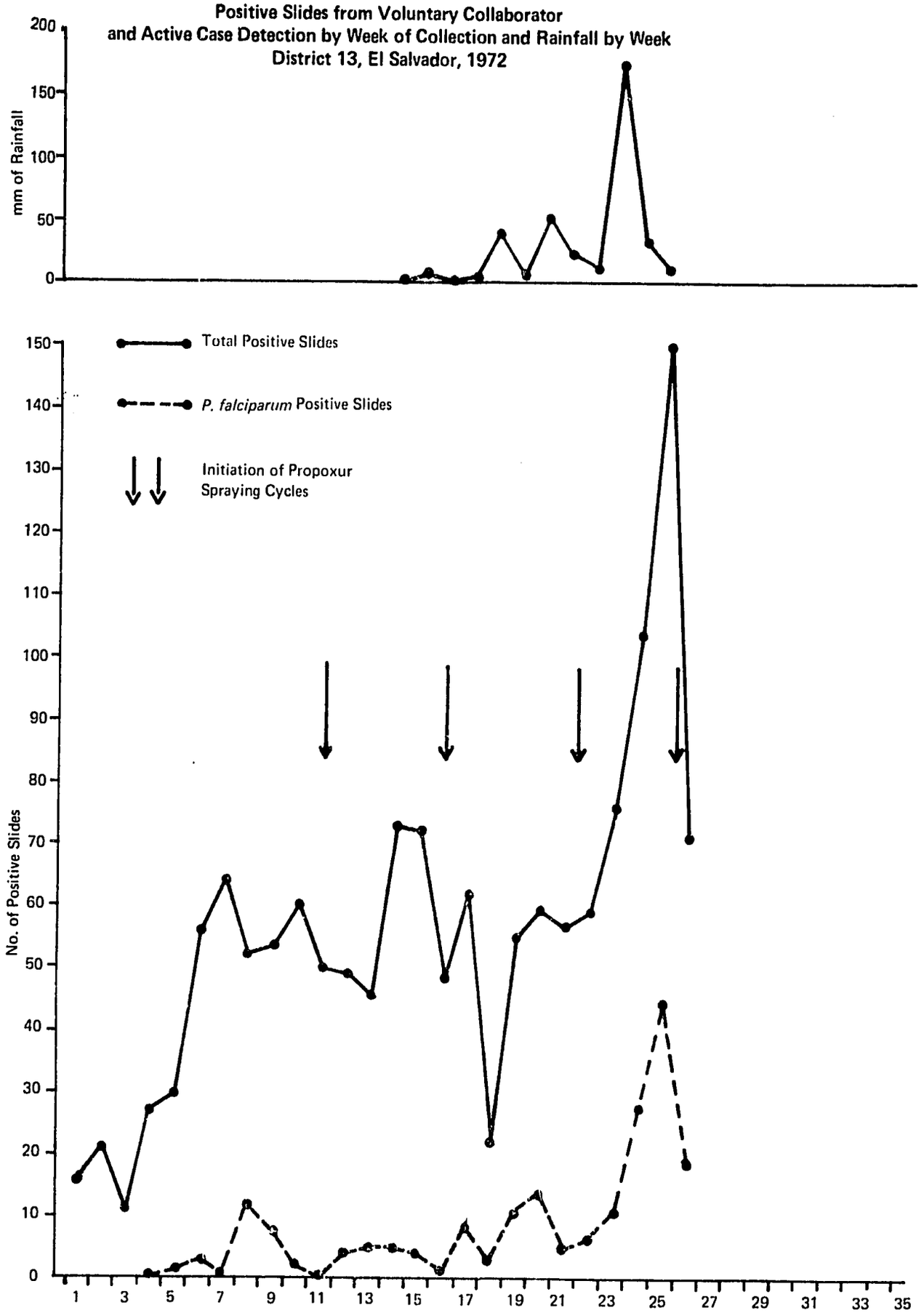


FIGURE 7
Latent Periods of *P. vivax* Cases Reported Positive During 1971, and Again During First Five Months of 1972

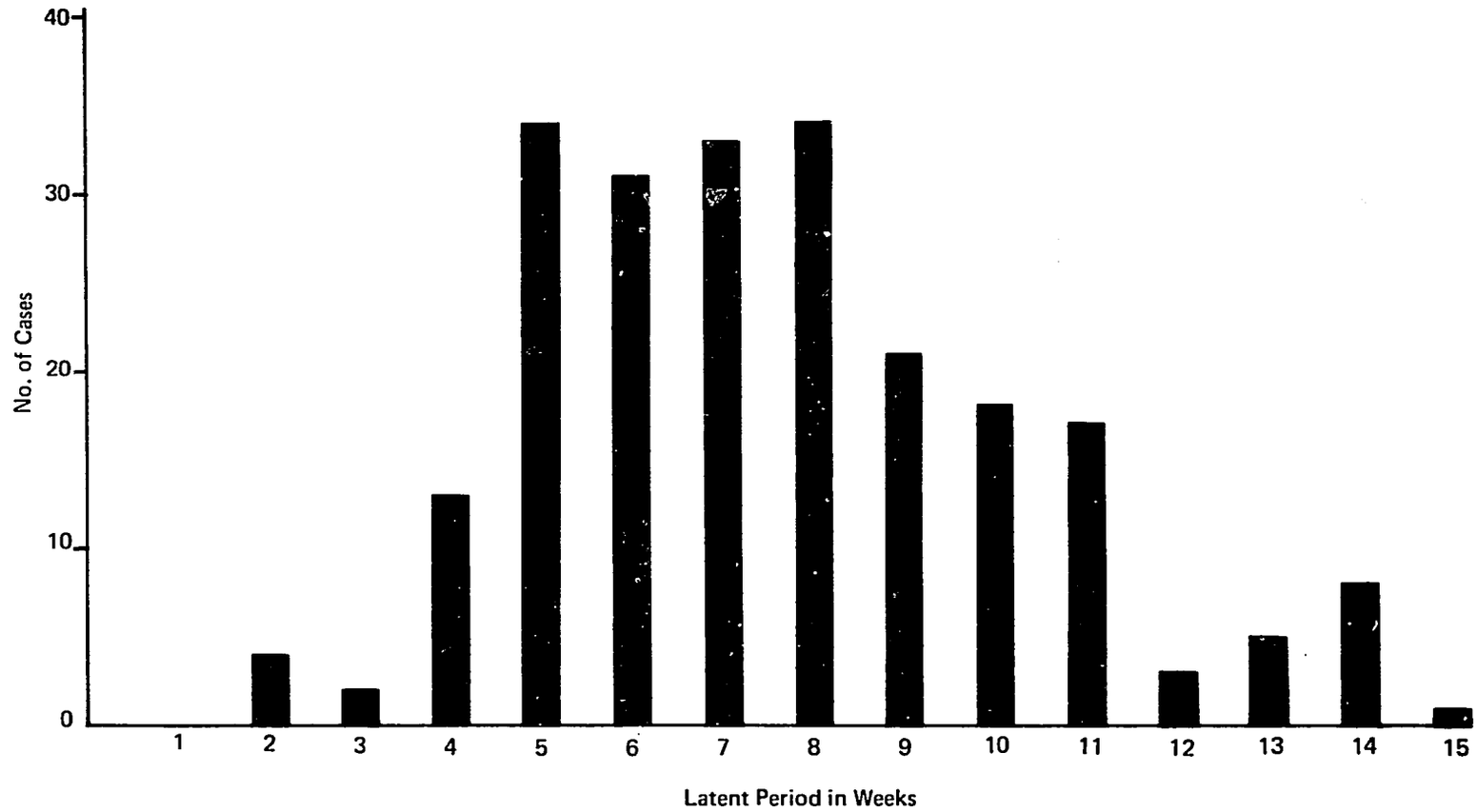
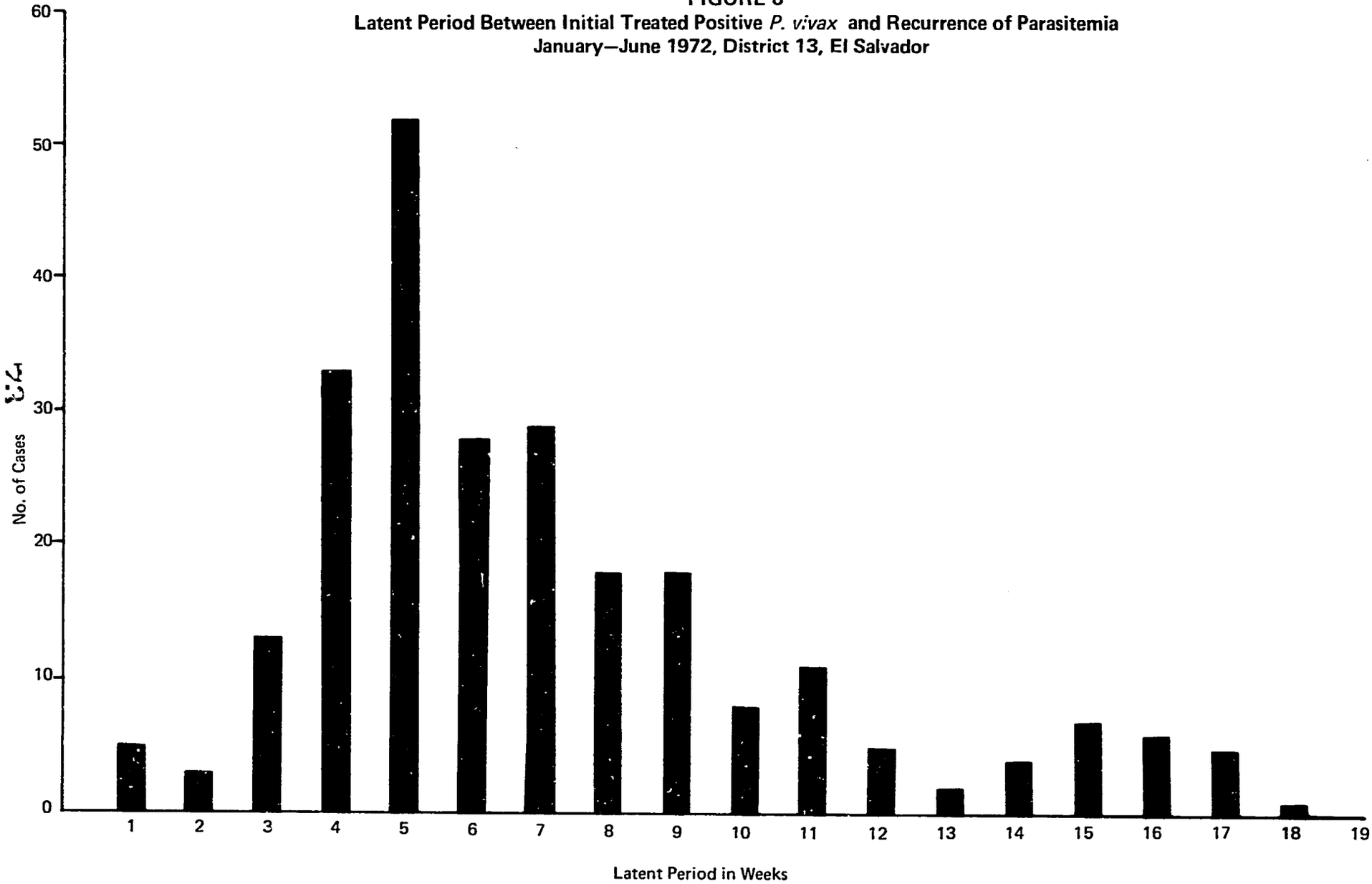


FIGURE 8
Latent Period Between Initial Treated Positive *P. vivax* and Recurrence of Parasitemia
January—June 1972, District 13, El Salvador



studies to epidemiologic investigations. Patterns of incidence will be scrutinized for the remainder of the year to determine the patterns of recurrence resulting from early season (May-July) *P. vivax* transmission now being seen in the District.

