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Malaria in the Peruvian Amazon: A Review of the Epidemiology, Entomology, and Insecticide Resistance of Vectors

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by

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TABLE OF CONTENTS

APPENDICES

FIGURES

PART 2: ENTOMOLOGICAL ASPECTS OF MALARIA TRANSMISSION
L. Philip Lounibos

PART 3: INSECTICIDE RESISTANCE BY MALARIA VECTORS (EHP)
Darjaniva Molina de Fernández

TABLES

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ACRONYMS

ACD	active case detection
API	Annual Parasite Index
BTI	Bacillus thuringensis israeliensis
CDC	U.S. Centers for Disease Control and Prevention
CL	clindamycin
CQ	chloroquine
DIGESA	National Environmental Health Office *(Direccion General de Salud Ambiental)*
EHP	Environmental Health Project
GIS	geographic information systems
IIAP	Institute for Investigations of the Peruvian Amazon *(Instituto de Investigaciones de Amazonia Peruana)*
INS	National Health Institute *(Instituto Nacional de Salud)*
MOH	Ministry of Health
NAMRID	Navy Medical Research InstituteCDetachment (now NAMRC or ANavy Medical Research Center@)
OEM	Otras Enfermedades Metaxenicas
OGE	Oficina General de Epidemiologia
PASA	Participating Agency Service Agreement
PCD	passive case detection
PNCM	National Malaria Control Program *(Programa Nacional de Control de Malaria)*
PQ	primaquine
Q	quinine
SP	sulfadoxine pyrimethamine
T	tetracycline
US HHS	U.S. Department of Health and Human Services
USAID	United States Agency for International Development
USAMRIID	U.S. Army Medical Research Institute for Infectious Diseases (now USAMRCD or AU.S. Army Medical Research CentersCDetachment@)

WHO	World Health Organization

Map
Peru and Neighboring Countries

FOREWORD

Andrew A. Arata

Background

In the spring of 1997, USAID/Lima requested assistance from the Environmental Health Project (EHP) to review the recent disturbing increase in malaria in the Peruvian Amazon. In collaboration with CDC, EHP was asked to provide technical assistance to help the Ministry of Health (MOH) focus its resources in a more practical fashion and, possibly, by working with other potential donors, to increase the resources available to the MOH in dealing with the escalating number of malaria cases in the Amazon region.

After a period in the 1960s and 1970s in which malaria was well controlled in Peru (2,010 cases were reported in 1968, with an Annual Parasitic Index (API) of only 0.16), the disease has returned to new high levels and has become a major public health problem. In 1996, over 200,000 cases of malaria were reported, with over 50,000 cases of *P. falciparum*; the API had increased to 30.9. In the Department of Loreto (Amazonas) alone, approximately 95,000 cases (over 30,000 *P. falciparum*) were recorded. Loreto, the largest administrative entity in the Amazon, has only 3% of the national population. In Loreto the incidence rate was 11,905/100,000 in 1996, and in early 1997, during the initiation of this project, over 1,000 cases had been reported in the major city, Iquitos. Overall, in 1997, a total of 155,435 malaria cases were reported from Loreto (54% of the national total, with an API of 29.43). Although the El Nino reduced the intensive breeding by the major anopheline vectors, the number of reported cases was equally high in 1998. (PAHO does not have a final figure from the 1998 malaria surveillance. As of the date of this report (January 1999), the most current information from PAHO is for 1997, as shown in Table 1.)

Other countries in the Amazon basin are having similar increases in malaria, but the figures reported from Peru are especially severe. In addition, heavy rains in the highlands during the past year and El Nino produced local variations resulting in more runoff in some areas leaving extensive vector breeding sites when the flood waters reside. The situation is further exacerbated by the large number of immigrants into the Amazon area resulting in further deforestation for agricultural expansion and exploitation of natural resources (primarily minerals and lumber). There are numerous species of anopheline vectors in the Amazon area, with divergent habits that are not well known (biting habits, place/time, competence and/or efficiency as vectors, etc.). This lack of information limits the choice of control measures in these areas.

In addition, information provided to consultants in 1997 in Lima indicated that *P. falciparum* in the Peruvian Amazon region has shown resistance to chloroquine and possibly to other antimalarial drugs. Similarly, the only insecticide reported tested (Baythroid=cyfluthrin, a synthetic pyrethroid) showed poor results in wall (cone) bioassays against *An. darlingi*, a very efficient vector that appears to be moving into the area in the wake of man-made changes that increase the distribution and frequency of suitable breeding sites.

PAHO has undertaken initial investigations in the Peruvian Amazon with national scientists, as have members of the MOH malaria control program. Preliminary reports were made available to EHP for additional background information.

Table 1
Malarious Areas in Peru at High Risk of Transmission and Control Priorities, 1997

Provinces (8 of 33)	Population	km^2	Reported Cases	Control Measures Applied in Different Areas	Main Vectors	Causes of Persistence of Transmission

Loreto	819,037	368,851	83,950		A. pseudopunct	Heavy migration and new colonization of the rain forest areas
Jaen	554,060	44,409	11,811		A. benarrochi	
Cusco	1,117,311		25,158		A. albimanus	
Ucayali	380,620	102,410	5,558		A. darlingi	
Ayacucho	518,528	43,814	7,629			
San Martin	667,414	51,253	8,010			
Junin	1,147,328	44,409	12,530	API=29.43/1000		
Madre de Dios	76,610	85,182	789	AFI=14.9/1000		
Total	5,280,908	740,328	155,435	(Pop. at P. fal. Areas=3,016,269)		

Source: PAHO 1999

Objectives

Severe outbreaks of falciparum malaria have emerged and continue in the Amazon areas of Peru (Loreto). In part because of rapidly increasing man-made environmental changes, the ecology of malaria in the area is poorly known. The activity described in this report was a collaborative effort of EHP and CDC to reach a better understanding of the mechanisms of malaria transmission in the tropical Amazon including:

- a review of diagnosis and treatment procedures and the status of antimalarial drug resistance by *P. falciparum* in the area **(Task 1**);
- collecting better information on the ecology of vector mosquitoes and the status of insecticide resistance in the area **(Tasks 2 and 3)**.

It was anticipated that information in this review, along with Aon-the-job training@ of Peruvian counterparts, would enable national authorities to develop more effective plans for malaria control in high-risk areas and assist them in obtaining the additional resources required to implement these plans.

Tasks

This foreword provides a brief overview of each task. The details are in the individual author/consultant reports which are presented in their entirety, as Parts 1, 2, and 3 of this document. Each part bears the tone and writing style of its author(s). The report on vector susceptibility or resistance to insecticides, Part 3, was originally written in Spanish and has been translated.

To help the USAID Mission in Peru assist the national malaria control efforts, the technical assistance provided by EHP and CDC was focused on needs assessments in three areas, as described below.

Task 1: Epidemiology: Diagnosis and Treatment of Malaria

a) Review existing microscopic diagnostic facilities and capabilities such as the possible use of immunological diagnostic (dip-stick) technologies for *P. falciparum*.

b) Review antimalarial drug resistance data and make recommendations for monitoring over time and in various geographic areas.

c) Determine the availability of alternate/appropriate preventive measures and treatment(s).

This task was carried out over a three-week period, June 22-July 9, 1997, by CDC staff (Drs. Ruebush, Porter, and Stein) and forms Part 1 of this report. It was funded by a PASA between the U.S. Department of Health and Human Services and USAID and coordinated by EHP. The report on this task (Part 1) presents national epidemiological data for the timeframe of the study and makes numerous observations and recommendations. Among the more important points are the following:

- Studies should be undertaken to describe the epidemiology of severe and complicated malaria cases, including those during pregnancy, and physicians should be instructed in the WHO guidelines for their management.
- Drug-resistance appears in 10-20% of *P. falciparum* patients; studies should be conducted to improve treatment regimes.
- More critical evaluation of the number of blood smears taken and evaluated could reduce the work load of the heavily overloaded microscopists.
- Most malaria transmission appears in the rural and peri-urban areas, where diagnosis and treatment facilities are weak and should be strengthened and concentrated.
- Improved entomology studies and vector control activities are critical but are presently neglected.

Task 2: Entomological Aspects of Malaria Transmission

a) Identify important local anopheline vectors.
b) Observe vector biology/ecology to improve control activities.

This task was carried out from July 13 to August 2, 1997, by an EHP consultant, Dr. Lounibos, who is a medical entomologist (see Part 2 of this report). The full range of observations and recommendations for improvement are included in his report, but among the most important are the following:

A. Taxonomy

- *Anopheles darlingi* appears to be the most important vector, but others (*An. evansae, An. oswaldoi*, *An. nuneztovari*) are present and known to be competent, if secondary, vectors, often transmitting malaria at different times or in different habitats than *An. darlingi.*

B. Biology/Ecology and Control

- *An. darlingi* was not known in the Iquitos area prior to 1993, and its abundance at present is associated with the man-made changes in the overall Amazonian environment (e.g., deforestation and agriculture).
- Studies on the population dynamics and behavior (biting, breeding, etc.) of *An. darlingi* and other vectors are essential to improvement or development of vector control measures.
- Basic studies and human resource development are necessary to determine the most cost-effective methods of vector control (spraying, breeding site reduction, use of impregnated bednets, etc.).

Task 3: Vector Susceptibility or Resistance to Insecticides Used for Malaria Control

a) Use standard WHO testing kits, with insecticide-impregnated papers of various concentrations according to WHO specifications, to test efficacy (% mortality) of insecticides in use to local malaria vectors.
b) Plot the results of the testing by location and type of insecticide (organochlorine, organophosphate, carbamate, synthetic pyrethroid).

This task was carried out by an EHP consultant, Dra. Molina de Fernández. For various reasons, the assignment was carried out in October 1998, a year after Tasks 1 and 2. Dra. Molina was prepared to carry out the assignment earlier; the work was delayed, however, at the request and on the advice of the USAID Mission and the Peruvian counterparts, due to El Nino and an absence of significant numbers of vectors in the Iquitos area. The susceptibility/resistance trials require a large number of female vectors for each insecticide and location being tested and for the replicates required for each trial. When the consultant arrived in Lima, she was asked by the USAID Mission to do the work in the Tumbes/Piura area of northern Peru, where a different vector, *An. albimanus*, is predominant. Nevertheless, she carried out the work very thoroughly, as can be seen in the report (Part 3). She provided initial training for her Peruvian counterparts as well.

Tests were carried out on five insecticides from the four classes mentioned above, with vectors from three to six localities each (see Table 2). The Anewest@ compounds (the pyrethroids l-cyhalothrin and cyfluthrin) showed the poorest efficacy, while the organophosphate (fenitrothion) and the carbamate (propoxur) proved to be very effective. Even DDT showed greater efficacy than the pyrethroids. Of course, these results cannot be extrapolated over the whole country or to different vectors, but the importance is clear: in malaria or other vector control programs based on insecticides (this includes house and space spraying, larviciding and impregnated bednets), one must be certain of the efficacy of the compounds selected and purchased. If there is not high efficacy, the control measures will not be effective, and it is likely that a large amount of money will be spent on ineffective chemical compounds.

Table 2
Preliminary Summary Results of Insecticide Susceptibility Trials in Northern Peru *(Anopheles albimanus)*

Insecticide	Localities	Range of Results	Average Mortality
DDT (4%)	4 localities	77-95%	87%
Fenitrothion (1%)	5 localities	100%	100%
Propoxur (0.1%)	3 localities	99-100%	99%
l-cyhalothrin (0.1%)	5 localities	15-76%	41%
Cyfluthrin (0.1%)	6 localities	28-89%	62%

N.B. Controls tested simultaneously with the above trials showed no significant mortality

Part 1
Epidemiology:
Diagnosis and Treatment of Malaria

June 22 - July 9, 1997

Trenton K. Ruebush II
Charles H. Porter
John D. Stein

TABLE OF CONTENTS

FIGURES

EXECUTIVE SUMMARY

Since 1991, the Amazon basin of Peru, and Loreto Region in particular, have experienced an unprecedented increase in the incidence of malaria, associated with the arrival and rapid spread of *Anopheles darlingi*. Currently, half of all malaria infections and two-thirds of all *Plasmodium falciparum* infections reported from Peru occur in Loreto Region. This trend has continued during the last 12 months in spite of aggressive malaria control efforts by the Ministry of Health.

Given the favorable climatic and environmental conditions, a very efficient vector that will probably be difficult to control, a highly mobile and very dispersed human population, and the spread and intensification of antimalarial drug resistance, malaria can be expected to remain an important public health problem in the region for the foreseeable future. It is extremely unlikely that even the best-designed and implemented malaria control program will succeed in reducing malaria transmission to pre-1992 levels and sustaining that reduction. Based on the evidence available at the present time, malaria transmission appears to be limited primarily to peri-urban and rural areas, with little if any transmission in more densely populated areas of the city of Iquitos. Furthermore, because of the apparent absence of suitable breeding sites for *An. darlingi*, the risk of an outbreak in Iquitos is minimal.

The malaria control program in Loreto Region has two urgent and critical needs. First, a strong, well-supported vector control unit should be established, led by someone with specialized training in integrated vector control. Second, because of the numerous gaps in knowledge about malaria in the Amazon basin, applied and operational research is needed to guide program interventions in the following areas:

- antimalarial drug resistance patterns,
- alternative second- and third-line drugs,
- the cost-effectiveness of rapid malaria diagnostic tests for use in isolated communities,
- the epidemiology of severe and complicated malaria and malaria in pregnant women,
- the behavior of adult *An. darlingi* and secondary vectors,
- sites of larval development, and
- the relative cost and effectiveness of specific vector control strategies.