Chemotherapy of Malaria

The presence of *P. falciparum* cases in the District provided an opportunity to assess the sensitivity of strains to amodiaquine, and a special effort was made to follow up all cases treated presumptively with 600 mg. of drug to determine parasite clearance. Thirty cases have been followed after treatment, and in no case could abnormal response to the drug be demonstrated, suggesting that the *P. falciparum* seen was normally sensitive to the 4-aminoquinolines. Cases of *P. vivax* were similarly followed and also demonstrated normal amodiaquine sensitivity.

Because a 5-day regimen of primaquine had been used extensively in District 13 in 1971 for treatment of *P. vivax* cases an effort was made to determine the efficacy of this method as a curative treatment. In most cases the drug was distributed to the patients with the first dose of 15 mg. to be taken with the evaluator present and the remaining four 15 mg. tablets to be taken once each day independently of supervision. This was a standard procedure of the national antimalaria program at the time. Of the persons who received this regimen (about 1500) about 21.5% had subsequent positive blood films, suggestive of relapses. Of about 500 who had not received the primaquine therapy, but who also remained in the observation group, about 22.2% had a subsequent relapse. In a third and smaller group of 87, the primaquine was administered by program personnel on a daily basis for the 5-day course, to ensure consumption of the drug. Of this group, 19 (21.8%) had subsequent positive blood films. There appears to be little difference among these three groups, and that the 5-day primaquine course may have little value as a curative. However, these studies were done during periods when transmission may have been a factor so the results cannot be considered conclusive. Additional controlled studies are underway and it should be possible to clarify the usefulness of the method, at least insofar as it is possible to do so under field conditions.

One factor which must be considered in epidemiologic or chemotherapeutic investigations in this area is the easy availability of antimalarial drugs to the population from commercial sources. To assess the extent of this factor, a study was made of the possible drug sources in District 13, other than through voluntary collaborators, active case detection and other official program activities. At present there are about 20 variety shops in the District which sell 4-aminoquinolines, primarily Aralen (chloroquine). Six of these shops also provide chloroquine injections. Aralen tablets containing 150 mg. of base sell for 15-20 centavos (6-8 cents U.S.) each and the usual dose sold is three tablets for an adult. On injection a dose of 150-250 mg. (base) of chloroquine is usually administered to an adult, at a cost of about ¢2 per injection ($0.80). It is estimated that some 5-10 thousand tablets are sold each year in the District and some 500 injections are given.

Studies in San Diego, El Salvador

This coastal village has been under study by various members of the CAMRS staff for approximately three years. The area is a popular beach resort where, during holiday periods, thousands of people spend from one to several days and nights in temporary shelters constructed just above the high-tide line, between the sea and an estuary. Malaria is a constant threat both to these visitors and to the permanent population. Studies on the ecology of *Anopheles albimanus* at this site during the last three years have included a continuing light trap operation to measure mosquito densities.

Between March 1971 and March 1972, three blood surveys were carried out in this area to provide malariometric data which could be correlated with information on the biology of the vector. Blood specimens on filter paper for fluorescent antibody determination as well as routine thick films were collected and analyzed. The initial survey in March 1971 (Table 24) showed that 43% of the population over 15 years of age had titers of 1:20 or higher to the *P. falciparum* antigen, but the percentage was only 19% for the whole population. The paucity of positive reactions with this antigen in younger age groups suggested that many of the positive titers might have been associated with malaria experience at some time in the past and not with current, active transmission. This interpretation was supported by the fact that the routine surveillance system maintained by the national malaria program reported very few cases of *P. falciparum* from this area. Reactions with the *P. vivax* antigen produced a somewhat different distribution. While the overall percentage positive was similar to that seen with the *P. falciparum* antigen, there were more positive *P. vivax* titers in lower age groups. There seemed to be little doubt that *P. vivax* was more active in this population than *P. falciparum*. This was confirmed both by surveillance data and by the appearance of ten positive cases of *P. vivax* in the survey specimens (Table 25). Some reactions to the *P. malariae* antigen were found but titers were low and primarily from the older age groups indicating that transmission of this parasite was either very low or absent in San Diego at the time of the study.

TABLE 25
Parasite Positives by Age in Three Surveys
San Diego, El Salvador, 1971-1972

Age Group Years	Parasite Positive*/Slides Examined		
	March 1971	November 1971	March 1972
≤ 4	0/9	0/15	0/0
5-9	3/63	6/48	1/48
10-14	6/91	7/88	5/85
≥ 15	1*/112	1/65	0/15
Total	10/275	14/216	6/148

* One *P. falciparum* infection in 43-year-old female; all others *P. vivax*.

TABLE 24
IFA Titers to *P. vivax* and *P. falciparum* Antigens
San Diego, El Salvador, 1971-1972

Age in Yrs.	No. of Sera	Anti-gen	GMRT	Positives (≥1:20) %	Positives (≥1:20) GMRT	Anti-gen	GMRT	Positives (≥1:20) %	Positives (≥1:20) GMRT
				March 1971					
≤ 4	9		5.8	-	-		6.8	11	(20.0)
5- 9	63		5.8	3	(20.0)		5.6	5	(25.1)
10-14	91	*P.f.*	5.8	3	(39.8)	*P.v.*	7.1	15	29.5
≥ 15	112		16.6	43	67.6		10.2	32	30.2
Total	275		8.9	19	63.1		7.6	20	29.5
				November 1971					
≤ 4	15		Neg	-	-		7.9	27	(28.2)
5- 9	48		5.8	4	(28.2)		9.1	27	39.8
10-14	88	*P.f.*	6.3	8	24.5	*P.v.*	15.1	42	61.7
≥ 15	65		25.1	58	64.6		28.8	68	57.5
Total	216		9.1	22	53.7		15.8	45	53.7
				March 1972					
≤ 4	0		-	-	-		-	-	-
5- 9	48		5.4	4	(20.0)		6.5	10	(45.7)
10-14	85	*P.f.*	5.2	1	(20.0)	*P.v.*	9.3	21	85.1
≥ 15	15		5.2	-	-		6.3	7	(20.0)
Total	148		5.2	2	(20.0)		7.9	16	70.8

GMRT = geometric mean reciprocal titer.
Neg = 5.0 = all titers <1:20.
( ) indicates mean based on 5 or fewer observations.

A second survey was conducted on the same population approximately seven months later (Table 24). The *P. falciparum* responses were essentially unchanged but a dramatic difference was seen in responses with the *P. vivax* antigen. The percentage of the whole population with positive *P. vivax* responses had more than doubled in the interval between the two surveys. The change in the lower age groups was even more striking. Both the geometric mean reciprocal titer for all specimens and that for the positives were increased. It was clear that an increase in *P. vivax* activity had occurred between March and November 1971. Considering only positive titers of 1:20 or greater, in the March 1971 survey 11% of the population had maximum titers to the *P. vivax* antigen and 19% to the *P. falciparum* antigen. There were five dual (*P. falciparum* - *P. vivax*) maximum titers, all in people above 15 years of age. In November 1971, 38% had maximum titers to *P. vivax* and only 15% to the *P. falciparum* antigen. Dual maximum titers (*P. falciparum* - *P. vivax*) were seen in 12 persons above 15 years of age. These results indicated an increase in *P. vivax* activity in this population, and this was supported by a slight increase in positive blood films (Table 25). There was no serologic or parasitologic evidence of an increase in the amount of *P. falciparum* malaria in the area.

In March 1972 a survey was conducted in the school at San Diego (Table 24). The majority of the specimens came from the 5-14 age group. The percentage with titers of 1:20 or higher for *P. vivax* had dropped and the geometric mean titers were lower in this survey than in the previous one. These results would seem to indicate that the high level of *P. vivax* transmission encountered between March and November 1971 had diminished by March 1972. A fourth survey will be conducted in the same area in November 1972 in order to learn if the increase recorded in 1971 was a sporadic occurrence or is a seasonal phenomenon.

From a review of the parasitemias encountered during the three surveys, it is apparent that the attack rate was higher in the 5-14-year-olds (Table 25). There is no ready explanation on the basis of exposure for the higher parasite rate in children. The sample size was higher in this age group, and this would account for the greater number of cases but not for the higher positivity rate. It is interesting that the pronounced increase in *P. vivax* shown in the serologic studies would not have been apparent if information had been available only from point blood surveys. Only one case of *P. falciparum* was diagnosed in a 43-year-old female in the March 1971 survey.

The ecology of malaria in San Diego has traditionally been associated with the production of *Anopheles albimanus* from the estuary which forms one boundary of the village. During the wet season, the quantity of water entering the estuary is sufficient to maintain an opening to the sea, thus subjecting the entire area to the daily wash of the tides. In this period there is no anopheline production from the estuary. In the dry season, the quantity of fresh water is greatly reduced and the action of the sea gradually erects a sandbar which closes the estuary to tidal changes and consequently to the daily influx of salt water. Fresh water continues to enter the estuary at a slow rate, gradually building a very extensive breeding area for *A. albimanus*, and malaria transmission has long been associated with this mosquito population. In 1969 a light trap was established at San Diego and weekly counts were made on the *A. albimanus* population (Table 26). In 1969 and 1970 the

TABLE 26
Mosquito Densities and Positive Malaria Cases*(PCD) by Month
San Diego, El Salvador, 1969-1971

Months	1969		1970		1971	
	Slides Pos./Exam.	Mosquito Density	Slides Pos./Exam.	Mosquito Density	Slides Pos./Exam.	Mosquito Density
January	0/3	N.I.	0/1	5	6/10	25
February	0/2	57	2/3	5	4/13	5
March	0/3	622	4/7	14	8/19	3
April	0/1	1064	0/4	15	1/5	30
May	1/3	1129	1/2	588	1/1	722
June	3/6	1350	0/3	3961	3/5	991
July	2/5	86	0/1	160	5/8	828
August	0/4	24	5/5	151	3/8	96
September	0/3	27	2/2	43	14/35	38
October	0/3	13	0/2	14	35/57	10
November	0/3	2	2/8	18	6/32	4
December	0/6	10	1/13	30	5/13	1
Totals	6/42	4384	17/51	5004	91/206	2753

* All *P. vivax* except 2 *P. falciparum* in July 1969 and 1 in March 1971.

estuary opened to the sea in June and its impact on the adult mosquito population was reflected in the light trap counts. In 1971 the estuary did not open until later and the mosquito populations did not markedly decrease until August.

Results of slide examinations from the voluntary collaborator post at San Diego showed an increase in malaria between March and November 1971. However, the peak appearance of new cases does not correlate with peak mosquito populations. At present we cannot say if these "cases" are actually the result of new infections or are relapses. The population curves for the estuary mosquito population are so sharp that it would seem likely that the seasonal curve for active malaria cases would follow the same pattern, but there is no clear association between the two. The possibility must be considered that the estuary mosquito population is not primarily responsible for malaria transmission in this area. As the wet season progresses and the estuary breeding sites disappear, flooded pastures become common in the area, although not immediately adjacent to the estuary. *Anopheles albimanus* are found breeding in these flooded areas in lesser numbers than in the estuary, and somewhat later in the season, usually at about the same time as the increase in cases is seen.

This question is currently under investigation. A mosquito colony from the estuary has been established and is under study. When available, a colony will also be established from the flooded pasture area. These colonies will be studied morphologically and with reference to malaria susceptibility. Distribution maps of positive malaria serologies and parasitemias are being prepared to elucidate possible orientation toward the estuary or toward the flooded pastures. The epidemiologic significance of the spatial distribution of positive malaria serologies in other study areas is now being investigated through the use of detailed locality maps.