In the short term, i.e., during the next one to two years while this information is being collected, the objective of the malaria control program in Loreto Region should be to halt the rapid increase in transmission and prevent severe and complicated malaria and malaria mortality. Emphasis should be placed on improving rapid diagnosis and treatment, passive rather than active case detection, training health care workers in the management of severe and complicated malaria, and improving direct supervision of health workers at all levels. Then, as better information becomes available, antiparasitic and antivector control measures can be refined and targeted in the most cost-effective fashion. The program should aim to reduce malaria transmission to acceptable levels.

At the national level, efforts should be made to ensure better coordination and communication among the institutions that have primary responsibility for malaria control in Peru. In particular, with the striking change in the epidemiology of malaria in the Amazon basin, national malaria control strategies and guidelines will need to be tailored to the local situation.

1 BACKGROUND

Between 1991 and 1996, the total number of malaria cases reported from Peru increased dramatically**C**from 33,705 to 211,561 (Figure 1). Most of this increase was due to the emergence of malaria in the Amazon basin, particularly Loreto Region, which reported an increase from 850 cases in 1991 to 102,000 in 1996, in association with the introduction and spread of the efficient vector, *Anopheles darlingi*, in the region (Figure 2). Even more alarming has been the increase in *Plasmodium falciparum* cases in Loreto Region from 140 in 1991 to 34,000 in 1997. (See Figure 3 for an illustration of this change from 1990 to 1996.) *P. falciparum* now makes up one-third of all malaria cases in the region, and of all *P. falciparum* cases in Peru, two-thirds are from the region.

From July 1996 to June 1997, the incidence of both total malaria cases and *P. falciparum* infections continued to rise in spite of an aggressive control program in Loreto Region, including house spraying, space spraying, larviciding, an extensive passive case detection network, and active case detection with mass drug administration. Because of the failure of these measures to control the epidemic, the Ministry of Health requested technical assistance from USAID, and the Centers for Disease Control and Prevention in Atlanta and the Environmental Health Project in Washington, D.C., were contacted to provide epidemiologic and entomologic assistance. A team of three malaria specialists from CDC visited Peru from June 22 to July 9, 1997. The purpose of their trip was to review the malaria situation and malaria control activities in Loreto Region and assist regional and national authorities in developing effective plans for dealing with the malaria problem in the Amazon basin of Peru.

Figure 1
Malaria Cases and Blood Smears - Peru 1960-96

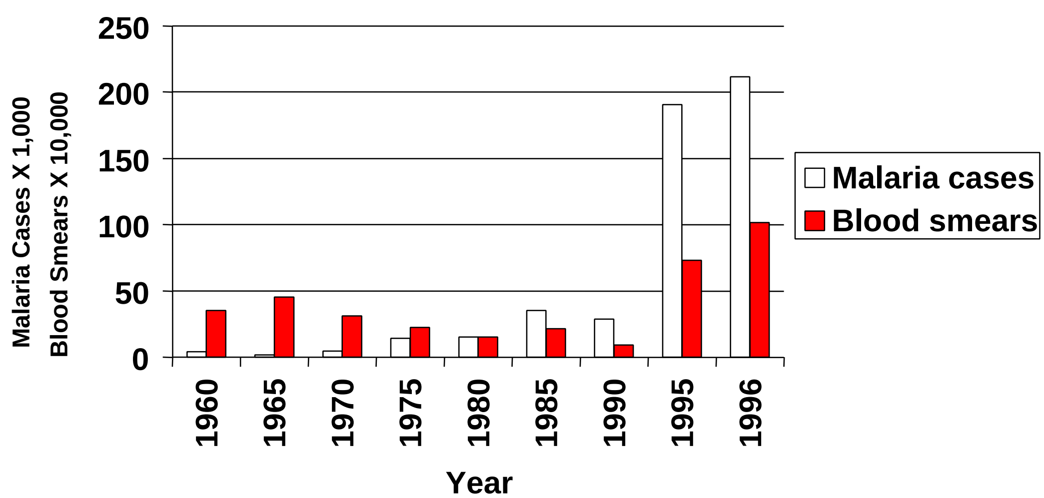


Figure 2
Population and Malaria Cases - Peru and Loreto Region 1960-96

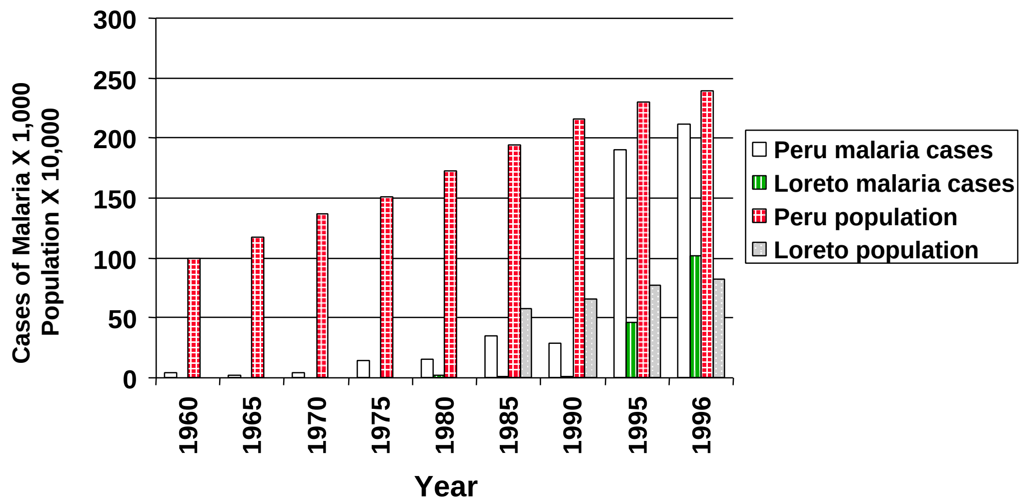
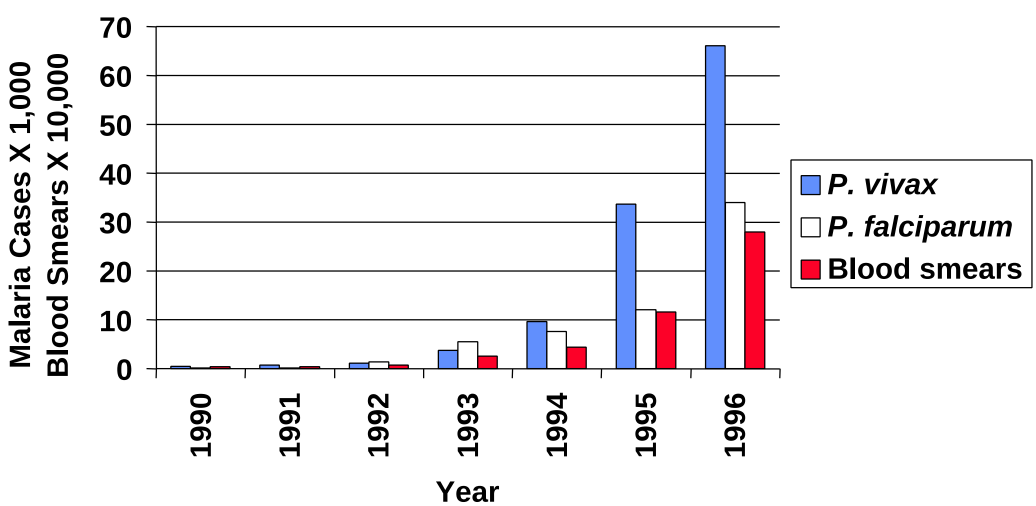


Figure 3
Cases of *P. falciparum* and *P. vivax* - Loreto Region, Peru 1990-96



2 ACTIVITIES

When they first arrived in Lima, the team reviewed the malaria recordkeeping and data management systems at the national level in the offices of the Programa Nacional de Control de Malaria (National Malaria Control Program; PNCM) and the Direccion General de Epidemiologia (National Epidemiology Office). The laboratory facilities at the headquarters of both Instituto Nacional de Salud (National Health Institute; INS) and the Direccion General de Salud Ambiental (National Environmental Health Office; DIGESA) in Lima were toured. At the Loreto Regional Health Office, malaria records were reviewed in both the malaria and epidemiology program offices. The mapping facility at the Instituto de Investigaciones de la Amazonia Peruana (Institute for Investigations of the Peruvian Amazon; IIAP) in Iquitos was also visited. (The team was based in Iquitos June 25 to July 4, and was in Lima again July 5 to 8.)

Visits were made to two Ministry of Health (MOH) hospitals and eight health centers and health posts in the urban and peri-urban areas of Iquitos and surrounding areas on the Nanáy, Momon, Itaya, and Manati Rivers and along the new highway under construction between Iquitos and Nauta. Several health posts can be distributed along a river (see map on following page). During these visits, physicians, nurses, and laboratory workers were interviewed about the malaria situation in their catchment area, the procedures they used for patient diagnosis, treatment, and follow-up, and the malaria control activities they had undertaken in the previous 12 months. Health facility malaria recordkeeping and reporting systems were reviewed, the malaria microscopy laboratory was inspected, and antimalarial drug stocks in the pharmacies were checked. Whenever possible, visits were then made to five or six homes in the vicinity of the health facility to interview residents about their experiences with malaria during the past several years, their health-care-seeking behavior, the availability of antimalarial drugs in the town or village, and their use of personal protection measures for malaria. Three large commercial pharmacies in Iquitos were also visited to assess the availability of over-the-counter antimalarial drugs in the region.

Vector control activities conducted during the previous 12 months in Loreto Region were reviewed in detail with regional entomology/vector control staff. Residual house spraying was observed in the village of Santa Cecilia on the Manati River, approximately three hours by boat from Iquitos. A pilot study of *Bacillus thuringensis israeliensis* (BTI) was reviewed in the outskirts of Iquitos. Night-time landing collections of adult mosquitoes were made in Santa Cecilia and in the village of Santa Clara on the outskirts of Iquitos. Larval collections were made in Santa Cecilia, as well as in several communities around the periphery of Iquitos. Habitats sampled included both natural ponds (*Acochas@*), man-made ponds used for raising fish (*Apiscigranjas@*), and various sites along the banks of the Nanáy River.

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Map
Rural Localities long the Rio Nanáy

3 OBSERVATIONS/FINDINGS

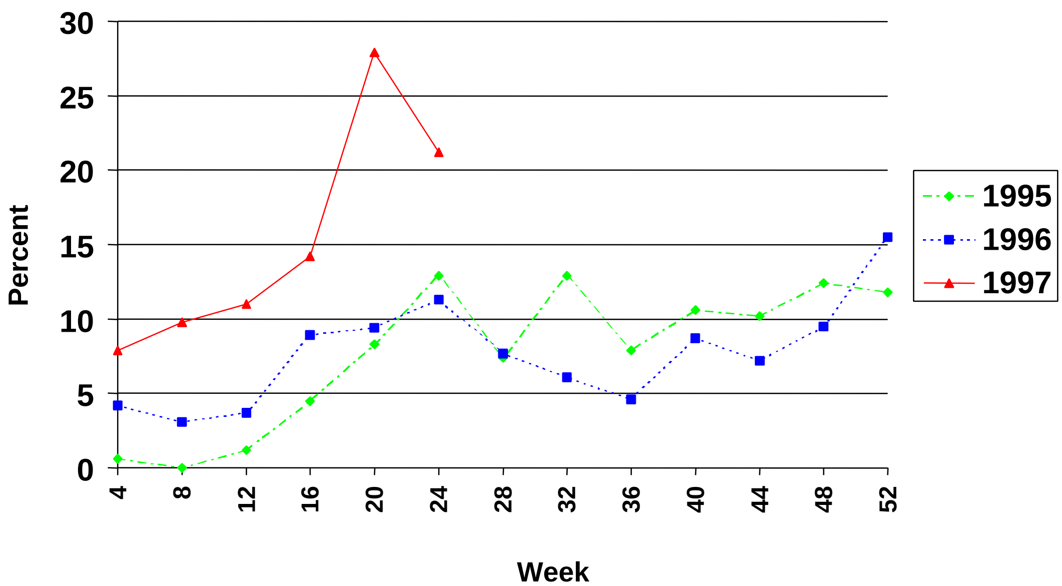
3.1 Current Malaria Situation in Loreto Region

Malaria transmission has reached very high levels in Loreto Region. In fact, the prevalence of malaria found in general blood smear surveys is as high or higher than any other site in the Americas. A general blood smear survey in the approximately 35 communities along the Nanáy River and 50 communities along the Momon River in May 1997 showed malaria prevalences of 38% and 50%, with two-thirds of the cases caused by *P. falciparum*. In these surveys, approximately 30% of the infected patients were reported to be Aasymptomatic.@ A similar survey in May-June 1997 along the Iquitos-Nauta highway showed an average prevalence of 14% with a range from 3% to 38% in different villages. In a general blood smear survey that was ongoing in the village of Santa Cecilia on the Maniti River on the day the team visited, 53 (78%) of the first 68 blood smears examined were positive, with approximately 40% due to *P. falciparum*. Although these are not random blood smear surveys, the slide positivity rates are extremely high, and the high proportion of *P. falciparum* infections is particularly striking. In the majority of health facilities the team visited, slide positivity rates were > 50% among patients seeking treatment for a febrile illness. The age distribution of patients with malaria appeared to differ in the city of Iquitos and rural areas, with a predominance of adults among patients in Iquitos and greater numbers of children in rural areas. This may be because most malaria patients in Iquitos are persons who travel and acquire their infections elsewhere, while residents of all ages are at risk in rural villages.

Although the number of deaths attributed to malaria in Loreto Region has increased from 5 in 1994 to 40 in 1996, with 34 in the first four months of 1997, the reported mortality rate is lower than might be expected, considering the high prevalence of *P. falciparum* infections. Further information will be needed to determine if this is due to under-reporting of deaths from isolated communities or to the ready availability of effective antimalarial drugs through health promotors and health facilities in nearly all communities. A similar situation exists with respect to malaria in pregnancy. Although nearly all health workers who were interviewed reported occasional adverse pregnancy outcomes, their frequency appears to be lower that expected.

Because malaria reporting from health facilities in Loreto Region combines both active and passive case detection data and there has been a striking increase in the number of blood smears taken for malaria diagnosis, it is difficult to define clear regional, provincial, or district trends in malaria incidence either seasonally or over longer periods of time. A review of data from the Hospital de Apoyo in Iquitos, which receives patients from a large area surrounding the city and where little active case detection is conducted, provides the best available information on malaria trends (Figure 4). Based on data from January 1995 through May 1997, malaria transmission appears to occur year-round, with a peak in transmission between March and May and a smaller and more variable peak from September to December. Malaria prevalence appears to correlate better with rainfall data for Iquitos than with the height of the Amazon River as measured at the Port of Iquitos; however, these data are very preliminary, and seasonal trends may well vary along smaller

Figures 4
***P. falciparum* Cases by Week - Loreto Region, Peru 1995-97**



rivers which experience greater variation in water level and, thus, flooding of surrounding areas.

Although not stated as such, the approach used to control malaria in Loreto Region during the past 12 to 18 months has apparently been based on the belief that with sufficient resources and effort, transmission can be reduced and the incidence of disease returned to pre-1992 levels. With this objective, intensive active case detection and vector control operations have been undertaken. Regional staff estimated that 25% of their health budget is currently spent on malaria.

3.2 Organization of Malaria Control Activities

Four governmental institutions based in Lima share responsibility for malaria control throughout Peru:

1. The National Malaria Control Program (*Programa Nacional de Control de Malaria* or PNCM) is responsible for overall malaria surveillance, for establishing standards for malaria treatment nationwide, and for most purchases of antimalarial drugs. It has a staff of six persons, including two physicians/epidemiologists, one biologist, and a computer programmer.
2. The National Epidemiology Office (*Oficina General de Epidemiologia* or OGE) is responsible for weekly surveillance and reporting of *P. falciparum* cases from a network of approximately 2,900 health facilities throughout the country.
3. The National Environmental Health Office (*Direccion General de Salud Ambiental*, DIGESA) is responsible for the selection and purchase of insecticides and provides general technical assistance for vector control activities.
4. The National Health Institute (*Instituto Nacional de Salud*, INS) has primary responsibility for establishing standards for laboratory diagnosis of malaria, for training of microscopists, and for quality control of malaria microscopic diagnosis in government health facilities in Peru. In addition, INS has a well-trained biomedical research staff and the capacity to perform serologic and molecular diagnostic assays for malaria, mosquito identification, colonization, and insecticide resistance testing.

The relationship between the four institutions is shown in the attached organogram (Figure 5). Although these groups maintain contact, they do not have regularly scheduled meetings to discuss the current malaria situation, the progress of malaria control activities, or to plan for future activities.

At the regional level in Loreto, the activities of the four groups are more closely integrated. A single person oversees both the regional malaria and epidemiology offices and is responsible for analysis and interpretation of all malaria surveillance data in the region. DIGESA and INS coordinate their activities at the regional level with the Regional Director and Sub-Director.

Plans and schedules for malaria control activities within Loreto Region, such as larviciding and adulticiding, general blood smear surveys, active case detection, and mass drug administration, are discussed in Iquitos among regional malaria control, epidemiology, and vector control staff and final programmatic decisions are made by the Regional Director or Sub-Director with technical input from the PNCM, DIGESA, and INS in Lima.

With the continued increase in malaria transmission in the Amazon basin in 1996 - 1997 and recognition that the malaria problem is not limited to Loreto Region, a task force made up of representatives of Loreto, San Martin, and Ucayali Regions was formed in March 1997 to coordinate malaria control activities in the Macro-Region of the Amazon basin. At the time of the team's visit, it was not clear what influence this task force had on the malaria control program in Loreto Region.

3.3 Diagnosis

The diagnosis of malaria in Peru is based, in nearly all instances, on a blood smear examined by a trained microscopist. Both thick and thin smears are taken, but only the thick smear is used for diagnosis and species identification. Giemsa stain is used in all Ministry of Health (MOH) facilities. In visits made to eight health facilities in Loreto Region, some differences were noted in staining times, and the quality of staining varied from laboratory to laboratory. All microscopes are binocular; although several new microscopes have been received, nearly all of the older microscopes are in need of cleaning and/or repair. The microscopists interviewed did not report any shortages of materials for taking or staining blood smears during the past six months. Since most blood smears taken in health facilities are made by persons *other* than the microscopists, the quality of the smears is quite variable and slides are often dirty and difficult to examine.

Microscopists interviewed reported that they examined from 50 to as many as 120 blood smears per day during peak transmission season or following large-scale blood smear surveys. Parasite density is estimated in a semi-quantitative manner using a scale of one to four, with four being the highest density. Quality control for microscopic diagnosis is conducted at all levels of the health care system. Health centers and health posts send 100% of positive and 10% of negative blood smears to the Regional Reference Laboratory in Iquitos for re-examination. In turn, these laboratories send 100% of blood smears with discordant results and 10% of positive and 10% of negative blood smears remaining to the Regional Reference Laboratory in Iquitos. Every three months, the Regional Reference Laboratory sends the same proportion of smears with concordant and discordant results to INS for re-examination. Since January 1996, the accuracy of microscopic diagnosis has improved markedly in Loreto Region. Between January and March 1996, approximately 10% of positive blood smears and nearly 6% of negative blood smears examined in health centers and health posts were incorrectly reported. In comparison, during the same period in 1997, < 2% were incorrectly reported. Of blood smears from the Regional Reference Laboratory in Iquitos

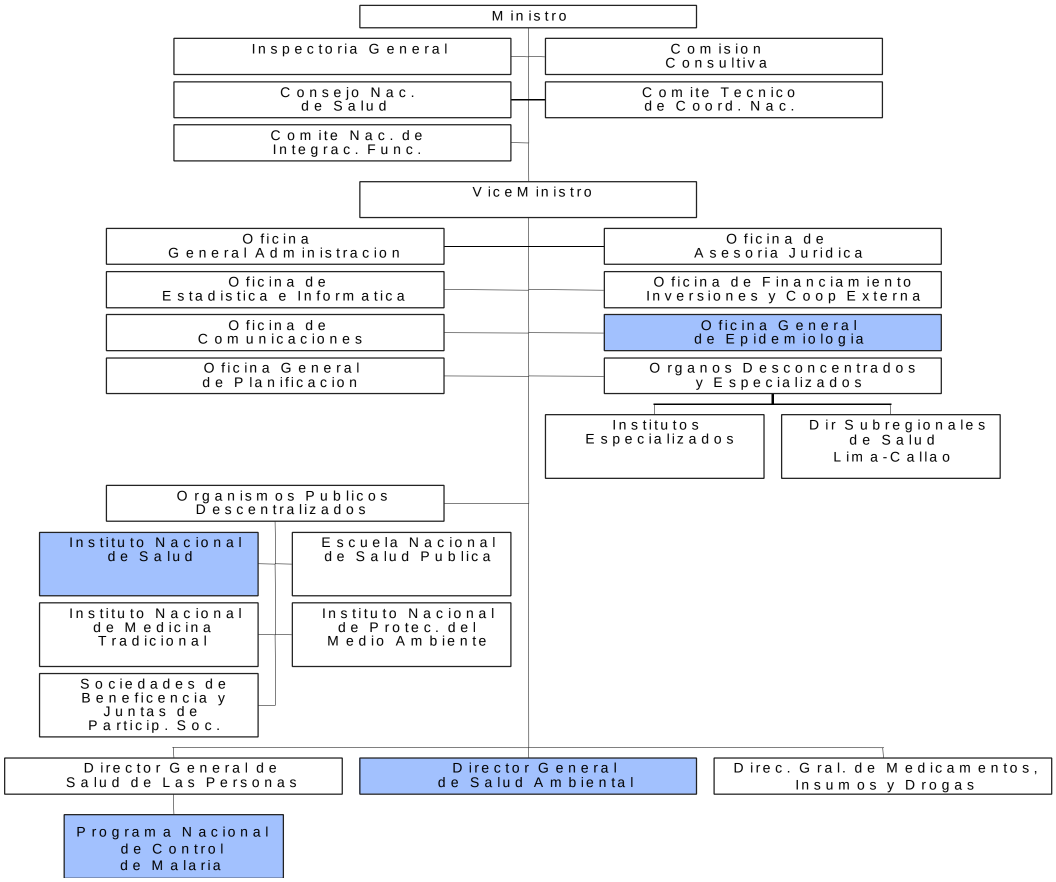


Figure 5
Organogram, Ministry of Health, Peru

re-examined by INS, errors were identified in approximately 5%. There has been no direct supervision of health center and health post microscopists outside the Iquitos area by staff of the Regional Reference Laboratory since January 1996 due largely to the distances involved and the cost of transportation.

3.4 Treatment

The PNCM has established clear guidelines for the treatment of malaria, including first-, second-, and third-line drugs for *P. falciparum* infections. In addition, it has published recommendations for the treatment of severe and complicated malaria and for malaria in pregnant women. All treatment for malaria provided by the MOH is free of charge. There was only one instance (a health post several hours by boat from Iquitos) of a reported stockout of antimalarial drugs during the six months preceding the team=s visit.

The recommended regimen for the treatment of *P. vivax* infections is chloroquine (CQ) 25 mg (base)/kg over three days plus primaquine (PQ) 0.25 mg (base)/kg daily for three days. In 1996 the duration of PQ treatment was reduced from 14 to 7 days. Although no formal studies have been conducted to assess the efficacy of CQ against *P. vivax*, follow-up of treated patients shows no evidence of resistance.

The first-line treatment for *P. falciparum* infections is CQ 25 mg/kg/day over three days plus PQ 0.75 mg/kg in a single dose. If the patient fails to respond within three days, sulfadoxine-pyrimethamine (Fansidar**7**; SP) is administered in a single dose of 25 mg/kg (sulfadoxine component). If there is no clinical improvement after SP treatment or parasitemia continues beyond three days, the third-line treatment of quinine (Q) plus tetracycline (T) or Q plus clindamycin (CL) for seven days is administered.

Until 1995, CQ was considered generally effective for the treatment of *P. falciparum* infections. Currently, based primarily on the frequency of CQ treatment failures in patients treated by health promotors and at MOH facilities who have adequate follow-up, moderate to high levels of *P. falciparum* resistance to CQ appear to be present in most of the region. The situation with respect to SP is much less clear. Based on results of follow-up of patients with *P. falciparum* treated with SP in health centers and hospitals, between 5% and 30% require treatment with Q because of failure to clear their parasitemia, recurrence of parasitemia by day 14, or deterioration in their clinical condition. Several in vitro and in vivo drug efficacy trials carried out in the last three years have shown varying levels of resistance, ranging from 10% to 20% moderate to high (RII/RIII) resistance. These trials, however, have not used standardized techniques, and it is difficult to interpret their results or to compare the results of one trial to another.

Wall charts showing the PNCM treatment schedules were clearly displayed in all health facilities visited, and health staff were well informed about malaria treatment. According to PNCM policy, all doses of the drugs are to be given under direct observation. In general, both health promotors and workers in MOH facilities seem to comply with this rule, except when a patient is leaving the area for several days and requests the drug to take with him/her. In some situations, exceptions to the general treatment guidelines are made. In the case of village-level volunteer health promotors, a presumptive treatment with CQ plus PQ is started when the patient first comes for diagnosis and treatment. If the blood smear is positive, the treatment is continued; if negative, treatment is terminated. Some variation was also observed in the criteria used for initiating the second- or third-line treatments, but in general, if the patient=s condition deteriorates or if blood smears taken routinely on days 3, 7, or 14 after treatment is initiated are positive, the patient will be treated with another drug regimen. In Angamos District on the border with Brazil in southeastern Loreto Region, Q plus T or CL is used as the first-line treatment by health promotors and in all health facilities because of data (based on routine follow-up blood smears of treated patients) that suggest very high levels of SP resistance.

In approximately 20% of cases in Loreto Region, malaria treatment is administered by a health worker without a blood smear diagnosis. These are referred to as Aprobable@ cases and are recorded and reported to monitor total consumption of antimalarial drugs, but are not included in the total counts of malaria cases reported for the region. Interviews with health promotors confirmed that they also occasionally administer treatment (with drugs left over from patients who fail to complete their treatment regimens) without taking a blood smear, but these cases are not reported to their health post or health center.

As a routine practice of the PNCM, all patients with confirmed malaria are asked to return to the health promotor or health facility for follow-up blood smears following treatment. For *P. vivax* infections, a single follow-up blood smear is taken when treatment is completed (currently day 7) and for *P. falciparum* infections on days 3, 7, and 14 after treatment is initiated. The total number of these follow-up blood smears is quite large. In a major health center in Iquitos, 22% of all blood smears examined at the facility during 1997 were for follow-up visits. In spite of the importance placed by the PNCM on these follow-up blood smears in cases of *P. falciparum* as a means of identifying resistance cases, few patients have all three blood smears taken. In one of the health centers in Iquitos, of 324 patients with *P. falciparum* treated in May 1997, only 42% had all three follow-up blood smears, while 21% had no follow-up blood smears. In comparison, of 394 patients with *P. vivax* infections, 83% had the required follow-up blood smear.

Side effects of antimalarial drugs have not been a problem for the malaria control program in Loreto Region. Health workers report that some patients complain of pruritis with CQ and of ringing in the ears and transient loss of hearing with Q, but more severe adverse drug reactions, such as Stevens-Johnson syndrome with SP have not been seen.