Studies on Surveillance Methods, Zapotitán, El Salvador

This area is located approximately 20 miles from the city of San Salvador. Originally a swamp, it has been recently undergoing extensive development including drainage and the establishment of an irrigation system. This activity has increased the population of the area and the irrigation ditches have brought these people much closer to potential *Anopheles albimanus* breeding sites. CAMRS began studies in this area in April 1971 in order to establish baselines for evaluation potential increases in the amount of malaria. An active case detection system was established as an additional means of discovering active malaria cases and to compare the efficiency of this surveillance activity with the traditional passive case detection system already in existence. Active case detection, in this case, consists of visits to all houses in an area once every two weeks. All people with fevers, encountered on these visits, receive a blood examination and are treated presumptively with a 4-aminoquinoline drug. Passive case detection consists of a volunteer in each locality to whom people with fevers can go to receive a blood examination and similar presumptive therapy. The results of these two surveillance techniques with the same population in Zapotitán from April 1971 through July 1972 indicate that the passive case detection system is considerably more efficient in discovering active malaria cases than the active surveillance technique (Table 27). The results indicate that the amount of malaria was somewhat higher in Zapotitán for the months April-July in 1972 than was seen during the same period in 1971. This is probably associated with a countrywide increase in malaria in 1972.

Theoretically all persons with fevers encountered in the active surveillance activity should have attended the voluntary collaborator. Over a period of approximately six months each person with a fever was asked why he had failed to go to the voluntary collaborator. The answers to this question are reviewed in Table 28. We had thought that answers 14 and 15 would be the most common, but it is clear that the voluntary collaborator system had been established for malaria and if the symptoms accompanying the fever did not resemble malaria then this service was not used. More than 28% of the people said that they did not go to the V.C. post because they did not think that they had malaria. In addition, more than 5% (answer #6) of the people said that they were waiting to see if the fever was caused by malaria. This level of self-diagnosis is surprising and if the positivity rate from the V.C. examinations is any indication the accuracy is quite high. Answers 3,4,9,13 and 16 are probably, for the most part, evasions and not valid.

The relative efficiency of the voluntary collaborator system, at least in this area, is high and these results suggest that such an activity, when well supervised, may constitute an effective malaria control measure in low incidence areas.

This study is still underway and it is hoped that specific recommendations can eventually be made on criteria for selecting a voluntary collaborator and the most effective spacing of these people in rural areas. It should be noted that these voluntary collaborators are better supervised than most and this level of efficiency may not be true of the entire system in El Salvador.

TABLE 27
Passive and Active Surveillance for Malaria by Month
Zapotitán, El Salvador, 1971-1972

	1971				1972			
	Passive		Active		Passive		Active	
Months	Exam.	Pos.*	Exam.	Pos.*	Exam.	Pos.*	Exam.	Pos.*
January					29	8	87	1
February					22	4	106	3
March					30	8	96	5
April	28	9	135	5	50	18	120	13
May	37	11	147	15	57	23	135	8
June	36	13	224	11	49	27	169	15
July	87	22	234	12	41	30	203	29
August	69	34	148	15				
September	106	50	181	25				
October	142	72	220	44				
November	108	71	192	25				
December	71	20	119	8				
Totals	684	302	1600	160	278	118	916	74

* All *Plasmodium vivax*

TABLE 28
Reasons for Failure of Febrile Persons to Visit Voluntary Collaborator Post in Zapotitán, El Salvador

Reasons	Male	Female	Total No.	Total %
1. Did not think symptoms due to malaria	95	120	215	28.5
2. Took self-purchased medication	49	54	103	13.7
3. Lack of time	55	36	91	12.1
4. Did not want to go (unqualified reason evasive)	30	43	73	9.7
5. Waiting for CNAP personnel to come	18	28	46	6.1
6. Waiting to see if symptoms due to malaria	23	19	42	5.6
7. Recency of symptoms	17	17	34	4.5
8. Poor relationship with V.C.	12	15	27	3.6
9. Location of post not known	18	6	24	3.2
10. Bad weather or road conditions	8	10	18	2.4
11. Too sick to go	8	7	15	2.0
12. Used other health facility	8	5	13	1.7
13. V.C. Post too far	5	7	12	1.6
14. Did not want finger stick for blood smear	3	6	9	1.2
15. Afraid of side effects from medication	2	6	8	1.1
16. V.C. not available at convenient time	5	3	8	1.1
17. Nobody available to take sick child	2	2	4	0.5
18. Other.	3	8	11	1.4
Total	361	392	753	100.0

Sero-epidemiologic Studies

Sero-epidemiologic studies on malaria have been carried out in Panama and Costa Rica as well as in El Salvador. Efforts have been made to explore as many different epidemiologic situations as possible in order to refine the interpretation of indirect fluorescent antibody (IFA) responses for malaria. Some of the basic problems being explored in these studies include the search for an "indicator population", definition of transmission areas, assessing the extent of malaria epidemics, evaluation of antimalaria measures, persistence of antibodies to different malaria species in naturally infected human populations, association of IFA level to malaria and splenic enlargement in areas of different endemicity, and IFA changes in the same population by means of serial studies. Six different field studies are presented in detail primarily according to the geographic area involved. Geometric mean reciprocal titers have been used in evaluating these results because the arithmetic mean would be inequitably weighted by high titers. All IFA studies have been done in cooperation with the Unit on Primate Malaria, LPD, NIAID, NIH, in Chamblee, Georgia.

Panama Studies

Sero-epidemiologic investigations have been carried out in the Lake Gatún area of the Republic of Panama. The population living on the shores of this lake have continued to have serious problems with malaria in spite of long-term antimalaria measures which include both residual insecticides and the mass distribution of drugs. *Plasmodium falciparum* is the dominant parasite in the area and *Anopheles albimanus* is presumably the primary vector, although the potential role of other anophelines has not been fully evaluated.

Three villages on the western side of Lake Gatún have been included in the study. One serologic parasitologic survey has been conducted in Escobal which can be reached by road from the town of Gatún in the Panama Canal Zone. Two similar surveys eight months apart, have been carried out in the villages of Cuipo and Ciricito which are approximately one and two hours, respectively, by boat from Escobal. The data from these surveys have been evaluated with reference to (a) serologic evidence of malaria experience, (b) changes in population serologic responses for malaria over a period of eight months, (c) the impact of a severe outbreak of *P. falciparum* on serologic responses to malaria, and (d) influence of mass distribution of antimalaria drugs on malaria serology.

The data on general incidence for the villages of Cuipo and Ciricito for August 1971 are summarized in Tables 29 and 30. The number of children under ten years of age with serologic evidence of recent experience with falciparum malaria is higher in Ciricito than in Cuipo. However, a difference does not exist in the 10-14 age group or in the adults. The higher incidence of positive titers ($\geq$1:20) in the younger age groups probably indicates a higher level of transmission in this village at the time of the initial surveys in August 1971. Titers $\geq$1:10 showed similar differences between the two localities; however, at the present time a titer of 1:10 is considered to be only suggestive of malaria experience, while titers of 1:20 or higher are considered firm evidence of such experience. Similar surveys were conducted among the school children in the same villages eight months later in April

TABLE 29
IFA Titers to Three Plasmodial Antigens
Cuipo, Panama, August 1971

Antigen	Age Group Years	IFA Serum Dilution Endpoint								No. Sera	GMRT	Positives (≥1:20)	
		≤1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640			%	GMRT
P. falciparum	≤ 4	40	3							43	5.2	0	-
	5-9	31	1							32	5.1	0	-
	10-14	22	3	3	0	0	2			30	7.8	17	(45.9)
	≥ 15	20	9	15	5	5	6	3	1	64	20.2	55	53.7
	All	113	16	18	5	5	8	3	1	169	9.3	24	52.7
P. vivax	≤ 4	41	2							43	5.1	0	-
	5-9	32								32	Neg	0	-
	10-14	29	1							30	5.1	0	-
	≥ 15	38	8	12	3	3				64	8.9	28	28.3
	All	140	11	12	3	3				169	6.3	28	28.3
P. malariae	≤ 4	42	1							43	5.1	0	-
	5-9	32								32	Neg	0	-
	10-14	30								30	Neg	0	-
	≥ 15	51	6	3	2	2				64	6.6	11	36.2
	All	155	7	3	2	2				169	5.6	4	36.2

Neg = 5.0 = All titers ≤1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 30
IFA Titers to Three Plasmodial Antigens
Ciricito, Panama, August 1971

Antigen	Age Group Years	IFA Serum Dilution Endpoint									No. Sera	GMRT	Positives (≥1:20)	
		<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280			%	GMRT
P. falciparum	≤ 4	30	2	0	1	1					34	6.0	6	(56.4)
	5-9	61	10	7	1	2	1	2			84	7.7	15	47.0
	10-14	45	5	2	2	3	2				59	7.8	15	58.8
	≥ 15	31	5	6	3	2	5	5	1	1	59	17.0	39	98.9
	All	167	22	15	7	8	8	7	1	1	236	9.0	20	71.1
P. vivax	≤ 4	31	3								34	5.3	0	-
	5-9	79	2	3							84	5.4	4	(20.0)
	10-14	47	8	4							59	6.0	7	(20.0)
	≥ 15	37	11	6	3	2					59	7.9	19	31.1
	All	194	24	13	3	2					236	6.1	8	26.2
P. malariae	≤ 4	33	0	0	1						34	5.3	3	(40.0)
	5-9	80	3	1							84	5.2	1	(20.0)
	10-14	54	4	1							59	5.4	2	(20.0)
	≥ 15	50	4	4	1						59	6.0	8	(22.9)
	All	217	11	6	2						236	5.5	3	23.7

( ) indicates mean based on 5 or fewer observations.

1972 (Tables 31 and 32). That the children 5-9 years of age in Cuipo showed an increase in malaria experience with *P. falciparum* during the period between the two surveys is reflected both in the percent who had positive IFA responses and in the geometric mean reciprocal titer of the positives. The second examination in Ciricito also included only school children and here there was recorded a decrease both in the percent of the children with positive IFA titers and in the geometric mean positive titer.

Serologic and parasitologic surveys were also carried out in April 1972 in the village of Escobal where a severe local outbreak of *P. falciparum* malaria had occurred between November 1971 and March 1972. This village is located at the end of the road and is also on the shores of Lake Gatún. Communication between Ciricito and Cuipo and other parts of Panama depends primarily on private and public boat traffic and Escobal is the terminus for most of this activity.

The results of the Escobal serologic survey are presented in Table 33. The impact of the outbreak is apparent both from the percentage positive titers and the geometric mean positive titers for the different age groups. The three positive titers (1:20, 1:40, and 1:80) for *P. falciparum* among children four years of age and under, and the absence of titers of 1:20 or higher to the *P. vivax* antigen in the same age group, indicates that the outbreak was a *P. falciparum* problem and was not associated with a concomitant increase in *P. vivax* transmission. The apparent increase in *P. falciparum* experience in Cuipo, noted above, may be associated with the outbreak in Escobal. Cuipo is closer than Ciricito to Escobal and may be reflecting, serologically, a direct extension of the falciparum outbreak from the latter area.

Table 34 summarizes the distribution of serologic responses according to a history of microscopically diagnosed falciparum malaria. Positive IFA responses were seen in 67% of the people who were recorded as having positive blood examinations during the outbreak while 24% of the people who did not have a positive diagnosis during the same period had an IFA titer of 1:20 or higher for *P. falciparum*. Mean titers are higher in the group with positive blood examinations than in those who were either not examined or did not show a demonstrable parasitemia during the period of intense malaria activity. Second surveys are planned for the same people in six to eight months. Since the outbreak was still underway, some of the known infections may have been too current to have produced detectable levels of antibody. In addition, a wide variety of drugs were used on a mass distribution basis during the outbreak and many infections were undoubtedly aborted early, before antibodies had developed. The next survey from this same population will provide a clearer picture of the serologic aspects of a massively treated outbreak of malaria.