Although according to standards established by the PNCM, blood smears are to be taken only from patients with fever or a history of fever, most health promotors and health facility staff interviewed by the team said that they took blood smears from anyone who suspected they had malaria. The blood smears are then carried by the health promotor, a member of their families, or another village resident to the nearest health facility laboratory for examination. If the blood smear is positive, a message is sent back to the health promotor to complete the full treatment and collect the follow-up blood smears. Based on a small sample of health promotors from villages within a 2-hour boat ride of Iquitos, the length of time between taking the blood smear and its examination and reporting of results was rarely more than two weeks.

PCD for malaria is also carried out in all MOH health posts, health centers, and hospitals in patients who come seeking treatment.

The cost of antimalarial drugs to the control program appears to be quite high, and savings could probably be made by buying only generic drugs in bulk. According to information provided by the regional office, the first-line treatment for *P. falciparum* of CQ plus PQ costs Soles/ 2.24 ($0.47), the second-line treatment with Fansidar**7** costs Soles/ 6.22 ($2.39), and the third-line treatment with Q plus T costs Soles/ 8.56 ($3.29). When generic SP (Fansidar**7**) is purchased in bulk, the World Health Organization has stated that the cost should be just slightly more than that of CQ.

3.5 Epidemiologic Surveillance

Both active and passive surveillance for malaria cases are conducted in Loreto Region.

3.5.1 Passive case detection (PCD)

PCD is carried out by health promotors (sometimes referred to as volunteer collaborators). These are village residents who are trained by local MOH staff to take thick blood smears, record limited demographic data, and administer a presumptive treatment for malaria to any person who comes to them with symptoms of malaria. The diagnosis and treatment are free to the patient, and the volunteer receives no payment for his/her services. Most villages or towns with population >100 have a health promotor.

3.5.2 Active case detection (ACD)

With the rapid increase in malaria in Loreto Region in the last three to four years, the MOH has greatly expanded its ACD activities for malaria. Blood smears are taken in a variety of different settings and from different subgroups of the population:

1. Persons with fever or a history of fever in the last 7-14 days. This is usually done by house-to-house visits, taking a blood smear from anyone who currently has a fever or gives a history of one.
2. Generalized surveys in which blood smears are taken from all residents of a town or village who are present at the time of the survey. This type of survey is usually reserved for those areas which

have a very high incidence of malaria, and in most such surveys, blood smears are taken on >80% of the population. General blood smear surveys were carried out in villages along the Nanáy and Momon Rivers in March and May 1997.

3. General blood smear surveys in schoolchildren between the ages of 5 and 17. As with the community surveys, these are reserved for towns or villages at particularly high risk of malaria.
4. Family members of patients with confirmed malaria. When a patient attending a health promotor, a health post, or a health center is found to have a positive blood smear, smears are then taken from any family members accompanying that patient whether or not they are febrile. In some communities, health facility staff may visit the patient=s house to take blood smears on all family members.
5. Patients attending MOH facilities who come from selected high-risk areas. Beginning in March 1997, health workers in Iquitos started taking blood smears from any resident of towns/villages along the Nanáy or Momon Rivers, regardless of their reason for attending the health facility.

Blood smears from these sources are examined either at the local health post or health center or, if the quantity of blood smears is too high, at the Regional Reference Laboratory.

When selected samples of blood smears taken at health posts and health centers are re-examined at higher levels for quality control and differences in results are found, no corrections are made in the database.

Most malaria cases reported from health facilities in Iquitos are recorded as Aimported,@ because the patient gives a history in the past month or two of having traveled outside the city. A small number of patients, however, give no history of travel outside Iquitos and thus presumably acquire their infection in the most densely populated areas of the city.

3.6 Health Information System for Malaria

Each time a blood smear is taken by a health promotor, a small form with basic demographic information is completed on the patient and sent along with the blood smear to the nearest MOH malaria laboratory. MOH health facilities maintain a register with a line listing for each patient on whom a blood smear is taken. They record patients seen by the health promotors in their catchment area as well as those seen at the health facility itself. If the blood smear is positive, a separate register for patient follow-up is completed, along with a card on which the daily treatment doses are recorded.

Results of blood smear examinations on febrile patients and other residents are hand tabulated at each health post and health center for their entire catchment area. Larger health centers, particularly those in the Iquitos area, and hospitals have computer facilities. Each week these facilities send a line listing of all confirmed *P. falciparum* cases by district to the Epidemiology Office at the regional level (see Appendix 1). No data are recorded on *P. vivax* cases or on total blood smears examined. Monthly, each health facility sends a tabulation of the number of febrile patients, number of positive blood smears by patient age and parasite species, number of adverse reactions to antimalarial drugs and number of deaths, number of first-, second-, and third-line treatments administered and remaining stocks of each drug to the Malaria Office at the regional level (Appendix 2).

Using a separate series of forms (Appendix 3), the same health facilities report every three months the number of patients treated and cured and treatment failures for both *P. vivax* and *P. falciparum* with the first-, second-, and third-line treatments. This information is used as a way to monitor resistance to antimalarial agents. In mid-1997, the PNCM introduced an additional form for weekly reporting of *P. vivax* and *P. falciparum* cases by district (Appendix 4).

On a weekly or monthly basis, the regional Epidemiology Office and Malaria Control Program Office enter the data in a computer and send the information to their respective offices in Lima by fax, as well as mailing a copy of the database on diskette. The National Epidemiology Office collects and reports nationwide data from approximately 2,900 reporting sites on a weekly basis in its Epidemiologic Bulletin.

All health facilities visited in Iquitos and surrounding areas had posted on their walls either typed or hand-drawn tables and/or figures of the trends in malaria cases by species by month for January to May 1997. Some health facilities also had tables for each community in their catchment area.

It is clear that health facility staff at all levels devote an excessive amount of time to recordkeeping and reporting; in spite of this, most staff remain very conscientious about their responsibilities. In particular, health workers pointed out that the information recorded in the patient follow-up register and the treatment follow-up card are very repetitive; one or the other could be eliminated without jeopardizing patient care or recordkeeping. In addition, because of the separate reporting systems for the PNCM and the National Epidemiology Office, considerable duplication occurs, requiring extra work by health care workers at all levels.

It was not possible to double check the accuracy of reporting from the peripheral to the national level, however, differences do occur in the counts of *P. falciparum* cases between the National Epidemiology Office and the PNCM, in part because the Epidemiology Office does not collect information from all health facilities. Relatively large differences were noted within the PNCM database at the regional and national levels. In 1994 for example, the Loreto Region malaria control office reported 9,621 cases of *P. vivax*, while the PNCM office in Lima reported just 6,908 cases.

3.7 Influence of Residents= Behavior on Malaria Transmission

Brief interviews with local residents in each of the towns/villages visited suggested several ways in which their behavior might influence malaria transmission and control efforts in Loreto Region. Unlike the situation in many Latin American countries, self-medication of malaria seems to be quite uncommon in Loreto Region; the vast majority of residents apparently seek malaria treatment at health facilities. House construction in rural areas greatly favors transmission. In most houses, enclosed sleeping areas occupy less than one-third to one-half of the total floor space of the house; all remaining space is taken up by an open area (Asala@) which usually has only a waist-high wall around it. According to our informants, most family members remain in this area until 7:00-8:00 pm, before retiring for the night. Since the sun sets between 6:00 and 6:30 pm, there would appear to be ample opportunity for a vector to enter, feed, and escape without resting on any sprayed surface. In nearly all of the residences visited in rural areas, all family members slept under mosquito nets; however, in most cases, several family members share the same bed and net, increasing the chance that a vector could feed on a sleeping person through the net . The nets are made of a thick cotton cloth that allows very little air movement. The nets cost from Soles/ 16 to 20 ($6.15 to $7.70) each in Iquitos. In a visit made to the Zungarococha area about 30 minutes by car from Iquitos, one possible source of infection was noted that might not be reported routinely by a permanent resident of Iquitos as travel outside the city. This area has several moderate to large lakes which are extremely popular swimming resorts on weekends. Although signs are posted warning of the dangers of malaria transmission after 5:00 pm, it is likely that many visitors stay until 6:00-7:00 pm.

3.8 Entomologic Observations

3.8.1 Adult mosquitoes

Anopheles darlingi was apparently first identified in the Peruvian Amazon in 1993-94. Review of the results of human attractant catches made by the entomology group of the Regional Reference Laboratory in Iquitos (1996-97) indicate that *An. darlingi* has now become the predominant anopheline mosquito attracted to man in the areas surrounding Iquitos. These observations are supported by the standardized series of mosquito collections being carried out by NAMRID (U.S. Navy Medical Research Institute Detachment) in Puerto Almendras and neighboring areas. *Anopheles darlingi* appears to exhibit marked seasonal fluctuations in population density. Review of the records of human attractant collections made by the regional entomology group show sharp declines in population density in June and early July. Even in the team's human attractant collections made in Santa Clara, a suburb of Iquitos adjacent to the Iquitos airport, 62 *An. darlingi* were collected on June 26, compared with only three on July 3. Interestingly, *An. darlingi* was the only anopheline captured on June 26, but the collection on July 3 contained specimens of *An. oswaldii* (1), *An. triannulatus* (1), and *An.* near *evansi* (2). Night catches made at Santa Cecilia, on the Maniti River, several hours by boat from Iquitos, yielded 74 *An. darlingi* and 1 *An. evansi*.

Throughout its extensive distribution, *An. darlingi* is considered to be highly anthropophilic (preferring to feed on humans) and somewhat more exophilic (feeding outside houses) than endophilic (feeding inside). However, it has been shown to have a greater level of indoor biting activity than any other neotropical anopheline mosquito. Biting activity by *An. darlingi* has been found to be bimodal (early evening and early morning hours) in some regions of the Amazon basin and unimodal (around midnight) in other areas. Such variation has been attributed to genetic polymorphism and the existence of a sibling species complex. A substantial number of collections would be required to define this species biting pattern in the Loreto Region as individual nightly catches are influenced by a variety of environmental factors, such as wind, rain, etc.

Very little is known about adult *An. darlingi* behavior, especially with regard to survival (age structure), dispersion, and resting sites. Seasonality of transmission could be studied using circumsporozoite ELISA assays. While *An. darlingi* is being accurately identified by the Regional Reference Laboratory staff, significant difficulties remain in differentiating other species of the *Nyssorhynchus* family, such as *An. benarrochi* and *An. evansi*. This appears to be due more to inadequacies in the keys used for mosquito identification than the abilities of the local entomology staff.

3.8.2 Larval stages

Not surprisingly, larval development sites for anopheline mosquitoes are abundant and varied in the Iquitos region. One such habitat is small to medium sized ponds utilized for raising local fish for consumption or exportation of exotic species. These ponds are locally known as *Apiscigranjas,@* or fish farms and have become common in the communities surrounding Iquitos. In 1996, an estimated $1 million of tropical fish were exported from Iquitos, and this industry is growing with support from the Government of Peru. These ponds are often partially covered by aquatic vegetation and become favorable sites for anopheline larvae. Preliminary samples revealed the presence of *An. triannulatus.* Before costly larval control measures are directed at these potential breeding sites, a thorough survey of the anopheline species developing in piscigranjas is warranted in different locations and in different seasons. A somewhat similar habitat to the piscigranjas is that of natural ponds (known locally as *Acochas@*). These ponds tend to have an abundant growth of aquatic plants and emergent grasses and sedges around the periphery and significant densities of natural predators (both insects and fish). Anophelines are present in these ponds but usually at relatively low densities, and with early instars predominating. The team also carried out some sampling on the Nanáy River and its backwaters. Anopheline larvae were found in sampling along the edge of the Nanáy River and its backwaters, where water movement was minimal and aquatic vegetation (*Salvinia, Pistia, Eichornia*) abundant. Although larval densities were low, the magnitude of the river systems in the Iquitos area suggests that they may be an important source of anophelines. In addition, during the rainy season when the river levels are much higher and adjoining areas become inundated, it is likely that sites suitable for anopheline larval development are greatly increased. Once the rivers begin to recede at the end of the rainy season, many small, transitory ponds may be created. These likely would be good sites for mosquito larval development and would tend to have fewer predators than the natural ponds. The relationship of the river systems to the population density of *An. darlingi* warrants further study.

3.9 Vector Control Activities

Extensive, emergency vector control measures were carried out in the 12 months preceding the team=s visit (i.e., 1996-97) in the city of Iquitos and along all of the major river systems entering the Amazon near the city in an attempt to control the epidemic. These measures included residual house spraying, ULV fogging, and larviciding. Vector control activities increased in intensity from March 1997 onward. As an example, from June 3 to June 22, 1997, an insecticide spray program was carried out at the periphery of Iquitos and in adjacent small communities. In this operation, a total of 265 kg of the synthetic pyrethroid, cyfluthrin, was sprayed in houses using motorized backpack sprayers. In addition, 133 liters of malathion were applied by ULV using motorized sprayers and by fogging with a London Aire fogger. Abate was also applied for larval control, but this appeared to be directed primarily at sites used by *Aedes aegypti*. Currently, houses in towns and villages along several of the river systems are being sprayed with cyfluthrin, but as noted above, the design of the houses with only limited enclosed wall space, raises questions about the effectiveness of this control measure.

The concentration of cyfluthrin applied in intradomiciliary spraying has varied significantly over the past months and there is concern about the longevity of its residual effectiveness. Supervision and evaluation of the spraying program appears to be sporadic. In one case, a village resident commented that because of the small doors in many houses, spraymen using backpack sprayers could not enter the house and had to carry out their spraying from the doorway. Since there has been relatively little agricultural development in the region, insecticide resistance is likely minimal. A review of records of the major distributor of insecticides in Iquitos revealed that the most commonly used insecticides for agricultural purposes are Tameron (metamidofos), Sevin (8% carbaryl) and Lorsban (chloropirifos).