The villages of Ciricito and Cuipo have been under a collective treatment program; the acceptance on the part of the people has varied. Some are very cooperative while others routinely refuse to take the drugs. The majority of the people fall into an interim category who participate in the antimalaria drug program on a sporadic basis. An effort was made to review the household records, for the preceding 18 months, of the people examined in the surveys in order to determine at what level they participated in the drug program. Only those persons for whom information was available were included in this analysis. The geometric mean IFA titers for *P. falciparum* are seen

TABLE 31
IFA Titers to Three Plasmodial Antigens
Cuipo, Panama, April 1972

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	No. Sera	GMRT	Positives (>1:20) %	GMRT
P. falciparum	5-9	50	1	1	2	1				55	5.9	7	(40.0)
	10-14	13	0	1	0	0	0	0	1	15	7.6	13	(113.2)
	5-14	63	1	2	2	1	0	0	1	70	6.2	9	56.6
P. vivax	5-9	54	1							55	5.1	0	-
	10-14	15								15	Neg	0	-
	5-14	69	1							70	5.1	0	-

Neg = 5.0 = All titers <1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 32
IFA Titers to Three Plasmodial Antigens
Ciricito, Panama, April 1972

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	No. Sera	GMRT	Positives (>1:20) %	GMRT
P. falciparum	5-9	62	4	4	1	71	5.8	7	(22.9)
	10-14	20	0	1	1	22	5.9	9	(28.2)
	5-14	82	4	5	2	93	5.8	8	24.3
P. vivax	5-9	69	2			71	5.1	0	-
	10-14	21	0	1		22	5.4	5	(20.0)
	5-14	90	2	1		93	5.1	3	(20.0)

Neg = 5.0 = All titers <1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 33
IFA Titers to Three Plasmodial Antigens
Escobal, Panama, April 1972

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	No. Sera	GMRT	Positives (≥1:20) %	GMRT
P. falciparum	≤ 4	20	3	1	1	1					26	6.9	12	40.0
	5-9	72	5	12	8	4	1				102	8.2	25	33.9
	10-14	18	1	3	0	3					25	8.5	24	40.0
	≥ 15	19	2	10	5	5	6	4	4	3	58	36.1	64	100.0
	All	129	11	26	14	13	7	4	4	3	211	12.0	34	61.5
P. vivax	≤ 4	25	1								26	5.1	0	-
	5-9	94	5	3							102	5.4	3	(20.0)
	10-14	22	2	1							25	5.6	4	(20.0)
	≥ 15	30	11	4	4	9					58	11.2	29	49.0
	All	171	19	8	4	9					211	6.6	10	41.3

Neg = 5.0 = all titers <1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 34
IFA Titers to *Plasmodium falciparum* Antigen by History of Microscopically Diagnosed *P. falciparum* Infection
Escobal, Panama

Age Groups Years	No. Sera	Previous Infection Recorded					No Previous Infection Recorded				
				IFA>1:20					IFA>1:20		
		No.	%	No.	%	GMRT	No.	%	No.	%	GMRT
≤ 4	26	3	12	1	33	12.6	23	88	2	9	6.5
5-9	102	23	23	14	61	17.8	79	77	11	14	6.6
10-14	25	4	16	1	25	6.8	21	84	5	24	7.9
≥ 15	58	18	31	16	89	79.4	40	69	21	53	24.0
All	211	48	23	32	67	26.9	163	77	39	24	9.5

TABLE 35
Mean IFA Titers in Relation to Participation in the Drug Distribution Program During the 18 Months Prior to August 1971

Antigen	Sex	No. Sera	Percentage Participation in Drug Program					
			0 - 50		51 - 75		76-100	
			No.	GMRT	No.	GMRT	No.	GMRT
P. falciparum	Male	93	21	11.5	44	7.9	28	8.7
	Female	113	32	13.8	40	12.0	41	6.5
Total		206	53	12.9	84	9.8	69	7.2
P. vivax	Male	93	21	7.2	44	5.6	28	5.6
	Female	113	32	7.8	40	6.5	41	5.4
Total		206	53	7.6	84	6.0	69	5.4
P. malariae	Male	93	21	6.9	44	5.4	28	5.2
	Female	113	32	6.0	40	5.5	41	5.4
All		206	53	6.3	84	5.4	69	5.4

to range from 12.9 in the group who took the drugs 50% or less of the time to 7.2 in those who participated in the drug program more than 75% of the time (Table 35). The titers are so low to both the *P. vivax* and *P. malariae* antigens that no trend can be recognized. These analyses are preliminary but do indicate that serologic techniques may be quite useful in evaluating drug distribution programs in malarious areas. Such an evaluation could be quite precise if the evaluation were planned at the beginning of the program. With this technique it is believed possible to eventually determine the level of population participation in antimalaria drug distribution programs required to produce a reduction in malaria transmission that is compatible with the cost.

Costa Rica Studies

Malaria programs in Costa Rica have achieved a greater level of success than in most other parts of Central American region. Active cases are sporadic and primarily confined to areas along the Pacific coast, and *P. falciparum* has essentially disappeared. These conditions present a series of problems to the national malaria programs which are somewhat different from those found in other Central American countries and Panama, but are probably indicative of what these latter countries will face in the future. Transmission undoubtedly continues in some areas, and it becomes very important to define specifically these localities in order to concentrate attack measures in appropriate places. Therefore, it is necessary to determine if active cases are autochthonous. To determine the applicability of serology to this problem, serologic and parasitologic surveys have been conducted in six villages in the Provinces of Puntarenas and Guanacaste. Occasional cases of *P. vivax* have recently been reported from all of these areas. In three of these villages the results suggest a possibility of active malaria transmission (Tables 36, 37, 38). Cuajiniquil (Table 36) would require some specific case investigations before the possibility of active transmission can be eliminated. The 1:40 *P. vivax* titer in the ≤ 4 age group does indicate recent malaria experience and it would be important to know if this child has been out of the area recently. In addition, three adults from the same village had relatively high titers to *P. vivax* (one 1:40 and two 1:80) which are also suggestive of recent parasitemias with this parasite. The falciparum titers from Cuajiniquil could represent older infections, as it is unlikely that this parasite would be active in the population and not appear in the organized field surveillance system maintained by the country program.

Tarcoles and El Palmar (Tables 37 and 38) also require some specific case investigations. The 1:20 *P. vivax* titer in the 5-9 age group in Tarcoles is probably the result of a recent infection with this parasite. Because of this, combined with the presence of eight adult titers $\geq$ 1:20 for the same parasite, the possibility of some local transmission must be considered in this village. El Palmar gave two low (1:10) titers for *P. vivax* in children under ten years of age. These are suspicious and should be investigated, however, there is little additional serologic evidence of recent malaria transmission in this village.

From the serologic results obtained, the other three villages in the study appeared less likely to have current transmission problems. The village of Guardia showed no serologic evidence of malaria transmission. No positive

TABLE 36
IFA Titers to Three Plasmodial Antigens
Cuajiniquil, Costa Rica

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	1:80	1:160	No. Sera	GMRT	Positives (≥1:20) %	GMRT
	≤ 4	16						16	Neg	0	-
	5-9	19	0	1				20	5.4	5	(20.0)
P. falciparum	10-14	25	1					26	5.1	0	-
	≥ 15	31	2	1	1	0	1	36	6.3	8	(50.3)
	All	91	3	2	1	0	1	98	5.6	4	(40.0)
	≤ 4	15	0	0	1			16	5.7	6	(40.0)
	5-9	20						20	Neg	0	-
P. vivax	10-14	25	1					26	5.1	0	-
	≥ 15	30	3	0	1	2		36	6.6	8	(63.1)
	All	90	4	0	2	2		98	5.4	4	(56.6)
	≤ 4	16						16	Neg	0	-
	5-9	20						20	Neg	0	-
P. malariae	10-14	26						26	Neg	0	-
	≥ 15	30	1	1	2	1	1	36	7.1	14	(52.7)
	All	92	1	1	2	1	1	98	5.7	5	(52.7)

Neg = 5.0 = all titers <1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 37
IFA Titers to Three Plasmodial Antigens
Tarcoles, Costa Rica

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	1:80	1:160	1:320	No. Sera	GMRT	Positives (≥1:20) %	GMRT
	≤ 4	23	1						24	5.1	0	-
	5-9	38	3	1					42	5.4	2	(20.0)
P. falciparum	10-14	27							27	Neg	0	-
	≥ 15	55	9	4	7	3	4	3	85	10.0	25	67.8
	All	143	13	5	7	3	4	3	178	7.2	12	64.0
	≤ 4	24							24	Neg	0	-
	5-9	41	0	1					42	5.1	2	(20.0)
P. vivax	10-14	26	1						27	5.1	0	-
	≥ 15	56	10	8	5	6			85	8.5	22	37.2
	All	147	11	9	5	6			178	6.5	11	36.1
	≤ 4	24							24	Neg	0	-
	5-9	42							42	Neg	0	-
P. malariae	10-14	25	1	1					27	5.4	4	(20.0)
	≥ 15	69	11	5					85	5.9	6	(20.0)
	All	160	12	6					178	5.5	3	20.0

Neg = 5.0 = all titers <1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 38
IFA Titers to Three Plasmodial Antigens
El Palmar, Costa Rica

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	1:80	No. Sera	GMRT	Positives (≥1:20) %	GMRT
	≤ 4	30	1				31	5.1	0	-
	5-9	17					17	Neg	0	-
P. falciparum	10-14	13					13	Neg	0	-
	≥ 15	41	3	6	0	3	53	7.1	17	31.6
	All	101	4	6	0	3	114	5.9	8	31.6
	≤ 4	30	1				31	5.1	0	-
	5-9	16	1				17	5.1	0	-
P. vivax	10-14	13					13	Neg	0	-
	≥ 15	45	5	2	0	1	53	5.9	6	(31.7)
	All	104	7	2	0	1	114	5.5	3	(31.7)
	≤ 4	31					31	Neg	0	-
	5-9	17					17	Neg	0	-
P. malariae	10-14	13					13	Neg	0	-
	≥ 15	47	5	1			53	5.5	2	(20.0)
	All	108	5	1			114	5.2	1	(20.0)

Neg = 5.0 = all titers <1:10.
( ) indicates mean based on 5 or fewer observations.

titers were found in 81 persons under 15 years of age. Every child encountered during the survey was examined, including all of the students in the local school. The absence of both parasites and positive serologic responses would suggest that malaria transmission does not occur here. Of 55 adults ($\geq$ 15 years of age) one had a positive titer of 1:20 and another of 1:160 for *P. vivax*. The higher titer requires specific investigation. Likewise no evidence was found of active transmission in Irigaray or Charcarita. Titers were 1:10 or less to *P. falciparum* in the 199 children under ten years of age from both of these villages; in addition, no falciparum case has been diagnosed in these areas for at least three years. Only four of 40 adults in Irigaray and none of 81 in Charcarita had minimal positive titers (1:20) for *P. vivax*. Consequently these two villages also offer little evidence of active transmission.

Previous investigations have strongly indicated that *P. vivax* antibodies do not persist for as long as those for *P. falciparum*. These studies in Costa Rica have added circumstantial evidence. The surveillance system in Costa Rica is well organized and it is not likely that significant numbers of active *P. falciparum* cases would appear in the populations of these villages without at least some of them being detected in routine examinations. We are, therefore, prone to consider these *P. falciparum* positive IFA responses as the result of infections at some time in the past and not the result of recent active transmission. The problem of persistence of titers to different malaria species is now under investigation.

The series of relatively high *P. malariae* titers in Cuajiniquil (Table 36) is somewhat surprising. However, it is known that infections with this parasite persist at a chronic level for many years. These data suggest the possibility that *P. malariae* was active in the area in the past, and/or some chronic infections persist in the adults.

The study furnishes added support to the use of serology in detecting areas of transmission, especially through sampling a target population -- school children.