The Loreto Region entomology evaluation group is composed of a field supervisor and five entomology technicians. The supervisor and three of the technicians are temporary employees, i.e., contracted by month. In general, their technical skills are adequate, but it was clear that the group lacks leadership and support. This group is currently supervised by a physician. The laboratory facilities used by the entomology group are being moved to the Regional Reference Laboratory. At present (1997) they occupy a room approximately 3 x 2 m in size. Transportation appears to be a major difficulty for the group as they are dependent upon the availability of vehicle and boats from the regional office. Equipment and supplies also are very limited; e.g., the single stereo microscope used for species identifications lacks an ocular micrometer, which is essential for accurate identification of many the *Nyssorhynchus* species.

House spraying, ULV space spraying, and larviciding are carried out by temporary workers hired by the region through a local contractor. The spray teams are supervised by a former national malaria eradication program worker with many years of experience in vector control, but with the magnitude of the malaria problem in the region and the variety and extent of vector control activities, more field supervisory staff is needed. In addition, entomologic evaluation of the vector control measures has been very sporadic, apparently due to limited financial resources. Thus, there is little assurance that these measures are achieving their desired effect.

4 CONCLUSIONS AND RECOMMENDATIONS

4.1 General Comments

1. Beginning in 1992-93, apparently in association with the introduction and spread of *Anopheles darlingi* in the Peruvian Amazon, there has been a striking and progressive increase in the incidence of malaria in Loreto Region. Of particular concern is the rapid increase in the incidence of *P. falciparum* infections in spite of aggressive control efforts.

2. Given the favorable climatic and environmental conditions, an efficient vector that will be difficult to control, the mobility and dispersed nature of the human population, and the spread of antimalarial drug resistance, the malaria problem in Loreto Region will probably prove extremely difficult to control and it is unlikely that even a well-designed and costly effort will reduce and sustain transmission at pre-1992 levels.

3. Most malaria transmission appears to be occurring in rural areas and in newer settlements on the outskirts of Iquitos, rather than in densely populated areas in the center of the city. Furthermore, because of the limited number of suitable breeding sites for *An. darlingi* in and near urban areas, the risk to the urban residents of Iquitos is probably very low, unless they travel outside of the city.

4. The serious nature of the malaria problem in Loreto Region requires close coordination and communication at the national level between the four key groups responsible for malaria control: the National Malaria Control Program, DIGESA, the National Epidemiology Office, and INS, and between the national and regional levels.

   The National Malaria Control Program, DIGESA, the National Epidemiology Office, and INS should consider holding monthly meetings to discuss the malaria situation, review progress of malaria control activities, and plan future interventions. All four groups should maintain close contact with Loreto Region Health Office.

4.2 Case Management/ Epidemiology

1. The quality of microscopic diagnosis of malaria in MOH facilities is generally quite high; however, because of the increased number of malaria cases and the heavy reliance on active case detection by the malaria control program, the network of microscopists is severely overextended.

   The National Malaria Control Program and Loreto Region malaria control program should consider eliminating the following blood smears to reduce microscopists= workload: (1) blood smears on asymptomatic family members; (2) follow-up blood smears on patients with P. vivax *infections; (3) day 3 and day 14 follow-up blood smears on patients with* P. falciparum *infections (day 7 smears should continue to be taken); and (4) blood smears on patients attending health facilities for reasons other than malaria who come from high risk areas.*

2. In patients infected with *P. falciparum*, accurate determination of parasite density is extremely important for good case management.

   The INS should consider training malaria microscopists in standard procedures for calculating parasite density and require such counts on all cases of P. falciparum *malaria.*

3. Adherence by Ministry of Health personnel to National Malaria Control Program standards and procedures for the diagnosis and treatment of malaria is excellent and the overall quality of care provided to outpatients with malaria is very high.

   Given the increasing incidence of P. falciparum *infections in the region, all physicians involved in patient care should receive training in the management of severe and complicated malaria. The World Health Organization has developed excellent training materials for such courses.*

4. Drug resistance of *P. falciparum* to chloroquine already appears to be widespread. The situation with respect to sulfadoxine-pyrimethamine (Fansidar**7**) is less clear but evidence suggests that 10 to 20% of patients may fail to respond parasitologically. The third-line treatment of quinine plus either tetracycline or clindamycin remains effective but is costly and is a difficult regimen to administer to outpatients because of side effects and the requirement for a seven-day treatment.

   Staff from the central and regional levels should be trained in the current WHO standardized in vivo drug efficacy testing procedure and sulfadoxine-pyrimethamine efficacy should be assessed at five or six geographically distinct sites in Loreto Region. In at least some of these sites, mefloquine (an alternative single-dose treatment to quinine plus tetracycline/clindamycin) should be evaluated.

5. Recent community-wide blood smear surveys in high transmission areas have identified a high proportion of residents who apparently have asymptomatic infections.

   Since the presence of large numbers of residents with asymptomatic malaria infections might modify approaches to case detection, an evaluation should be undertaken to determine the prevalence of such infections in Loreto Region.

6. The health information system for malaria is excellent and is providing critical information for following the course of the epidemic in Loreto Region. Health workers are extremely conscientious in their malaria recordkeeping and reporting responsibilities; however, as a result of the large number of forms required for malaria case reporting and duplication of effort between the National Malaria Control Program and the National Epidemiology Office, these workers are spending an excessive amount of time on paperwork.

   The groups responsible for malaria epidemiologic surveillance should review the current data collection and reporting forms with the aim of eliminating repetitious forms and simplifying the remaining forms and recordkeeping and reporting procedures as much as possible.

7. The Loreto Region passive case detection network seems to be functioning very well. Passive case detection will provide the most representative and consistent picture of malaria transmission in the region.

   The reliance of the malaria control program on active surveillance, which is more appropriate to a disease elimination effort, should be reduced. An assessment should be made of the sensitivity of the passive case detection network in Loreto Region to detect cases of malaria occurring at the community level. Passive case detection data should be tabulated and analyzed separately from active case detection data. Trends in the incidence of malaria and slide positivity rates from the passive case detection network can be used to identify high transmission areas and target control interventions.

8. Several aspects of residents' behavior that may increase the risk of malaria or influence malaria transmission, treatment, and prevention were identified. These behaviors and residents' attitudes towards malaria and malaria control deserve further study by a social scientist with experience in the health field.

9. Currently, only limited direct supervision of health care workers and microscopists in peripheral health facilities is conducted in Loreto Region. Direct supervision of workers in peripheral health facilities not only helps to improve their performance, but also serves to motivate the workers.

   Loreto Region should seek additional funds to cover transportation and per diem costs so that regional clinical and laboratory staff can visit health centers and health posts and the staff at those peripheral health facilities can visit the villages in their catchment area on a regular basis.

10. Given the rapid spread and high incidence of *P. falciparum* infections in the region, malaria mortality and complications of malaria in pregnancy are reported less frequently than might be expected.

    Studies should be undertaken to describe the epidemiology of severe and complicated malaria and malaria during pregnancy in Loreto Region, including both mestizo and Amerindian populations.

4.3 Entomology/Vector Control

1. Vector control is a critical but neglected component of the Loreto Region malaria control program. The lack of a specialist in integrated vector control to organize and lead vector control activities in Loreto Region has severely hampered control operations. The current entomology evaluation team is capable of carrying out routine entomology surveillance and other basic tasks, but lacks supervision, leadership and direction. The dependence of the entomology team on vehicles and boats from other groups, the limited laboratory space, and the lack of adequate microscopes and other equipment have made it impossible for the team to carry out its mission.

   Loreto Region health authorities should identify a person who is already trained or can receive short-term training in integrated vector control for malaria. This person should have leadership qualities and be willing to spend 30% to 50% of his time in the field supervising entomologic surveillance, evaluation of insecticide use, and other activities. The candidate should have a permanent post in the Regional Health Office and a salary commensurate with his responsibilities. Ideally, a similar individual should be identified at the national level. The Loreto Region vector control specialist should work closely with a unified team of entomology technicians that has its own vehicles and boats and adequate laboratory space and equipment.

2. Insufficient information is available about the biology and behavior of the major malaria vector, *An. darlingi*, to plan cost-effective vector control operations.

   Applied research should be carried out on An. darlingi *adult survival, flight range, resting sites, and feeding behavior as well as on defining sites of larval development over a range of geographic locations and during different seasons.*

3. In spite of extensive vector control activities in Loreto Region, there has been little or no evaluation of the impact of those activities. This information is needed to refine and target vector control measures in the future.

A standard approach to the monitoring and evaluation of vector control activities should be developed for the region and workers trained to conduct that evaluation on a routine basis.

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Weekly Register of Immediately Notifiable Diseases

Monthly Operational Record for Malaria

Malaria Patients Receiving Different Treatments by Drug and P.f./P.c.

Weekly Malaria Summary

Part 2
Entomological Aspects of Malaria Transmission

July 13 - August 2, 1997

L. Philip Lounibos

TABLE OF CONTENTS

1 PURPOSE OF THIS CONSULTANCY

To provide support leading to a reduction in human malaria in the Department of Loreto in the Peruvian Amazon, the Environmental Health Project (EHP) arranged for visits of consultants with experience in malaria epidemiology, vectors and control. My visit followed that in late June and early July of a three-person CDC team which extensively reviewed the epidemiological situation and initiated studies on malaria vectors (see Part 1). My assignment was to undertake entomological observations, submit perspectives and recommendations based on extensive interviews, and review data and personal field experiences during my three weeks in Peru, integrated with previous research on malaria vectors elsewhere in South America. Where this report overlaps with topics discussed by the CDC team (Part 1 of this report), the purpose is to emphasize and/or elaborate on particular features of vector biology and control, and the capacity to regulate them, considered to be important for malaria reduction in the Iquitos region.

In brief, the seriousness of the malaria situation around Iquitos is largely due to the unfortunate coincidence of *Plasmodium falciparum* and *Anopheles darlingi*, the latter being the most dangerous vector of malignant tertian malaria in South America. Numerous factors complicate resolution of the malaria problem, including a high incidence of *Plasmodium vivax*, the presence of many species of potential secondary vectors, and the vast aquatic systems that impede access to human settlements and provide diverse larval habitats.

2 ACTIVITIES

From July 13 to 16, 1997, meetings, interviews, and tours of facilities in Lima were coordinated with the USAID office. Particular emphasis was placed on the entomological capacities, output, and activities of the *Instituto Nacional de Salud* (INS) where malaria and molecular biology laboratories were also toured. Staff of DIGESA, OGE, PNCM, and OEM involved in malaria and/or vector research and control met with me to describe their respective responsibilities as relating to malaria in the Iquitos region.

In Iquitos, most meetings and interviews, and all coordination of field trips, were accomplished through the Regional Hospital of Loreto and its reference laboratory, the latter which has space allocated for entomology. Discussions and reviews of data were conducted with both Peruvian physicians and biologists employed at these facilities. Additionally, I visited the research facilities of Navy Medical Research Center (NAMRID) in Iquitos and discussed entomological observations and data with researchers of both NAMRID and U.S. Army Medical Research Center (USAMRIID) currently engaged in research on the vector fauna of the Iquitos region. A visit to the Instituto de Investigaciones de Amazonia Peruana (IIAP) was made to review its remote sensing and GIS capacities, and a subsequent meeting was held with IIAP personnel to discuss collaboration between IIAP and malaria control workers.

Field trips were made to investigate malaria vectors and human behavior in the following communities on the Rio Nanáy: San José and San Pedro de Lupuna; Santa Maria de Nanáy and Padre Cocha (two trips). Because numbers of anopheline females captured at human baits were increasingly low on these trips (always << 1.0 mosquito/man-hour), emphasis was placed on identification of larval breeding sites. When possible, collected larvae were reared to adulthood in my hotel room, where provisional species identification was done. To augment the low number of mosquitoes caught at human baits, mosquitoes were collected at night from cattle and pigs stabled in a corral at Shushuna, which allowed trapping of secondary vector species. Biologists from INS, OGE, and/or DIGESA accompanied me on field trips, as did local biologists and field assistants who participated in mosquito collection and subsequent identification.

3 OBSERVATIONS, PERSPECTIVES, AND RECOMMENDATIONS

3.1 Vector Biology

3.1.1 Taxonomy in relation to malaria transmission

All of the likely vectors of human malaria in the Peruvian Amazon belong to the anopheline subgenus *Nyssorhynchus* which, for decades, has caused problems for taxonomists because of the inadequacy of morphological characters, especially in the adult female stage, for accurate species identification. In spite of their morphological similarity, these cryptic species have distinctive behaviors and habitats that determine their importance as malaria vectors. No taxonomic key currently available satisfactorily separates many important species of *Nyssorhynchus* in the Peruvian Amazon region, whose mosquito fauna has been poorly characterized.