Serologic Changes in an Indicator Population: El Salvador

The use of sero-epidemiologic techniques to evaluate specific antimalaria measures has been investigated in a coastal area of El Salvador where residual propoxur (OMS-33) was to be applied once every 35 days as a partial coverage spray (two 1-meter swaths) on the interior walls of houses.

One person from each of 269 houses, which were scattered throughout the study area, was selected for the investigation. During the previous two years these houses had experienced at least one active case of malaria. No person who was febrile on the day of the first visit was included. Thick blood films and blood specimens on filter paper were taken from each of the 269. All then received the equivalent of an adult dose of 1500 mg. chloroquine base over the first three days as well as 15 mgs. (adult dose) primaquine per day for 14 days. The therapeutic regimen was completed for all members of the study group. A second specimen for serologic analysis was taken after six months, which included the period of highest malaria transmission in the area. These second examinations included 236 of the original study population of 269.

Analyses of the fluorescent antibody results include only those 236 individuals available for both samplings. In the initial survey 22% of the population had titers of 1:20 or higher to the *P. falciparum* antigen and 11% and 6% to the *P. vivax* and *P. malariae* antigens, respectively (Table 39). The existence of six positive titers (1:20) to *P. vivax* and no positive titers to *P. falciparum* in children under ten years of age, indicates that the former parasite was more active in the area. In addition, three blood smears were positive for *P. vivax* parasites. There was little serologic evidence of recent *P. malariae* transmission in this population.

The surveys in October 1971 (Table 40) showed little change in the number of people who had positive (≥1:20) titers (21% vs. 22% *P. falciparum* and 13% vs. 11% *P. vivax*); however, the geometric mean titers of the positives were higher for both antigens suggesting that there had been some malaria activity in the area during the interval between the two surveys. The number of persons with positive titers for *P. malariae* decreased during the interval from 15 to 8. The positive malariae titers were, with one exception in each survey, confined to adults and probably represent either old infections or a cross-reactivity with other antigens, since there is no evidence, either parasitologic or serologic, to indicate the active transmission of *P. malariae* malaria in this coastal area.

No detectable IFA antibody to malaria was seen on the first examination in 136 of the 236 people who were examined during both surveys, and 110 remained non-reactors in the second examination (Table 41). During the six-month interval between the two examinations 26 people converted from non-reactors to having a demonstrable titer to one or more plasmodial antigens (17 at 1:10 and 9 at 1:20 or higher). The rate of conversion was lower in the 10-14 age group than in the other groups. There is no apparent explanation for a possibly different risk of exposure to malaria in this age group.

The serologic conversions from non-reactive to positive are detailed in Tables 42 and 43. Fluorescent antibody titers of 1:10 (Table 42) may be related to malaria experience but are not considered positive; titers of 1:20 or higher are considered positive. In this study, where two consecutive specimens six months apart revealed a change from non-reactive to a titer of 1:10, it is possible that some of these low titers reflect early infections. In fact, one (Case No. 18) was parasite positive at the time of the second survey. Some clarification of this problem will be obtained when a full analysis is made of the data collected on the third survey of the same population made in May 1972.

The conversions from non-reactive to titers of 1:20 or higher (Table 43) is considered to be clear evidence of malaria experience in the six-month interval between the two examinations. The serologic changes came with one, two or even all three human plasmodial antigens used in the test. At present, only the antigen providing the maximum titer is considered to be specifically associated with malaria experience. Lower titers with second or even third antigens are difficult to interpret, but may be partially explained by the fact that all plasmodial antigens are, to some extent, similar and may produce heterologous as well as homologous antibody responses.

TABLE 39
IFA Titers to Three Plasmodial Antigens
Las Isletas, El Salvador, May 1971

Antigen	Age Group Years	IFA Serum Dilution Endpoint <1:10	1:10	1:20	1:40	1:80	1:160	1:320	No. Sera	GMRT	Positives (≥1:20) %	GMRT
	≤ 4	14							14	Neg	0	-
	5-9	61	4						65	5.1	0	-
P. falciparum	10-14	44	1	1					46	5.2	2	(20.0)
	≥ 15	44	17	21	14	10	1	4	111	14.5	45	41.7
	All	163	22	22	14	10	1	4	236	8.4	22	41.1
	≤ 4	13	0	1					14	5.5	7	(20.0)
	5-9	56	4	5					65	5.8	8	(20.0)
P. vivax	10-14	42	3	0	1				46	5.5	2	(40.0)
	≥ 15	75	17	11	6	2			111	7.5	17	28.8
	All	186	24	17	7	2			236	6.5	11	26.8
	≤ 4	14							14	Neg	0	-
	5-9	64	1						65	5.1	0	-
P. malariae	10-14	46							46	Neg	0	-
	≥ 15	83	13	11	2	2			111	6.8	14	27.7
	All	207	14	11	2	2			236	5.8	6	27.7

Neg = 5.0 = all titers <1:10.
( ) indicates mean based on 5 or fewer observations.

TABLE 40
IFA Titers to Three Plasmodial Antigens
Las Isletas, El Salvador October 1971

Antigen	Age Group Years	IFA Serum Dilution Endpoint									No. Sera	GMRT	Positives (≥1:20)	
		<1:10	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280			%	GMRT
P. falciparum	≤ 4	14									14	Neg	0	-
	5-9	58	6						1		65	5.8	2	(640.0)
	10-14	43	0	3							46	5.5	7	(20.0)
	≥ 15	45	20	25	4	4	8	3	1	1	111	14.6	41	48.6
	All	160	26	28	4	4	8	3	2	1	236	8.7	21	48.5
P. vivax	≤ 4	13	1								14	5.2	0	-
	5-9	56	5	4							65	5.8	6	(20.0)
	10-14	44	1	1							46	5.2	2	(20.0)
	≥ 15	69	17	10	6	8	1				111	8.9	23	40.0
	All	182	24	15	6	8	1				236	6.9	13	35.9
P. malariae	≤ 4	14									14	Neg	0	-
	5-9	59	5	1							65	5.4	2	(20.0)
	10-14	46									46	Neg	0	-
	≥ 15	93	11	6	1						111	5.9	6	21.6
	All	212	16	7	1						236	5.5	3	21.6

Neg = 5.0 = all titers <1:10
( ) indicates mean based on 5 or fewer observations.

TABLE 41
Sero-conversions (IFA) for Malaria from Negative to Positive
Las Isletas, El Salvador

Age Group	No. with Two Examinations	No. Non-Reactors First Examination	No. Non-Reactors First and Second Examination	No. Conversions Negative to 1:10	No. Conversions Negative to ≥1:20
≤ 4	14	12	11	1	0
5-9	65	57	44	10	3
10-14	46	38	36	1	1
≥ 15	111	29	19	5	5
ALL	236	136	110	17	9

TABLE 42
Conversions of IFA Titers for Malaria from Negative to 1:10 between May and October 1971 in Las Isletas, El Salvador

No.	Age	Sex	Fluorescent Antibody Titer October 1971		
			P.f.	*P.v.*	*P.m.*
18*	7	F	-	1:10	-
74	6	M	-	1:10	-
78	7	F	1:10	-	-
87	7	M	1:10	-	-
103	17	M	-	1:10	-
104	8	F	-	1:10	-
105	6	F	-	-	1:10
107	30	F	1:10	-	-
112	9	M	-	-	1:10
127	7	M	-	1:10	-
132	12	F	1:10	-	-
137	8	F	-	1:10	1:10
156	32	F	-	1:10	-
177	18	F	1:10	-	-
220	5	M	1:10	-	1:10
236	4	F	-	1:10	-
237	34	F	1:10	1:10	-

* A blood slide from Case No. 18 in the October survey was positive for *P. vivax*.

TABLE 43
Conversions of IFA Titers for Malaria from Negative to 1:20 or higher between May and October 1971 in Las Isletas, El Salvador

No.	Age	Sex	Fluorescent Antibody Titer October 1971		
			P.f.	*P.v.*	*P.m.*
4	20	F	1:40	1:80	1:10
28	19	M	-	1:20	-
33	8	F	1:640	1:20	1:20
86	13	F	-	1:20	-
115	6	F	1:10	1:20	-
128	18	F	1:40	-	-
231	15	F	-	1:20	-
233	7	M	-	1:20	-
253	54	F	1:20	-	-

Note: No parasites found in either survey.

A third category of titer change was observed in this study. More than 40% of the study group had IFA malaria titers of 1:10 or higher on the first survey. Fourteen of these people, all with titers of 1:10 or 1:20, were serologically non-reactive for malaria on the second examination; this change probably represents antibody decay in individuals who have had no recent exposure to the stimulating antigen. Seventy-eight individuals showed no change, a decrease in titer, or an increase of only one two-fold dilution. Increases of two or more two-fold dilutions in titer were seen in eight people (Table 44), and these marked changes probably represent malaria experience during the period of the study.

These data indicate that serologic techniques associated with an indicator population can be of value in determining the effectiveness of antimalaria methods in interrupting transmission. Thirty-four of 236 people in the study area gave some serologic evidence of malaria experience during the period of the study. Nine of these converted from non-reactive to titers of 1:20 or higher. Malaria experience is suggestive but less clear for the remaining twenty-five. Nevertheless, there is no doubt the malaria transmission was not completely interrupted by the insecticide. These results have been confirmed by blood examinations on both the study group and the area population as a whole. Therefore, in one six-month period specific information on the effectiveness of an antimalaria measure was obtained through the use of serologic studies on an indicator population. More recently insecticide resistance has been detected in the mosquito populations in the study area and may be at least partially responsible for the failure of propoxur to interrupt transmission.

TABLE 44
Increase of Two or More Two-fold Dilutions in IFA Titers
for Malaria between May and October 1971
Las Isletas, El Salvador

			Fluorescent Antibody Titer					
			1st			2nd		
No.	Age	Sex	*P.f.*	*P.v.*	*P.m.*	*P.f.*	*P.v.*	*P.m.*
45	50	F	1:80	1:20	1:20	1:1280	1:160	1:40
48	35	M	1:40	1:20	1:10	1:320	1:80	-
62	35	F	1:40	-	-	1:160	-	-
116	26	F	1:80	1:10	1:10	1:80	1:80	-
124	53	M	1:40	1:10	1:20	1:20	1:40	-
240	62	M	1:10	1:10	-	1:10	1:80	-
244	40	F	1:10	-	-	1:40	-	-
268	32	M	1:10	1:10	-	-	1:80	-

Note: No parasites found in either survey.

It should be noted that these results are dependent upon the effectiveness of the radical cure regimen employed, particularly with relapsing *P. vivax* infections. Studies on strains of parasites from this area have indicated that 14 days of primaquine does effect radical cures of *P. vivax* infections and to date all *P. falciparum* strains in El Salvador respond to 1500 mgs. of chloroquine.

Splenic Enlargement and Fluorescent Antibody Titers: El Salvador

Surveys of populations for splenic enlargement has been a traditional method of defining malaria endemicity. It is of interest to know if the more recently developed serologic techniques reflect similar levels of population malaria experience, since both splenic enlargement and antibody development theoretically represent host responses to a malaria infection.

Five different villages in different parts of El Salvador were selected for this study. In two of the study areas both adults and children were included in house-to-house surveys. In the remaining three areas only school children were examined. Three of the study areas are in the malarious coastal part of El Salvador. San Diego has a history of continuous transmission of *P. vivax*; La Zunganera is a developing area adjacent to the sea where trials of the carbamate insecticide Landrin have been conducted; Jocotal, near the large town of Usulután, is located on a permanent fresh water lagoon and has a notorious history of malaria transmission. The fourth study area, Zapotitán, is near Santa Tecla and is part of an extensive agricultural development project including a large irrigation effort. Tejutepeque is located in the mountainous interior in the Department of Cabañas. This village is located above 500 meters and previous studies have indicated little or no malaria transmission in the area.

Surveys were carried out in each of the study areas and included routine thick blood films, spleen examinations and blood specimens on filter paper for IFA determinations. All sera failing to show a reaction at a dilution of 1:10 are arbitrarily given a value of 1:5 since 1:10 was the lowest dilution examined.

That Tejutepeque is clearly little involved in active malaria transmission is evidenced by the presence of only two titers of 1:10 in the school sample from this village.

Jocotal is considered to be more malarious and this condition was reflected in the serologic responses. There were 12 children with titers of 1:20 or higher for *P. vivax* or *P. falciparum*, indicating recent malaria experience.

San Diego showed the expected group serologic responses associated with a persistent moderate level of malaria transmission with *P. vivax* as the dominant parasite. Approximately 17% of the children examined in this area gave evidence of recent experience with *P. vivax*.

Zapotitán revealed an interesting anachronism. Serologic responses in the 15 and over age group indicated some malaria experience in the population. Also, active malaria cases have been confirmed in the area. However, the absence of titers in the children would seem to reflect a low level of transmission and consequently a considerable amount of "imported" malaria. Investigations currently underway to clarify this situation include more extensive school surveys, mapping of positive responses and a thorough investigation of the transmission ecology in the area.

La Zunganera is an interesting coastal community that extends for a considerable distance along the beach. Serologic responses indicated that both *P. vivax* and *P. falciparum* are active in the area. The high positive GMRT shows current experience with both of these parasites, and the segment of the population involved was quite large. Positive responses are now being mapped in order to determine if transmission is more active in one part of this sprawling community than in another.

The relationships between serologic responses to malaria and splenic enlargement are presented in Table 45. Analysis of these data is still preliminary but generally the study seems to indicate little correlation between splenic enlargement and malaria endemicity as measured serologically. The proportion of persons with maximum titers to one or more malaria antigens was higher in the group with enlarged spleens in Tejutepeque, San Diego and La Zunganera but the reverse in Jocotal and Zapotitán. (Numbers were insufficient to further divide the individuals by degree of spleen enlargement). These latter two villages are currently being investigated in order to look for an explanation of this result. At present there is no apparent bias in sampling and there is no known disease entity in any of the study areas that would be prone to produce enlarged spleens other than malaria.

TABLE 45
Malariometric Indices in Five Villages of El Salvador

Village	Age Group	No. Exam.	Spleen not Palpable							Spleen 1 + or More						
			No.	%	Par. Pos.	Max. IFA Titers *P.f.*	*P.v.*	*P.m.*	%*	No.	%	Par. Pos.	Max. IFA Titers *P.f.*	*P.v.*	*P.m.*	%*
San Diego	< 4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5-9	48	35	73	0	1	2	0	9	13	27	1	0	3	0	23
	10-14	85	67	79	5	0	11	0	16	18	21	1	0	7	0	39
	≥ 15	15	10	67	0	0	0	0	0	5	33	0	0	1	0	20
	All	148	112	76	5	1	13	0	13	36	24	2	0	11	0	31
Tejutepeque	< 4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5-9	107	87	81	0	1	1	0	2	20	19	0	2	1	0	15
	10-14	105	82	78	0	0	0	0	0	23	22	0	0	0	0	0
	> 15	11	11	100	0	0	0	0	0	0	-	-	-	-	-	-
	All	223	180	81	0	1	1	0	1	43	19	0	2	1	0	7
Jocotal	< 4	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	5-9	30	21	70	0	1	6	0	33	9	30	1	0	0	0	0
	10-14	24	18	75	0	1	3	0	22	6	25	0	0	1	-	16
	≥ 15	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	All	54	39	72	0	2	9	0	28	15	28	1	0	1	0	7
Zunganera	< 4	19	12	63	0	0	1	0	8	7	37	1	0	1	0	14
	5-9	35	24	69	2	0	1	0	4	11	31	2**	3	2	1	45
	10-14	13	7	54	0	1	0	0	14	6	46	0	2	1	0	50
	≥ 15	62	41	66	3	11	11	1	27*	21	34	1	12	5	1	71
	All	129	84	65	5	12	13	1	30	45	35	4	17	9	2	53
Zapotitán	< 4	25	14	56	-	-	-	-	-	11	44	-	0	0	0	0
	5-9	37	23	62	-	0	1	0	4	14	38	-	0	0	0	0
	10-14	27	12	44	-	0	1	0	8	15	56	0	0	1	0	7
	≥ 15	80	55	69	-	9	8	9	33	25	31	0	3	4	0	28
	All	169	104	62	0	9	10	9	19	65	38	0	3	5	0	12

* % of population with maximum titer to at least one antigen.
** Dual infection - *P. vivax* + *P. falciparum*.

Longitudinal Studies in Guayabo: El Salvador

The incidence of malaria has historically been lower in the interior valleys of El Salvador than in the coastal area. However, occasional cases do occur and it has been difficult to determine how many of these were imported and how many were actually the result of local transmission. Such low incidence areas offer the greatest challenge to malaria surveillance systems. A longitudinal study was initiated in 1970 in seven villages near a hydro-electric project in the Rio Lempa valley in the departments of Cabañas and Chalatenango. This area was considered to be particularly advantageous for sero-epidemiologic studies since the low incidence would offer a greater challenge to the validity of these techniques in accurately assessing malaria incidence. Longitudinal investigations were considered important since interpretation of serologic responses could be better refined if population responses could be correlated with the known malaria situation over a period of time. Finally the establishment of serologic profiles for malaria in these areas would provide baselines for future studies, particularly with reference to new serologic techniques.

Surveys were conducted either through house-to-house surveys or on some occasions school populations were examined. Thick blood films for microscopic examination as well as blood specimens on filter paper for indirect fluorescent antibody determination were prepared. Voluntary collaborator posts in the area were visited frequently so that levels of fevers and diagnosed cases of malaria were known. At least two surveys have been conducted in each village and in one, three surveys have been completed. The time lapse between surveys averaged nine months ranging from three to 20.

A summary of the serologic profiles for malaria in these villages are presented in Table 46. San Jose Potrerillos showed an increase in malaria experience between March and December 1971, from 0 to 3.4% of the population with titers of 1:20 or higher to *P. falciparum* antigen. This increase was associated with the older age groups. The increase in *P. falciparum* responses is particularly interesting since there is no evidence, from routine surveillance data, of active transmission of this parasite in San Jose Potrerillos. However, all of the *P. falciparum* titers came from people over 20 years of age and four of the six were from men between 22 and 30 years, which is the group most likely to go to the more malarious coast for temporary agricultural work. Therefore the increase in malaria experience may not be associated with an increase in local transmission.

Three surveys have been conducted in San Benito and the two examinations in 1971 do indicate an increase in malaria experience over that seen in 1970, from 0 to 9.1% positive to *P. vivax* antigen. These serologic increases were again mostly associated with older age groups; however, some low titers did appear in children under nine years of age and in March 1971 one six-year old girl had a titer to *P. vivax* of 1:160. These serologic data are compatible with low level local transmission.

There is little evidence in San Nicolás of active malaria transmission. Titers of 1:20 or higher were all from older age groups and there was little change in the population response between July 1970 and March 1971.

TABLE 46
Repeated Serologic Surveys
Guayabo, El Salvador

Locality	Antigen	SURVEY 1 Date	SURVEY 1 No. Exam.	SURVEY 1 GMRT	SURVEY 1 % Pos(≥1:20)	SURVEY 2 Date	SURVEY 2 No. Exam.	SURVEY 2 GMRT	SURVEY 2 % Pos(≥1:20)
San Jose Potrerillos	*P.f.*	3/71	106	Neg	0	12/71	89	5.4	3.4
	P.v.		106	Neg	0		89	5.4	1.1
	P.m.		0	-	-		89	5.1	0
San Benito	*P.f.*	8/70	40	5.1	0	3/71	66	5.8	4.5
	P.v.		40	Neg	0		66	6.2	9.1
	P.m.		40	5.1	0		66	5.1	0
	P.f.					12/71*	143	5.6	4.2
	P.v.						143	5.5	3.5
	P.m.						143	5.1	0.7
San Nicolás	*P.f.*	7/70	58	5.5	3.4	3/71	73	5.5	1.4
	P.v.		58	5.2	1.7		73	5.1	1.4
	P.m.		58	5.2	1.7		73	5.1	1.4
Nombre de Jesús	*P.f.*	10/70	82	-	0	1/71	174	5.2	2.3
	P.v.		82	-	0		174	5.1	0.6
	P.m.		82	-	0		174	Neg	0
La Trinidad	*P.f.*	11/70	156	5.2	0.6	12/71	21	5.4	0
	P.v.		156	5.2	1.9		21	5.6	4.8
	P.m.		156	Neg	0		21	5.5	4.8
El Dique	*P.f.*	7/70	15	6.0	0	12/71	52	6.3	9.6
	P.v.		15	6.6	13.3		52	7.4	11.5
	P.m.		15	5.8	6.7		52	5.6	7.8
Sta. Rosa La Maraña	*P.f.*	12/70	117	6.0	5.1	6/71	135	5.4	2.2
	P.v.		117	6.5	9.4		135	5.6	4.4
	P.m.		117	5.2	0		135	Neg	0

* A third survey from San Benito is included.

Serologic responses for malaria in Nombre de Jesús were very low. The survey in October of 1970 was primarily in school children (72 of 82 samples from ages five to 14 years) and showed that the children from this village had no malaria experience. A follow-up survey in January 1971 confirmed the earlier results with reference to the children but did reveal a few, mostly low titers to both *P. falciparum* and *P. vivax*.

La Trinidad, in November 1970, revealed some evidence of malaria transmission with at least two children under 14 years of age with positive titers for *P. vivax*. A second survey in December 1971 represented a very limited sample but did show one child with a positive *P. vivax* titer.

El Dique is a small community occupied by the members of one familial group. There is no voluntary collaborator post in this locality and there are occasional cases of *P. vivax*. In July 1970 a small number of people from this village were examined and there were titers which indicated recent experience with *P. vivax* malaria. There was one titer to this antigen of 1:40 in a child under four years of age. This case was investigated and there was no history of trips outside the village. One titer of 1:40 for *P. malariae* was seen and may well indicate the continued active presence of this parasite in the area. In December 1971 a more complete survey was taken and the *P. vivax* activity was serologically quite evident. The positive *P. falciparum* titers were all in adults and probably represent exposure elsewhere.

Santa Rosa La Maraña is undoubtedly a transmission area. The serologic evidence of current malaria activity is confirmed by information from the voluntary collaborator post. This village is adjacent to the others under study in the area and does not appear to be different, ecologically, from nearby localities with less malaria activity.

These data reveal that malaria transmission continues in this interior area of El Salvador but is sporadic and varies not only with time but with locality as well. Residual DDT has been used until recently on a routine basis in these villages but, at present, the only antimalaria measure is the presumptive treatment, with chloroquine, of febriles by the voluntary collaborators.

Positive blood films were rarely found in these surveys. Data from all of the voluntary collaborators in the study area are now being analyzed in order to make specific correlations between the diagnosis of active cases and the serologic data from the periodic surveys. Vector studies are being undertaken in Santa Rosa La Maraña in an effort to ascertain why the level of transmission is higher in this village. The malaria picture in this area is complicated by the presence of imported malaria as revealed by positive *P. falciparum* titers in adult males but not in children. As yet, there is no evidence that these cases tend to produce secondary infections by means of local transmission but this situation may change now that residual insecticides are no longer in use.

Additional periodic surveys will be conducted in these villages to learn if the voluntary collaborator system alone is able to maintain the relatively low level of transmission now present in the area. These surveys will also help to learn if and when the general increase in malaria incidence in

coastal El Salvador begins to extend to what are now low-incidence interior valleys.