There should, in principle, be no difficulty in identifying specimens of *An. darlingi*, in all probability the most important malaria vector in the Loreto region. In spite of some geographic variation in biting schedules, *An. darlingi* is relatively homogeneous genetically throughout its range from southern Mexico to northern Argentina, and there are, at present, no grounds for regarding it as a species complex. Locally, *An. darlingi* is known as ›*pata blanca*= because of its entirely white fifth hind tarsomere. However, identification should not be based on this characteristic alone, since other species of the Argyritarsis Section of this subgenus known from Amazonian Peru also possess this same A*pata blanca.*@

There is malaria transmission in parts of the Departmento de Loreto where *An. darlingi* is absent, and the suspected vectors have been variously identified as *Anopheles evansae*, *Anopheles oswaldoi*, *Anopheles nuneztovari* and/or *Anopheles benarrochi*; field collections of each of these purported taxa have tested positive to sporozoite ELISAs for *P. falciparum* or *P. vivax*. However, confirmation of the identification of these suspected secondary vectors remains to be done. In particular, *An. evansae*, a relatively uncommon species originally identified in Argentina, has to date not been confirmed from Peru. The taxon *An. oswaldoi*, the principle vector of *P. falciparum* malaria in Acre State, Brazil, actually consists of two species, *An. oswaldoi* sensu strictu and *Anopheles konderi*, which cannot be separated in the adult female stage. Likewise, morphological keys to females will not accurately distinguish *An. nuneztovari* from *Anopheles dunhami*, both of which species are known to co-occur as close to Iquitos as Tabatinga, Brazil. Finally, R. Wilkerson, the foremost authority on this group of mosquitoes, believes that *An. benarrochi* occuring in Amazonian Peru may be different from the species of that name known elsewhere in South America.

In order to rectify this situation, link rearing should be conducted with progeny broods from females of each of the species of doubtful taxonomic status. Examples of all life stages should be preserved for morphological studies, together with specimens from the same progeny brood placed in 95% ethanol for DNA extractions. Through this dual approach to taxonomic identifications, assisted by collaboration between Peruvian and American investigators, a complete inventory of the *Nyssorhynchus* in the Loreto region would be achieved. Once this has been accomplished, taxonomic keys to the local anopheline fauna can be constructed based on the known species inventory.

3.1.2 Ecology

Origins

The vector species *An. darlingi* was unknown in the Iquitos area prior to 1993. Whether it was introduced accidentally or had previously existed undetected in the region is not known. However, investigations as to the source of *An. darlingi*, now the most abundant human-biting anopheline mosquito in malarious areas of Loreto region, have very practical implications. For one, the genetic background of the mosquito population determines its ecology and behavior, an understanding of which would allow inferences about expected niches and habits that might be occupied in the Iquitos area. If, for example, *An. darlingi* was accidentally imported from Amazonian Brazil, then it would be expected that its biting schedule and host preferences would correspond to the well-studied patterns of that region. If, furthermore, *An. darlingi* was recently imported and now occupies previously unused niches, then the probability of rapid evolution is higher as the species adapts to its new environment. Finally, if it can be determined how and when *An. darlingi* was introduced into the Iquitos area, then some measures to prevent its further spread might be undertaken. (Present ecological information on this species predicts that *An. darlingi* will not spread beyind *selva baja*, i.e., will not invade such areas as Alto Amazonas.)

The genetic origins of *An. darlingi* now in the Iquitos area can be explored by comparing hypervariable regions of DNA with patterns obtained from this species elsewhere in its range. Such a project could initially utilize the expertise of American investigators currently studying the molecular genetics of *An. darlingi* in Amazonian Brazil.

Population dynamics

Because *An. darlingi* was previously unknown in the Iquitos region, little or no information is available on its bionomics and the factors that influence such. It would seem, for example, that *An. darlingi* numbers are correlated positively with river levels and rainfall, but these relationships need to be established quantitatively in different zones, where larval habitats change seasonally (see, also, below). Trends derived from these studies could be used to predict the levels of vector population reduction needed through mosquito control measures. (Although the number of mosquitoes was low in all human-bait catches conducted in three communities on the Rio Nanáy, *An. darlingi* was the dominant species collected at all sites, indicating that malaria transmission may continue even at the driest time of year in the rural regions.)

A thorough study of *An. darlingi* dynamics should also examine age structure of adult populations, using one or more methods of parity determination. Such information allows not only estimates of adult survivorship, which is directly related to transmission capacity for malaria, but is useful for addressing the success of insecticide applications. For example, parity rates should plummet if insecticide fumigation is providing effective vector control.

Adult dispersal

It is very important to know how far *An. darlingi* fly from breeding (larval) sites to obtain blood meals, if larviciding is to provide protection from malaria transmission. Based on observations made on this trip and results from mark-release-recaptures of *An. darlingi* in Rondônia State, Brazil, it is reasonable to expect this species to travel 1 to 2 km in search of blood, including crossing narrow tributaries of the Amazon. Under such circumstances, larviciding limited to the peripheries of rural communities may not provide adequate personal protection.

Larval distribution

The limited larval surveys conducted during this consultancy recovered *An. darlingi* in virtually all aquatic habitats that were positive for *Anopheles*, such as stream margins, aquaculture ponds *(piscigranjas)*, ponds and wells. However, more systematic and quantitative surveys are needed to identify all important breeding sites in areas of malaria transmission, in order to implement effective control of immature stages. This proposition is daunting because of the habits of *An. darlingi* that include occupancy of stream margins and calm waters inaccessible from land. In Belize, recent progress has been made in collecting immatures of *An. darlingi* from small boats and rubber rafts, permitting a more thorough mapping of its distribution in aquatic habitats. Describing the relationship between larval distribution and physical and biotic characteristics of habitats will be useful for a geographic information system (see below) that interrelates vector ecology, malaria epidemiology, and human settlement patterns.

Secondary vectors

Once secondary vector species have been identified by systematists (see Section 3.1.1, above), comparable studies on their population dynamics and niches should be undertaken in concert with investigations to establish vector competence (see Section 3.1.4, below). Priorities as to the relative importance of controls of these secondary vector species cannot be assessed until the more basic research is done.

Geographic information systems (GIS)

The presence in Iquitos of IIAP, and the interest of its staff in collaborating in the public health sector, presents an opportunity to use modern remote sensing and mapping tools to investigate and, ultimately, intervene in the many complex factors involved in the malaria epidemic. However, as discussed above, the important entomological components of a comprehensive GIS on malaria in the Iquitos region are currently unavailable. Nonetheless, meetings of local entomologists interested in achieving this goal are a step towards identifying the field data needed.

Similar mark-release-recapture techniques may be used to identify the distances and directions that *An. darlingi* are flying from breeding sites in order to take blood in and near houses. These results will influence which breeding sites must be treated with larvicides. To acquire adult densities that ensure sufficient recoveries, mark-release-recapture studies should be done in the rainy season using large numbers of human baits.

Host preference

3.1.3 Behavior

Biting cycles

Sufficient data are now available, both from Peruvian research and such investigations as the current NAMRID study at Puerto Almendras, to demonstrate that *An. darlingi* attacks humans throughout the night in the Iquitos area, with a broad peak in abundance between 1900 and 2400 hours; there are also some indications of a small dawn peak. Biting densities are approximately equal inside and outside of houses. More research is needed to characterize the biting cycles of secondary vectors, although preliminary data suggest that some (e.g., the species identified as *An. evansae*) prefer to seek blood at earlier hours (1800-2000 hours).

Resting

I could find no data on this very critical component of success for applications of residual insecticides. Particularly, it is apparently not known where or if *An. darlingi* rest inside houses before, and especially after, blood meals. Therefore, as soon as *An. darlingi* adult densities rebound in the Iquitos area, detailed research on house entry, resting, and exiting behavior should be initiated. Data should be taken not only when and whether, but also ***where*** *An. darlingi* rest indoors, since knowledge of specialized resting sites or heights may reduce quantities of insecticide used. Simple tools such as colored, fluorescent dusts and the ›Colombian curtain= may be useful for tracing movements of individual mosquitoes.

Flight

Although it is already appreciated that *An. darlingi* is the most anthropophilic malaria vector in the Loreto region, very little is known about the host preferences of potential secondary vectors. Blood-fed mosquitoes should be collected before dawn from vegetation outside houses using a power aspirator and preserved for analyses of host blood by appropriate immunological procedures. This research should be a priority only where species other than *An. darlingi* (e.g., *An. benarrochi)* are expected to be important in malaria transmission.

Human-vector contact

In rural areas on the Rio Nanáy, I observed a marked decrease in human activity outdoors after 1930 hours. However, residents remaining indoors often stayed up late. Villages with electricity usually stopped their usage at 2200 hours, which terminated television and influenced bedtimes. Many persons watch televsion, relax, and sleep in hammocks in sections of houses that have little or no walls. Because *An. darlingi* is attracted to man throughout the night with approximately equal intensity indoors and out, it is likely that substantial malaria transmission occurs indoors.

3.1.4 Incrimination and competence

Although the circumstantial evidence is strong that *An. darlingi* is the principal vector of human malaria in the Loreto region, positive incrimination of this species requires detection of parasites in salivary glands, either by dissection or an immunological method, such as sporozoite ELISA. This is important because ELISAs applied to whole specimens may yield positive reactions to oocysts that may not proceed to the infective sporozoite stage. Salivary gland dissections and/or immunological testing of salivaries should also be performed on secondary vectors caught at human baits, since the capacity of such species as *An. benarrochi*, *An. oswaldoi* and *An. nuneztovari* to transmit malaria remains uncertain. Abdomens of specimens of potential secondary vectors whose salivaries are tested should be preserved for later DNA extractions to corroborate species identifications.

The physical facilities, interested physicians, and the many malaria patients at the Loreto Regional Hospital also afford an unusual opportunity to investigate vector competence, i.e., the ability of a species to ingest, successfully develop, and transmit malaria parasites. Collaboration between an entomological investigator and the hospital, whereby uninfected mosquitoes feed on patients with diagnosed malaria, could yield data important not only to the local epidemic, but also answer questions about the relative competency of these same species elsewhere in South America.

3.2 Vector Control

Current control methods are largely chemical, based on applications of larvicides to anopheline breeding sites and/or adulticides applied by fumigation or as residual treatments to buildings. The text below suggests, among other things, that insecticide-impregnated bednets should be added on an experimental basis to local malaria control measures.

3.2.1 Control versus eradication

The issue of control versus eradication is mentioned because of the successful eradication of *Anopheles gambiae*, which was accidentally introduced by boat or airplane from West Africa to coastal Brazil. Eradication occurred after this species had vectored in 1938-40 the most serious malaria epidemic in South American history, with over 200,000 persons infected and more than 20,000 dead. The success of this eradication campaign was attributed to massive external funding, the dry and isolated landscape of northeastern Brazil, and the highly toxic Paris Green insecticide. None of these elements applies to the supposedly introduced *An. darlingi* in the Loreto region. Not only are the limits of distribution of *An. darlingi* unknown in the Loreto region, but its isolation is uncertain, i.e., *An. darlingi* in the Peruvian Amazon may be continuous in distribution with populations of the species in Colombia or Brazil. Thus, unlike the situation with *An. gambiae*, successful eradication of *An. darlingi* from the Loreto region seems unlikely because of the uncertainty of its isolation and the extent of the river systems that it inhabits.

3.2.2 Impregnated bednets

No single method for malaria reduction since DDT has been so cost-effective and produced such persisting results as the use of mosquito bednets impreganted with pyrethroid insecticides. Not only have bednets greatly reduced morbidity attributable to malaria in the African and Asian tropics, but they have also significantly decreased morbidity due to *other* causes. With community participation (which may require social workers) I believe that bednets will reduce malaria transmission by *An. darlingi*, about half of which may occur indoors in the Loreto region. Information on appropriate materials can be solicited from WHO. To convince rural communities to use bednets, lighter-weight nets than the cotton ones found in Iquitos markets will be needed. Evaluation of bednet efficacy in selected villages must be carefully designed (see Section 3.2.5, below) and monitored in treated and control sites for malaria case detection, mosquito biting densities, and net use.

3.2.3 Larval control

Perhaps the most crucial commentary on larviciding is that it should be done in the dry season when the breeding sites of *An. darlingi* are reduced and concentrated into a few manageable and treatable areas. This vector species was identified from all sampled larval habitats with anophelines in Lupuna, Santa Maria de Nanáy, and Padre Cocha. If these sites are left uncontrolled, the resident immatures provide the seeds for a rapid population explosion when rains begin and larval habitats expand. Larviciding in the dry season, using Temephos (=Abate) or BTI, may keep a treated village malaria-free several months into the rainy season before *An. darlingi* reinvade.

Although drainage of breeding sites would be best, where that measure is not possible, owners of *piscigranjas* and other permanent water bodies

Fogging (= fumigation) is, and should continue to be, the last resort of the insecticide program, used when other intervention techniques are not providing adequate malaria control.

3.2.5 Interventions, designs, and experiments

There has been little or no follow-up to date on most control measures against malaria vectors. It is imperative, especially for new interventions such as bednets, that their introduction be designed as experiments, with one or more nontreated sites selected for comparison. Ideally, entomological data (mosquito numbers) should be compared between pre- and post-treatment and followed temporally to chart recovery patterns after vector reduction. Experimental designs should be established so that treatments and controls can be compared by valid statistical procedures.

3.3 Manpower and Resources

Currently, entomology occupies one room under renovation at the Health Reference Laboratory in Iquitos. During my visit, this room functioned mainly as a processing center for mosquito

should be encouraged to cut and/or eliminate vegetation, both emergent and overhanging. The absence of plants or detritus renders habitats less suitable for most anopheline larvae.

Over the long term, native larvivorous fish suitable for *piscigranjas* and wells should be tested for capacity to detect and eat anopheline larvae, first in aquarium settings and, subsequently, in field trials. Such biological control research should be conducted apart from insecticide trials (see Section 3.3.3).

3.2.4 Adult spraying

Although Asusceptibility tests@ are currently done for residual spray treatments there seem to be no data to indicate whether *An. darlingi*, or other vector species, are actually being killed by adulticides used in natural situations. For example, a white drop cloth placed beneath sprayed walls should catch resting mosquitoes for the sake of tabulating anophelines killed by the insecticide. Such evaluations need to be done in both experimental (see Section 3.2.5) and control houses.

collecting supplies. There is no insectary, and the entomology staff share one heavily used field vehicle with many others from the Hospital. Biologists employed to do entomological work have little training in entomology. The following sections deal with upgrades needed at the regional level to accomplish at least some of the recommendations set forth under the discussion of vector biology and control.