Brazil Studies

The sero-epidemiologic study in the Cuiabá Sector of Mato Grosso State, Brazil was concluded with a fourth parasitologic/serologic survey carried out in September 1971. Surveillance data from the area have been collected and tabulated through December 1971.

During the fourth survey a total of 933 blood films and dual filter paper samples were collected in 15 localities. The results are presented in Table 47, and a summary of the four surveys between April 1970 and September 1971 is contained in Table 48. There was little change in the overall positivity in the final survey, with 1.4% of the slides being positive and 12.6% showing positive serology by the indirect fluorescent antibody (IFA) method.

The surveillance data available for the years 1966 through 1971 are summarized in Table 49. Surveillance in this area has been by both active and passive case detection methods, although, with the exception of the year 1970, the greatest yield of samples was from the passive detection network. For the year 1970, a fairly extensive program of active case detection was installed, which explains both the larger number of slides taken and the lower percent of positives. One striking difference during 1971 which cannot be similarly explained is the significant decrease in the numbers of *P. falciparum* positives which were detected during that year.

During the five previous years the proportion of *P. falciparum* positive had remained fairly stable, although there had been a progressive decrease from 80 to 66% between 1967 and 1970. The somewhat precipitous decrease to about 40% in 1971 was unexpected and difficult to explain. There was a moderate decrease in the number of maximum FA titers to *P. falciparum* between October of 1970 and September of 1971, from 91 to 77%, which may be a reflection of the same general decrease of *P. falciparum* prevalence in the area. There are a number of unknown factors which may have a bearing on this phenomenon. Many of the *P. falciparum* cases in this area may be imported from Amazonian regions to the north and west of the Cuiaba Sector, and during the past year the awareness of this problem may have increased and brought about more intensive case finding and treatment of these cases prior to their reaching the Cuiaba area in transient populations. Development of the Cuiaba Sector and of the areas to the north and west has been fairly rapid in recent years, and may have resulted in a reduction of the number of persons returning to or through the Sector. A high proportion of the *P. falciparum* cases in the past had been found by one or two laboratories in major municipios and represented truck drivers or other laborers returning from areas as far away as Porto Velho who apparently happened to reach these population centers at about the time malaria symptoms appeared. A number of unknown factors might well affect the yield of positives through these sources: the risk of malaria may have decreased in the areas of origin; any physical change in the diagnostic laboratories, such as change of location, change of personnel or change of diagnostic methods, may have decreased the yield; the availability of private sources of diagnosis and treatment may have increased. No additional control methods had been installed within the Cuiaba Sector during the period in

TABLE 47
Parasitologic and Serologic (IFA) Results of a Survey of 15 Localities in Cuiabá Sector, Mato Grosso, Brazil, September 1971

Locality	Parasitologic Examinations						Serologic Examinations (IFA)					
		Positive						Positive		Maximum Titers		
	Exam.	No.	%	*P.f.*	*P.v.*	Unk.	Exam.	No.	%	*P.f.*	*P.v.*	*P.f.-P.v.*
Tres Pedras	57	0	0.0				57	9	15.8	3	5	1
Fazendinha	26	0	0.0				26	4	15.4	0	4	0
Coxipo do Ouro	42	1	2.4	0	0	1	42	1	2.4	0	1	0
San Goncalo	57	0	0.0				57	1	1.8	0	1	0
N.S. de Guia	150	1	0.7	1	0	0	150	8	5.3	5	3	0
Guarita	78	1	1.3	1	0	0	78	2	2.6	2	0	0
Baro do Bugres	244	3	1.2	2	1	0	243	38	15.6	30	5	3
Porto Estrella	64	3	4.7	0	3	0	63	8	12.7	6	2	0
Joao Paulo	29	0	0.0				29	6	20.7	6	0	0
Pai Luis	54	1	1.9	1	0	0	54	10	18.5	8	1	1
Passagem Sociedade	27	1	3.7	0	1	0	26	1	3.8	1	0	0
Quitanda Faz.	19	1	5.3	1	0	0	19	2	10.5	2	0	0
Posto Indigena	49	0	0.0				49	22	44.9	16	5	1
Faz. Santana	14	0	0.0				14	1	7.1	1	0	0
Ouro Fino	23	1	4.3	1	0	0	23	5	21.7	5	0	0
Total	933	13	1.4	7	5	1	930	118	12.7	85	27	6

TABLE 48
Parasitologic and Serologic (IFA) Results,
Cuiabá Sector, Mato Grosso, April 1970-Sept. 1971

Survey Date	Parasitologic Examinations						Serologic Examinations			
		Positive						Positive		
	Exam.	No.	%	*P.v.*	*P.f.*	Unk.	Exam.	No.	%	% Max. *P.f.*
Apr '70	957	8	0.8	7*	2*	0	920	110	12.0	80.9
Oct '70	767	9	1.2	1	8	0	741	69	9.3	91.3
Feb '71	1134	26	2.3	15*	12*	0	1124	156	13.9	82.7
Sept '71	933	13	1.4	5	7	1	930	118	12.7	76.9

* 1 Mixed infection (*P.v.* + *P.f.*)

TABLE 49
Surveillance Data in Cuiabá Study Area,
Mato Grosso, Brazil, 1966-1971

Year	Slides	% Pos.	% *P.f.*
1966	7,109	15.0	74.9
1967	7,603	15.5	80.3
1968	6,590	24.0	78.2
1969	4,289	31.4	67.8
1970	9,473	22.1	66.4
1971	4,409	30.2	39.8

question. During 1970, when active case search was intensified, there would have been an associated increase in the presumptive treatment of cases, and it is likely the antimalaria drugs have become more easily available for self-treatment during recent years, and this may have reduced the *P. falciparum* reservoir in the area, while not greatly affecting the occurrence of *P. vivax* relapses and new transmissions. If some of the factors mentioned above are responsible for the decrease in the proportion of *P. falciparum* cases, the maintenance of a high total percent of positives in 1971 (30%) suggests that transmission within the area has not decreased, and may actually have increased over previous years. However, the serologic results during the two years of observations remained fairly stable, suggesting little change in transmission levels.

The studies in Mato Grosso have been terminated and the data is being assembled and analyzed for publication.

APPENDIX 3

Publications and Presentations

PUBLICATIONS

Breeland, S.G., 1970. The effect of test cage materials on ULV malathion evaluations. *Mosquito News*, 30:338-342.

Breeland, S.G., Pletsch, D.J., & Quarterman, K.D., 1970. Laboratory studies on the suitability of mud as an oviposition substrate for *Anopheles albimanus* Wiedemann. *Mosquito News*, 30:81-88.

Breeland, S.G., Kliewer, J.W., Austin, J.R., & Miller, C.W., 1970. Observations on malathion-resistant adults of *Anopheles albimanus* Wiedemann in coastal El Salvador. *Bull. WHO*, 43:627-631.

Miller, C.W., Austin, J.R., and Pletsch, D.J., 1970. An interesting survival adaptation of *Anopheles albimanus* Wiedemann. *Mosquito News*, 30:96.

Pletsch, D.J., 1970. A collapsible model of the "Red Box" for measuring mosquito population density. *Mosquito News*, 30:646-648.

Miller, C.W., and Funes A., F.M., 1971. Alkali-flame gas chromatography of Abate. *Journal of Chromatography*, 59:161-162.

Breeland, S.G., 1972. Studies on the diurnal resting habits of *Anopheles albimanus* and *A. pseudopunctipennis* in El Salvador. *Mosquito News*, 32:99-106.

Breeland, S.G., 1972. Studies on the ecology of *Anopheles albimanus*. *American Journal of Tropical Medicine and Hygiene*, 21:751-754.

Breeland, S.G., 1972. Methods for measuring anopheline densities in El Salvador. *Mosquito News*, 32:62-72.

Contacos, Peter G., Collins, William E., Jeffery, Geoffrey M., Krotoski, Wojciech A., and Howard, William A., 1972. Studies on the characterization of *Plasmodium vivax* strains from Central America. *American Journal of Tropical Medicine and Hygiene*, 21:707-712.

Breeland, S.G., 1970. The effect of test cage materials on ULV malathion evaluations. *Mosquito News*, 30:338-342.

This study shows that the type screening material used in test cages for evaluating ULV malathion treatments is important in making proper evaluations. Some types of screening inhibit the penetration of droplets to the specimens inside cages. In this work, aluminum, bronze and plastic screening materials interfered with penetration more than other materials tested, while nylon and galvanized materials interfered least. The latter materials allowed ULV droplet penetration almost equal to that of unscreened controls. Galvanized screening, when competitively compared to aluminum, consistently allowed better droplet penetration and was the material adopted for later ULV evaluations. While galvanized screening proved better for our use than other materials tested, other workers using cages for mosquito tests in ULV studies should select cage materials based on their own evaluations and requirements since different insecticides and spraying equipment might affect the results.

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Breeland, S.C., Pletsch, D.J., & Quarterman, K.D., 1970. Laboratory studies on the suitability of mud as an oviposition substrate for *Anopheles albimanus* Wiedemann. *Mosquito News*, 30:81-88.

In tests in Salvador in 1968, laboratory-reared and wild-caught females of *Anopheles albimanus* Wied. oviposited readily on mud in the laboratory. The source of the mud (from a known habitat of the Anopheline or from a garden) seemed to be unimportant. Ovipositing females preferred mud to either drinking water (from a bottle) or water from the known habitat. The length of survival of eggs deposited on mud was related to the rate at which the mud dried. The results of these studies indicated that eggs of *A. albimanus* are very resistant to desiccation. In limited tests, eggs of wild-caught females of *A. pseudopunctipennis* Theo. showed no signs of adaptation to a mud substrate or of resistance to desiccation.

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Breeland, S.G., Kliewer, J.W., Austin, J.R., & Miller, C.W., 1970. Observations on malathion-resistant adults of *Anopheles albimanus* Wiedemann in coastal El Salvador. *Bull. WHO*, 43:627-631.

In 1969, the Central America Malaria Research Station was conducting field trials of aerial ultra-low-volume sprays of malathion against *Anopheles albimanus* in coastal El Salvador. The treatments were assayed by dye cards, to measure droplet deposition, and by exposed cages of adults and bowls of larval mosquitoes. The insects were either locally collected *A. albimanus* or specimens from a laboratory colony. It was noticed that, while the adult kills showed reasonable correlation with droplet numbers, the kills of wild-collected mosquitoes were always below those of the colony insects (averages 19.7% and 71.1% respectively). It was suspected that incipient resistance was developing. Wild and colony *A. albimanus* were exposed on the deposits of pure malathion on plywood panels under WHO bioassay cones for one hour and mortalities checked next day. The results confirmed the higher tolerance of the wild insects. Finally, WHO test papers were used and provided additional verification. No evidence of resistance in larvae was observed. The resistance seemed to be due to the heavy insecticidal treatments against cotton pests in the coastal areas of El Salvador.

Miller, C.W., Austin, J.R., and Pletsch, D.J., 1970. An interesting survival adaptation of *Anopheles albimanus* Wiedemann. *Mosquito News*, 30:96.

Larvae of the malaria vector, *Anopheles albimanus* Wiedemann were observed in estuarine waters in El Salvador, Central America, to seek refuge in the water film covering partially submerged leaves. The authors consider this an effective adaptation in habitats where predacious fish may otherwise threaten larval survival.

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Pletsch, D.J., 1970. A collapsible model of the "Red Box" for measuring mosquito population density. *Mosquito News*, 30:646-648.

A collapsible model of the "red box" used for quantitative measurement of adult mosquito densities has been designed and its description and specifications are presented. The newly-designed unit, in collapsed form, occupies only one-eighth of the space required for the transport or storage of the conventional rigid box. Increased portability will facilitate the use of the box in off-road situations often involved in field studies of mosquito population density.

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Miller, C.W. and Funes A., F.M., 1971. Alkali-flame gas chromatography of Abate. *Journal of Chromatography*, 59:161-162.