3.3.1 Personnel

Two scientists are needed, permanently stationed in Iquitos, with post-graduate training in mosquito biology and the ability to make independent decisions about mosquito research and control. One person should be in charge of research, and the other, control. The two should have separate but complementary field-lab teams that facilitate exchanges of information.

3.3.2 Collaborations

Local, national, and international collaborations are necessary to make entomology a useful component of malaria control in the Iquitos region. One promising initiative is the interest of

entomologists at IIAP in undertaking investigations on anophelines in collaboration with personnel at the Regional Hospital. However, external guidance is needed to direct local investigators along fruitful research paths. Peru=s biologists with the most experience in mosquito biology and medical entomology are based in Lima, and several of these individuals have participated in investigations on malaria vectors in the Iquitos area. Thus, collaboration with, and transfer of knowledge from, specialists in Lima is critical to strengthen entomology in Iquitos and to achieve control of malaria vectors. Finally, international links are necessary both for training and introduction of new research tools. Many foreign biologists are interested in the insect
The present laboratory allocated for entomology at the Health Reference Laboratory is not adequate to meet malaria research and control needs. Ideally, a medical entomology laboratory with a climate-controlled insectary and bench and desk space designed for entomological work should be established on the grounds of the Loreto Regional Hospital. Insecticide storage, testing, and formulation should be done elsewhere, in a locality where there is no chance of contaminating and killing live mosquitoes being used for investigations.

vectors of the Iquitos area, and efforts should be made to secure their help, for example, in taxonomy (Walter Reed Biosystematics Unit of the Smithsonian Institution) and gaining access to local data on abundances and biting schedules (NAMRID and USAMRIID projects). It would also be valuable for entomologists in Iquitos to have more contact with their peers in neighboring Latin American countries, such as through the malaria network and the Internet listserve on vectors, both organized from the PAHO regional office in Brasilia.

3.3.3 Facilities

3.3.4 Resources

New microscopes, both dissecting and compound, are needed for malaria vector research in Iquitos, as well as a new 4-wheel drive vehicle and a boat of adequate size and durability to carry entomological teams and supplies on river trips. Inflatable, heavy-duty rafts (e.g., Zodiacs) would be useful in larval sampling efforts.

4 CONCLUSIONS

Entomology could play an important role in malaria control in the Iquitos area if initaitives were taken in the areas discussed below.

4.1 Vector Biology

As promptly as possible, research information is needed on the taxonomy of secondary vector species and on adult population dynamics, resting behavior, and the larval distribution of the primary vector species, *An. darlingi*. Over the long term, research on vector competence, dispersal, host preference and origins of the *An. darlingi* invasion would refine and strengthen an integrated control plan, as would a multi-component GIS system incorporating local epidemiological and entomological data.

4.2 Vector Control

As promptly as possible, materials for impregnated bednets should be acquired and delivered, with instructions for conducting community trials. Larval breeding sites identified as harboring *An. darlingi* should be treated with Temephos or BTI in the dry season. Pre- and post-treatment monitoring of vectors and malaria cases is critical for the evaluation of interventions. Long-term plans for vector control should integrate environmental management, such as drainage, removal of aquatic vegetation, and use of native predatory fish.

4.3 Manpower and Resources

To make vector biology and control an important component of malaria reduction in Iquitos, locally based entomological teams with leaders trained at the post-graduate level are essential. These teams and their leaders need local, national and international collaborations to achieve malaria control goals. They also need the use of facilities designed for entomological research and adequate land and field transport to carry out their work.

Part 3
Insecticide Resistance by Malaria Vectors

October 1998

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TABLE OF CONTENTS

TABLES

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SUMMARY

To update available information on the response of adult *Anopheles albimanus Wiedemann* mosquitoes to insecticides in the northern coastal region of Peru, an assessment was conducted on resistance to the organochlorine insecticide DDT (4%), the pyrethroids cyfluthrin and l-cyhalothrin (0.1%), the organophosphorus insecticide fenitrothion (1%), and the carbamate propoxur (0.1%). The method used consisted of residual exposure on paper, with the diagnostic doses for adult anopheline mosquitoes recommended by the World Health Organization used as points of reference. All of the populations of *Anopheles albimanus* evaluated in the provinces of Piura, Tumbes, and Sullana were classified as susceptible to the organophosphorus insecticide fenitrothion and the carbamate propoxur (99-100% mortality rate). With regard to DDT, these populations were classified as being Aunder surveillance@ (93-95% mortality rate), except in San Isidro de Tumbes where the results showed resistance (77% mortality rate). With regard to the pyrethroids, resistance was detected in the Tumbes and Sullana strains (<80% mortality rate), while the strains found in Piura were classified as being Aunder surveillance@ for cyfluthrin (86% mortality rate) and resistant to l-cyhalothrin (61% mortality rate).

The table below gives the range of results in all the localities where trials were conducted. The results in each specific locality are shown in Chapter 3.

Table 1
Preliminary Summary Results of Insecticide Susceptibility Trials in Northern Peru *(Anopheles albimanus)*

Insecticide	Localities	Range of Results	Average Mortality
DDT (4%)	4 localities	77-95%	87%
Fenitrothion (1%)	5 localities	100%	100%
Propoxur (0.1%)	3 localities	99-100%	99%
l-cyhalothrin (0.1%)	5 localities	15-76%	41%
Cyfluthrin (0.1%)	6 localities	28-89%	62%

N.B. Controls tested simultaneously with the above trials showed no significant mortality

1 BACKGROUND AND PURPOSE

Malaria is the most frequently occurring and most devastating disease found in the tropics; it poses a threat to approximately 40% of the world's population (WHO 1993). Malaria transmission has increased markedly in the Americas in recent years, particularly in the Amazon basin where approximately 65% of all cases of malaria occurring on the continent are recorded.

The worldwide strategy to eradicate malaria takes into account the fact that this disease is focally distributed and extremely variable with regard to its epidemiological characteristics, as a result of the various ways in which humans, vectors, and parasites have adapted to the wide variety of ecological environments as well as to environmental modifications, both natural and man-made. Despite the development of resistance to insecticides in malaria vectors in certain areas and despite the fact that *P. falciparum* has also developed resistance to the most common antimalarial medications in many areas, it is felt that it is still possible to prevent this disease in specific situations provided appropriate measures are taken on a timely basis (WHO 1993).

The most effective malaria control operations generally involve vector control measures aimed at reducing either the number or longevity of the vector or diminishing human-vector contact by physical, chemical, or biological means. However, the development and selection of the appropriate methods to be used in a given situation depend on a precise knowledge of the local epidemiology of the disease as well as on the biological characteristics and habits of the vector (Fleming 1996). In the Americas, *Anopheles albimanus* and *Anopheles darlingi* rank high as malaria-transmitting insects. In Peru, both species are primary vectors of this disease.

Information was gathered from local workers in the Province of Tumbes regarding the use of insecticides in the past. Table 2 shows the various insecticides applied in that locality, by date, for vector control. Unfortunately, information on volume (quantity) used per year was not available.

This report presents the results of a survey of resistance to chemical insecticides among adult *An. albimanus* mosquitoes in the northern coastal region of Peru. The survey was designed to determine the levels of susceptibility and/or resistance to the insecticides currently being used to control malaria as well as to other insecticides that might be used as alternatives. Integration and correlation of the results will provide information on the most appropriate insecticides to use, if a decision is made to use chemical measures as part of a control strategy.

Thus, the general objective of the consultancy in October 1998 was to assess resistance to insecticides among *Anopheles albimanus Wiedeman* in malarious areas of northern Peru. Two specific objectives were:

- To determine if there is resistance of *Anopheles albimanus* to the four classes of insecticides—organochlorine insecticides (DDT), organophosphorus insecticides, carbamates and pyrethroids—in various localities in northern Peru, and
- If there is, to carry out tests on the levels of resistance (and importance for vector control) of *An. albimanus* to these insecticides.

Information was gathered from local workers in the Province of Tumbes regarding the use of inscecticides in the past. Table 2 shows the various insecticides applied in the locality, by date, for vector control. Unfortunately, information on volume (quantity) used per year was not available.

Table 2
Insecticides Applied in the Province of Tumbes (1957-1998)

PERIOD	INSECTICIDE APPLIED
1957 - 1960	DDT
1980 - 1992	DDT 75% PM
1993	DDT Fenitrothion Cyfluthrin Chlorpyrifos
1994	Fenitrothion
1995	Fenitrothion Cyfluthrin
1996	Fenitrothion Cyfluthrin
1997	Cyfluthrin Cyfluthrin Alphacypermethrin
1998	Cyfluthrin Alphacypermethrin Cypermethrin L-cyhalothrin

2 MATERIALS AND METHODS

2.1 Areas of Study

In October 1998, an assessment was conducted of *Anopheles albimanus* mosquitoes in a number of districts in the northern Peruvian provinces of Piura and Tumbes. The selection of sites was based on the following parameters: existing insecticidal pressure, malaria transmission, and the existence of a high density of *An. albimanus*. As a result of the low density of mosquitoes in many areas, including areas characterized as being good collection sites, it was necessary to intensify the search to identify localities that would ensure a sufficient number of adult mosquitoes to properly conduct the insecticide resistance tests. Thus, an assessment was conducted of strains of *An. albimanus* at sites in the departments of Piura and Tumbes.

In the Department of Piura, mosquitoes were assessed in Salitral, located in the District of Salitral, and Somate, located in the District of Bella Vista, both in the Province of Sullana, and in Monterredondo, located in the District of La Union in the Province of Piura.

In the Department of Tumbes, located in the province of the same name, mosquitoes were assessed in San Isidro, in the District of Corrales, and San José and Puerto Pizarro, both located in the District of Tumbes.

2.2 Bioassays to Determine Resistance to Insecticides

In performing the bioassays, WHO standards for adult mosquitoes were used. Insecticides were assessed for resistance using filter papers

A control group was constructed using two additional replications, in which the mosquitoes

impregnated with technical-grade products at WHO-recognized diagnostic concentrations (%).

The technical-grade insecticides were provided by their manufacturers. The papers used were impregnated using the WHO technique. Selection of insecticides was based on the need to assess mosquito resistance to each of the four classes of insecticides.

Mosquitoes were collected at each site and the females were exposed to papers impregnated with insecticides at the preliminary diagnostic doses recommended by WHO. The diagnostic dose was defined as twice the concentration required to kill 99.99% of the exposed population of insects. It should be pointed out that, according to the latest WHO report (September 1998) on discriminative concentrations of insecticides for adult anopheline mosquitoes, diagnostic doses of 0.15% and 0.05% were established for cyfluthrin and l-cyhalothrin, respectively. In this study, both pyrethroids were assessed at a diagnostic dose of 0.1%, meaning that the l-cyhalothrin was assessed at a higher concentration, while cyfluthrin, at a concentration of 0.1%, was assessed at a dose slightly less than that more recently recommended by WHO. Each of the insecticides was evaluated by means of four replications, each of those were conducted with 25 mosquitoes. The insects were exposed to the paper, which had been placed in clear plastic exposure tubes (WHO kits) for a period of 60 minutes. Subsequently, a reading was taken of all insects Aknocked down,@ or affected, after which the mosquitoes were transferred to holding tubes containing cotton swabs soaked in a 10% glucose solution. AMortality@ was recorded after a period of 24 hours.

were exposed to paper not treated with insecticide. In each test, the

Table 3
Insecticides Used in Vector Susceptibility Trials

Insecticide	Concentration	Date of Impregnation	Source*

Organochlorine DDT	(4%)	08-29-98	1
Organophosphorus Fenitrothion	(1%)	08-28-98	1
Carbamate Propoxur	(0.1%)	08-28-98	1
Pyrethroid Cyfluthrin	(0.1%)	05-29-98	1
Pyrethroid L-cyhalothrin	(0.1%)	05-05-98	2

* 1 = Insecticide Evaluation Laboratory of the Research Division, School of Malariology and Environmental Sanitation, Venezuelan Ministry of Health and Social Welfare.
2 = World Health Organization.

environmental variables of temperature and relative humidity were recorded using a portable thermohygrometer.

Each test was repeated three times on consecutive days to verify the reproducibility of the results. This was done in locations where sufficient numbers of mosquitoes could be collected for testing. Analysis of the results was based on insecticide resistance criteria developed by Davidson and Zahar (1973) and WHO (1993). The reporting of vector response to insecticides used the following criteria and terminology:

- Mortality 99%-100%: vector is *susceptible*.
- Mortality 80%-97%: vector is *under surveillance* regarding resistance.
- Mortality < 80%: vector is *resistant*.

3 RESULTS

Results in the tables that follow are expressed as the percentage of insects knocked down as a rapid effect and percent of mortality observed 24 hours subsequent to exposure of the mosquitoes for a period of 60 minutes to the diagnostic dose recommended by WHO.

The results displayed in Table 4 show that mortality rates for *An. albimanus* in Monterredondo in the District of La Union, Province of Piura, recorded with regard to the organochlorine insecticide DDT and the pyrethroid cyfluthrin ranged between 86.1% and 93.5%. Based on these results, this strain is classified as being under surveillance for these insecticides. For the pyrethroid l-cyhalothrin, this strain reflected a mortality rate of 61.4%, thus giving evidence of resistance. With regard to the organophosphorus insecticide fenitrothion and the carbamate propoxur, the strain of *An. albimanus* evaluated was susceptible to both, with recorded mortality rates ranging between 99 and 100%. It was possible to observe the rapid Aknockdown@ effect that is characteristic of pyrethoid insecticides, followed by the recovery of a percentage of the insects after a period of 24 hours. With the insecticide propoxur, it was possible to observe the knockdown effect; only a single insect recovered after a period of 24 hours. The control group showed zero mortality.