Operating parameters and response of the alkali-flame detector in regards to analysis of Abate are presented. The technique provides a rapid, sensitive, and specific method of determination for this insecticide.

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Breeland, S.G., 1972. Studies on the diurnal resting habits of *Anopheles albimanus* and *A. pseudopunctipennis* in El Salvador. *Mosquito News*, 32:99-106.

Research objectives of the Central America Malaria Research Station (CAMRS in El Salvador require means of measuring anophelism apart from commonly used domicile methods. *Anopheles albimanus* and *A. pseudopunctipennis* are present in large numbers in natural diurnal resting sites, and are less abundant in artificial sites. Favored natural sites are "rock-oriented" and include crevices of large and small rocks, rock walls, and lava deposits in shaded areas. Tree trunks, cavities, and roots also offer favorable sites. From natural sites, 181 collections yielded 7,670 *A. albimanus* and 2,344 *A. pseudopunctipennis* for an average of 42.4 and 12.9, respectively. Of these, the sex ratio of *A. albimanus* was 24.5% males and 75.5% females, while that of *A. pseudopunctipennis* was 34.0% males and 66.0% females. *A. pseudopunctipennis* seemed to prefer darker, more protected niches than *A. albimanus*. *A. albimanus* females moved into resting sites at dawn, departed at dusk, and returned between midnight and 3 a.m., whereas *A. pseudopunctipennis* females were absent from resting sites throughout the period of darkness. The results emphasize the usefulness of diurnal resting sites for density measurements associated with control projects and biological studies, and as a source of specimens for experimental use.

Breeland, S.G., 1972. Studies on the ecology of *Anopheles albimanus*. *American Journal of Tropical Medicine and Hygiene*, 21:751-754.

Vector ecology studies conducted by the Central America Malaria Research Station have yielded more than 2000 field collections and associated observations. Results from diel and seasonal studies as well as studies on host preference, susceptibility to malaria parasites, and longevity, are reviewed in light of vector control implications. Collectively, an interpretation of the data presented would seem to indicate that *A. albimanus* must be more zoophilic than anthropophilic, more exophagic than endophagic, more exophilic than endophilic, not particularly long-lived, and infrequently found harboring malaria parasites. The studies should form a background for seeking needed answers to control problems and opportunities.

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Breeland, S.G., 1972. Methods for measuring anopheline densities in El Salvador. *Mosquito News*, 32:62-72.

Various methods for measuring adult densities of *Anopheles albimanus* and *A. pseudopunctipennis* in a coastal malarious area of El Salvador are evaluated from data collected between January 1968 and June 1971. Two New Jersey light traps, operated for a total of 358 trap-nights, yielded an average of 93.9 *A. albimanus* per trap-night, 96.9% of which were females. Collections of *A. pseudopunctipennis* averaged only 0.05 specimens, all females, per trap-night. Nighttime collections from two cattle corrals in the same area accounted for an average of 84.4 *A. albimanus* (99.9% females) and 1.1 *A. pseudopunctipennis* (100% females) in 107 collections. Early evening human bait captures yielded an average of 29.5 *A. albimanus* females and no specimens of *A. pseudopunctipennis* in 23 collections. Daytime collections from natural diurnal resting sites yielded an average of 20.2 *A. albimanus* and 5.8 *A. pseudopunctipennis*, represented by 18.9% and 43.7% females, respectively. Daytime counts from beneath four bridges in the area collectively averaged 7.2 *A. albimanus* (74.4% females) and 9.7 *A. pseudopunctipennis* (63.3% females). Early morning captures of anophelines resting on interior surfaces of dwellings yielded an average of 9.4 *A. albimanus* (65.1% females) and only a single *A. pseudopunctipennis* female in 72 captures. The relative usefulness of each method is discussed in light of the purpose of the collection.

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Contacos, Peter G., Collins, William E., Jeffery, Geoffrey M., Krotoski, Wojciech A., and Howard, William A., 1972. Studies on the characterization of *Plasmodium vivax* strains from Central America. *American Journal of Tropical Medicine and Hygiene*, 21:707-712

Four strains of vivax malaria from three Central American countries (Panama, El Salvador, Nicaragua) were studied in volunteers for characteristics of their relapse patterns and for their response to recommended dosage regimens of some standard antimalarial drugs. The relapse patterns of the four Central American vivax strains fell into the "temperate zone" category of relapse pattern; namely, an early primary attack (short prepatent period) followed by a long period of latency before appearance of frequent relapse activity. The characteristics and classification of relapse patterns are discussed.

PAPERS IN PRESS

Georghiou, G.P., Ariaratnam, V., and Breeland, S.G., 1971. *Anopheles albimanus:* Development of carbamate and organophosphorus resistance in nature. WHO/VBC 71.313 (WHO/MAL/71.751):1-5. In press, *Bull. WHO.*

Breeland, S.G., 1972. Seasonal breeding habitats of *Anopheles albimanus* and *A. pseudopunctipennis* in coastal areas of El Salvador. In manuscript.

Breeland, S.G., 1972. Studies on the blood feeding habits of anophelines in El Salvador. In manuscript.

Austin, Joseph R., 1972. Village trial of carbaryl against *Anopheles albimanus* in El Salvador, Central America. In press, *Mosquito News.*

Austin, Joseph R., 1972. Village trial of external application of propoxur against *Anopheles albimanus* in El Salvador, Central America. In press, *Mosquito News.*

Austin, Joseph R., 1972. Village trial of fenitrothion against *Anopheles albimanus* in El Salvador, Central America. In press, *Mosquito News.*

Georghiou, G.P., Ariaratnam, V., and Breeland, S.G., 1971. *Anopheles albimanus:* Development of carbamate and organophosphorus resistance in nature. WHO/VBC 71.313 (WHO/MAL/71.751):1-5, In press, *Bull. WHO.*

Natural populations of *Anopheles albimanus* in the area of La Libertad in coastal El Salvador demonstrate a broad spectrum of resistance to carbamate and organophosphorus insecticides, including propoxur, carbaryl, parathion, malathion, and fenitrothion. This development appears to be the result of extensive and long-term use of organophosphorus, and to a lesser extent carbamate, insecticides on agricultural crops, especially on cotton.

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Breeland, S.G., 1972. Seasonal breeding habitats of *Anopheles albimanus* and *A. pseudopunctipennis* in coastal areas of El Salvador. In manuscript.

Results from more than 2,000 collections of anophelines in coastal malarious areas of El Salvador indicate a sequential pattern of larval habitat availability and use on a seasonal basis. This pattern is graphically illustrated and discussed. Habitats favorable to both species, to varying degrees, during dry season include estuaries, rivers, irrigation waters and permanent water with stable margins. Rainy season habitats begin with flooded coastal lowlands, followed by inundations of more inland basins and overflows such as lake margins, pastures, ditches, etc., which in turn are followed by flooding of many kinds of miscellaneous habitats and the establishment of breeding in wet-weather lakes, ponds, and sloughs. Collectively, this sequential breeding explains year-round anophelism with ample overlap of habitat availability during transitional periods between wet-dry and dry-wet seasons.

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Breeland, S.G., 1972. Studies on the blood feeding habits of anophelines in El Salvador. In manuscript.

The results of microprecipitin tests to determine vertebrate host preference during 1970-71 show that 48.3% of 1,132 specimens of *Anopheles albimanus* collected from inside houses had fed on humans and 26.1% on cattle. In contrast, collections from outdoor natural diurnal resting sites included only 1.7% human feedings and 83.3% cattle. The remainder of the specimens are divided among other domestic animals. Double feedings occurred in nine combinations from intradomiciliary collections, five involving man. Five kinds of animals (humans, cattle, dogs, pigs, and horses) furnished about 97% of the feedings, regardless of the collection source. *A. pseudopunctipennis* almost invariably captured out-of-door and this species showed similar results to those from *A. albimanus* captured outdoors with 1.0% human feedings and 82.1% cattle feeding. Implications of results are discussed.

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Austin, Joseph R., 1972. Village trial of carbaryl against *Anopheles albimanus* in El Salvador, Central America. In press, *Mosquito News.*

Carbaryl was tested in El Salvador against *Anopheles albimanus* as a residual insecticide in several types of rural houses. At $2g/m^2$, mosquitoes confined to treated surfaces were killed for 15 weeks after application. However, mortalities of naturally entering and departing female mosquitoes were too low and, therefore, the material was judged to be unsatisfactory for malaria control under the conditions of these tests.

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Austin, Joseph R., 1972. Village trial of external application of propoxur against *Anopheles albimanus* in El Salvador, Central America. In press, *Mosquito News*.

Propoxur was compared with DDT as an exterior treatment of houses in El Salvador for control of *Anopheles albimanus*. a principal vector of malaria in Central America. Pole house exteriors were treated with $2g/m^2$ of propoxur, and compared with $4g/m^2$ of DDT. Although DDT had little or no effect, propoxur drastically reduced intradomiciliary biting for approximately four weeks. A second treatment several weeks after the first suggested an additive effect from the retreatment, thereby warranting further studies.

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Austin, Joseph R., 1972. Village trial of fenitrothion against *Anopheles albimanus* in El Salvador, Central America. In press, *Mosquito News*.

Fenitrothion, formulated as a 50% water-dispersible powder, applied as a residual treatment at the rate of $2g/m^2$ to houses in El Salvador, Central America, controlled DDT-resistant *Anopheles albimanus* adults for approximately four weeks on wood, thatch, pole, and tile surfaces. However, this period of residual activity was judged to be too short to test this material further in antimalaria programs in the coastal problem areas of El Salvador.

PRESENTATIONS AT SCIENTIFIC MEETINGS

Breeland, S.G. Studies on the dry season survival of anophelines in a coastal area of El Salvador. Presented at Entomological Society of America Annual Meeting, December 2-5, 1968.

Breeland, S.G. Laboratory studies on the suitability of mud as an oviposition substrate for *Anopheles albimanus* (Breeland, Pletsch, and Quarterman). Presented at the American Mosquito Control Association Annual Meeting, February 24-27, 1969.

Kilpatrick, J.W., Pletsch, D.J., Breeland, S.G., and Lewallen, L.L. Preliminary evaluation on the role of ULV aerial application of malathion for malaria control in Central America. Presented at the American Mosquito Control Association Annual Meeting, February 24-27, 1969.

Breeland, S.G. Observations of natural diurnal resting habits of *Anopheles albimanus* and *A. pseudopunctipennis* in El Salvador. Presented at the Entomological Society of America Annual Meeting, December 1, 1969.

Breeland, S.G. The effect of test cage materials on ULV malathion evaluations. Presented at the American Mosquito Control Association Annual Meeting, February 24, 1970.

Breeland, S.G. The diel resting habits of *Anopheles albimanus* and *A. pseudopunctipennis* in El Salvador. Presented at the Entomological Society of America Annual Meeting, December 2, 1970.

Breeland, S.G. Studies on the Ecology of *Anopheles albimanus*. Presented at the Inter-American Malaria Research Symposium, November 3, 1971.

Breeland, S.G. Preliminary studies on the blood feeding habits of anophelines in El Salvador. Presented at the Entomological Society of America Annual Meeting, November 29, 1971.

Jeffery, G.M., Warren, McW., Collins, W.E., and Mathews, H.M. Longitudinal sero-epidemiologic studies on malaria in the Mato Grosso area of Brazil. Presented at the American Society of Tropical Medicine and Hygiene, November 30-December 3, 1971.

Warren, McW. and Collins, W.E. Sero-epidemiologic techniques in the assessment of malaria program needs. Presented at the American Society of Tropical Medicine and Hygiene, November 30-December 3, 1971.

Miller, C.W., Shafik, M.T., and Biros, F.S. A method for sampling and analysis of propoxur in air. Presented at the American Chemical Society Meeting, April 1972.