Table 4
Resistance of Adult *Anopheles albimanus* to Insecticides in Monterredono, District of La Union, Province of Piura

Insecticides (%)	No. of Insects Exposed	Rapid Effect (% Insects Knocked Down)	24-Hour Mortality (%)
DDT (4)	296	68.58	93.5
Fenitrothion (1)	299	12.70	100
Propoxur (0.1)	299	99.6	99.3
Cyfluthrin (0.1)	299	95.6	86.1
L-cyhalothrin (0.1)	342	77.7	61.4
Control (no insecticide)	250	0	0

Table 5
Resistance of Adult *Anopheles albimanus* to Insecticides in Maran, Somate Sector, District of Bella Vista, Province of Sullana

Insecticides (%)	No. of Insects Exposed	Rapid Effect (% Insects Knocked Down)	24-Hour Mortality (%)
DDT (4)	100	68	95.5
Fenitrothion (1)	100	11	100
Propoxur (0.1)	100	100	100
Cyfluthrin (0.1)	112	98.2	77.6
L-cyhalothrin (0.1)	140	95.7	76.4
Control (no insecticide)	100	0	0

Average temperature: 26.5 EC
Average relative humidity: 70%

The results obtained in Maran are not conclusive in terms of detecting resistance, as it was possible to run only one test. However, the *An. albimanus* mosquitoes in Maran, Somate sector, showed a mortality rate of 95.5% for the organochlorine insecticide DDT. For the pyrethroids cyfluthrin and l-cyhalothrin, this strain showed mortality rates ranging between 77.6 and 76.4%, respectively. With regard to the organophosphorus insecticide fenitrothion and the carbamate propoxur, it was found that the strain of *An. albimanus* evaluated was susceptible to both insecticides, recording mortality rates of 100%. With regard to the pyrethroid insecticides, it was possible to observe the rapid knockdown effect characteristic of this group, with the subsequent recovery of a percentage of insects after a period of 24 hours. With the insecticide propoxur, it was also possible to observe the knockdown effect in 100% of the insects exposed, with no recuperation of insects after a period of 24 hours. The control group showed zero mortality.

Table 6
Resistance of Adult *Anopheles albimanus* to Insecticides in Salitral, District of Salitral, Province of Sullana

Insecticides (%)	No. of Insects Exposed	Rapid Effect (% Insects Knocked Down)	24-Hour Mortality (%)
DDT (4)	44	45.5	81.8
Fenitrothion (1)	100	0	100
Cyfluthrin (0.1)	159	89.3	89.3
Control (no insecticide)	215	0	0

Average temperature: 21.5 EC
Average relative humidity: 70%

The results obtained in Salitral are not conclusive in terms of determining resistance in *An. albimanus*, since it was not possible to repeat the tests due to low density of the vector in the sampling areas. The 44 mosquitoes evaluated with the organochlorine insecticide DDT, however, had a mortality rate of 81.8%, while the 159 mosquitoes tested for pyrethroid insecticide showed a mortality rate of 89.3%. With regard to the organophosphorus insecticide fenitrothion, the strain of *An. albimanus* evaluated had a mortality of 100%. With the pyrethroid insecticide, it was possible to observe the rapid knockdown effect characteristic of this group, with no recovery of insects observed after a period of 24 hours. The control group showed zero mortality.

Table 7
Resistance of Adult *Anopheles albimanus* to Insecticides in San Isidro, District of Corrales, Province of Tumbes

Insecticides (%)	No. of Insects Exposed	Rapid Effect (% Insects Knocked Down)	24-Hour Mortality (%)
DDT (4)	282	68.4	76.9
Fenitrothion (1)	297	44.1	100
Propoxur (0.1)	294	99	99
Cyfluthrin (0.1)	368	65.2	38.5
L-cyhalothrin (0.1)	456	36.1	25.4
Control (no insecticide)	400	0	0

Average temperature: 26.3 EC
Average relative humidity: 69%

The results presented in Table 7 show that *An. albimanus* in San Isidro recorded a mortality rate of 76.9% for the organochlorine insecticide DDT, meaning that this strain is classified as being under surveillance for this insecticide. Resistance was detected to both the pyrethroid insecticides cyfluthrin and l-cyhalothrin, with mortality rates ranging between 38.5% and 25.4%, respectively. The strain of *An. albimanus* evaluated was susceptible to the organophosphorus insecticide fenitrothion and the carbamate propoxur, with recorded mortality rates ranging between 99 and 100%. With the pyrethroid insecticides, the knockdown effect characteristic of this group was considerably reduced, with the subsequent recovery of a percentage of insects after a period of 24 hours. With propoxur, the knockdown effect was evident in 99% of the insects exposed, with 0% recovery after a period of 24 hours. The control group showed zero mortality.

Table 8
Resistance of Adult *Anopheles albimanus* to Insecticides in Puerto Pizarro, Province of Tumbes

Insecticides (%)	No. of Insects Exposed	Rapid Effect (% Insects Knocked Down)	24-Hour Mortality (%)
Cyfluthrin (0.1)	100	54	29
L-cyhalothrin (0.1)	84	29.7	15.4
Control (no insecticide)	50	0	0

Average temperature: 26 EC
Average relative humidity: 70%

Table 9
Resistance of Adult *Anopheles albimanus* to Insecticides in San José, District of Tumbes, Province of Tumbes

Insecticides (%)	No. of Insects Exposed	Rapid Effect (% Insects Knocked Down)	24-Hour Mortality (%)
Fenitrothion (1)	50	70	100
Cyfluthrin (0.1)	94	52.1	53.19
L-cyhalothrin (0.1)	62	35.4	30.6
Control (no insecticide)	50	0	0

Average temperature: 26 EC
Average relative humidity: 70%

The findings shown in Table 8 are the result of a preliminary exploration conducted in Puerto Pizarro; they are not conclusive, since they were not repeated. However, they show low mortality rates for the pyrethroids cyfluthrin and l-cyhalothrin. The control group showed zero mortality.

The findings shown in Table 9 are the result of a preliminary exploration conducted in San José. They are not conclusive, since they were not repeated and the number of mosquitoes evaluated per treatment was less than 100. However, they show low mortality rates for the pyrethroids cyfluthrin and l-cyhalothrin (53.2 and 30.6%, respectively), while the mortality rate for fenitrothion was 100%. The control group showed zero mortality.

4 DISCUSSION

As indicated in Tables 4 through 9, the response shown by the various strains of *An. albimanus* in the provinces of Piura and Tumbes with regard to resistance to insecticides was quite similar. All strains showed susceptibility to the organophosphorus insecticide fenitrothion and to the carbamate propoxur and tolerance to the organochlorine insecticide DDT; accordingly, these strains were classified as Aunder surveillance@ and Aresistant@ to the latter insecticide, and as Aresistant@ and Aunder surveillance@ to the pyrethroids cyfluthrin and l-cyhalothrin.

The results obtained might be explained by the fact that the study areas have a history of malaria infestation, having been subject in the past to strong pressure from organochlorine insecticides, specifically DDT, an insecticide to which *An. albimanus* demonstrated vulnerability.

Available information indicates that health authorities in the area of Tumbes have applied DDT by means of intradomicilliary spraying to control malaria, the most recent intervention occurring in 1993 with an initial application of organophosphorus insecticides and pyrethroids. The latter have been applied continuously since that time.

Taking into account the fact that the primary economic activity in the areas evaluated involves intensive monocultural agriculture, including musaceous (banana family) and gramineous (grass family) crops and to a lesser extent livestock production, it is assumed that this region is under strong insecticide pressure throughout the entire year from compounds applied for purposes of

In this study, it was also observed that resistance to pyrethroids was accompanied by an increased susceptibility to the organophosphorus insecticide fenitrothion that is consistent with the findings of a number of authors who have observed in insect populations an increased susceptibility to organophosphorus insecticides as a result of selection with pyrethroids and vice versa (Chapman and Penman 1979).

chemically controlling agricultural pests. During the past five years a significant amount of pyrethroid insecticides has been applied in this area for agricultural purposes and use of these products is currently on the increase as they provide a broad range of toxic activity, which for agricultural producers could represent a significant economic advantage. Thus, it can be concluded that the ongoing use of insecticides in agricultural production activities in the study areas has contributed to the development of resistance in *An. albimanus* and perhaps in other species as well which, together with *An. albimanus,* are constituent elements of a single ecosystem. Consequently, a large percentage of each generation of mosquitoes is frequently exposed to insecticides, with the potential for a significant degree of gene selection favoring resistance.

The tolerance of DDT and resistance to the pyrethroid insecticides observed in this study constitute a warning sign as to the possible presence of cross-resistance between organochlorine and pyrethroid insecticides. This is conferred by the small Kdr gene, since the Kdr factor (resistance to knockdown) in pyrethroids is responsible for resistance to these compounds in insects (Farnham 1973; De Vries and Georghiou 1981). Studies have shown that the application of pyrethroids in areas having a history of DDT application can lead to the rapid development of pyrethroid resistance. This has occurred in the case of DDT applications, which may have increased the percentage of individuals with the Kdr factor.

That said, once resistance has been detected in *An. albimanus*, the most appropriate action would be to establish a program for managing resistance to insecticides in that particular population with a view toward ensuring close surveillance, since such resistance apparently occurs in a very focalized manner and is manageable with an appropriate control strategy.

This would make it possible to take steps to implement chemical measures for purposes of controlling this species. However, it would be advisable to contact agricultural authorities with regard to the development of joint control programs, since agricultural producers also apply pressure through their use of insecticides and are no doubt unaware of the problem they are creating in insects such as *An. albimanus.* Thus, if pyrethroids continue to be applied during the coming years, in the absence of proper precautionary measures, resistance to these products could reach a level where the pyrethroids themselves would lose their operational efficiency with regard to vectors of disease in humans, including *An. albimanus.* Similar results have been reported for anopheline species in Venezuela, such as *An. albimanus* and *An. aquasalis* (Saume and Molina 1997).

The results show that the phenomenon of resistance in *An. albimanus* in Tumbes and Piura is not comparable to observations made in certain areas of Central America, such as Guatemala, where the species was reported as being resistant to malathion. Breeland et al. (1970) found that resistance to malathion occurred in areas of El Salvador where organophosphorus insecticides had been used intensively to control cotton pests. A 1971 report (Ariaratnam and Georghiou) provided conclusive evidence of resistance to organophosphorus insecticides and carbamates in *An. albimanus* in El Salvador.

A subsequent report described the emergence of resistance to organophosphorus insecticides and carbamates in a number of areas of Central America. Georghiou (1982) described the role of agricultural insecticides in the development of resistance as a result of a number of operating factors, since there was evidence based, among other things, on the appearance of resistance in mosquitoes prior to the application of chemicals for controlling vector insects and increased resistance of mosquitoes in agricultural areas as compared to those found in nonagricultural areas.

The above-cited evidence contributes to a better understanding of the phenomenon of resistance in *An. albimanus,* which must be taken into account when developing vector control strategies.

5 CONCLUSIONS

The study leads to a preliminary diagnosis of resistance to insecticides in *An. albimanus* in a number of villages and provinces to the north of Piura.

The best assessments were carried out in Monterredondo (Piura) and San Isidro (Tumbes), as an adequate number of mosquitoes were available to conduct the study.

In Monterredondo, the study detected incipient resistance to the pyrethroid cyfluthrin and DDT, as well as susceptibility to fenitrothion and propoxur.

In San Isidro (Tumbes), this study detected resistance to DDT and pyrethroids, while susceptibility to fenitrothion and propoxur still exist. With regard to the strains found in the other areas evaluated, the results yielded only preliminary information. They were not sufficiently conclusive to make a diagnosis of specific insecticide resistance.

Temperature and relative humidity data recorded during the performance of the bioassays were adequate.

Based on the limited amount of time available to evaluate the various strains, it was not possible to conduct additional laboratory or field bioassays, both of which are essential for confirming resistance detected in the first stage of the evaluation.

It is assumed that insecticide pressure applied by farmers in controlling pests affecting their crops is influencing the resistance detected in *An. albimanus*, as this species is a known inhabitant of agricultural ecosystems.

6 RECOMMENDATIONS

Based on the findings from this study, the following recommendations are made.

- Strategies should be established to control *An. albimanus* in the study areas with a view toward preventing resistance to DDT and pyrethroids from becoming a problem in the future. Accordingly, either organophosphorus insecticides or the carbamate propoxur could be used as alternatives.
- Work should continue in verifying the resistance to insecticides detected in the strains of *An. albimanus* found in San Pedro in the Province of Tumbes and in Monterredondo in the Province of Piura through laboratory and field bioassays.
- A program should be put in place immediately to monitor resistance to insecticides, with appropriate activities to be included among the routine activities performed by entomology reference laboratories, whereby the primary vectors of malaria would be evaluated, with priority attention to be given to studies to be conducted in areas showing the greatest endemicity.
- A national laboratory, staffed with trained personnel, should be established to conduct biological evaluations of insecticides and provide the technical information required for selection of insecticides to be used for controlling vectors that threaten public health. Such a central laboratory should establish guidelines for reference laboratories to conduct related studies and, in the future, to undertake research into possible mechanisms of resistance to insecticides.
- Using valid evaluation criteria, steps should be take to rationalize the selection of insecticides used in public health campaigns to control vectors. In order to improve control and to conserve alternative insecticide compounds, an insecticide should not be used unless it has been evaluated on native species.
- There needs to be increased supervision of vector control activities involving insecticides as a chemical tool.
- The work currently being undertaken in reference laboratories with regard to the ecology, biology, and behavior of pest species should continue, to ensure the effectiveness of control measures.

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