



PRELIMINARY NOTE ON THE EPIDEMIOLOGICAL STUDIES  
MADE IN EL SALVADOR TO DETERMINE THE CAUSES OF THE FAILURE OF  
RESIDUAL SPRAYING TO INTERRUPT THE TRANSMISSION OF MALARIA<sup>1</sup>

by

René G. Rachou (Epidemiologist-in-Charge)

Lewis A. Schinazi (Parasitologist)

and

Milton Moura-Lima (Entomologist)

Epidemiological Field Study Team, Malaria Eradication Branch,  
Pan American Health Organization/Regional Office for the Americas,  
World Health Organization

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1. General considerations

In July 1956, the Republic of El Salvador converted its extensive programme of malaria control to one of malaria eradication, using residual insecticides as the chief weapon of attack. Initially, the houses in the malarious part of the country were treated with dieldrin at intervals of 12 months, and later with DDT at intervals of six months.

In 1958, it was observed at a number of places that Anopheles albimanus, the vector of malaria in El Salvador, had developed resistance to dieldrin. The use of dieldrin was discontinued and beginning in August 1958 DDT was the only insecticide used in the malaria eradication campaign. Shortly after, it was demonstrated that A. albimanus had also developed resistance to DDT in several localities. However, for lack of any better insecticide the use of DDT was continued. It was soon established that there was a significant degree of correlation between the areas with insecticide-resistant A. albimanus and areas of cotton cultivation. The insect pests of the cotton are attacked by the use of very large quantities of chlorinated hydro-carbon and organophosphorus insecticides in great diversity, applied by aircraft during five or six months of the year.

The transmission of malaria had been greatly reduced in large areas of the country, but it was still relatively high for a programme of eradication, as shown by the malaria prevalence rates of 5.8, 9.3 and 5.3 per 1000 of the population in the malarious area, in the years 1958, 1959 and 1960, respectively. While the reduction in the total number of cases of malaria due to P. falciparum, and of the percentage of blood smears positive for that species in the years 1958 to 1960 indicated some improvement, the transmission of malaria persisted, not only in areas in which the vector was insecticide-resistant but also in areas in which it was still DDT-susceptible. This is shown in Table 1.

TABLE 1. PARASITOLOGICAL DATA FOR THE YEARS 1958 to 1960,  
AS REPORTED BY CNAP IN MAY 1961

Details	1958	1959	1960
Number of blood smears examined	56 095	71 295	76 287
Number of positive smears -			
Total	10 925	17 521	10 066
<u>Plasmodium vivax</u> only	5 854	13 470	7 107*
<u>P. falciparum</u> alone or mixed	5 071	4 051	2 959
Percentage of positive smears -			
Total	19.4%	24.6%	13.1%
<u>Plasmodium vivax</u> only	10.4%	18.9%	9.3%
<u>P. falciparum</u> alone or mixed	9.0%	5.7%	3.8%
Observed malaria prevalence, per 1000 inhabitants in the malarious area, based on positive blood smears	5.8	9.3	5.3

\* Includes one P. malariae infection

In January 1961, the Government of El Salvador and the Pan American Health Organization (PAHO), Regional Office of the World Health Organization (WHO), signed an agreement to undertake long-term studies in El Salvador with the cooperation of the Campaña Nacional Antipalúdica (CNAP) with the objective of elucidating and overcoming the obstacles which were impeding the success of the malaria eradication campaign in El Salvador. These studies were to be undertaken in two representative localities, one with DDT-resistant A. albimanus, and the other with DDT-susceptible A. albimanus during a period of one year.

Though the cost of these studies was borne almost entirely by PASB, they would not have been possible without the invaluable collaboration and assistance of Dr Carlos Diaz del Pinal, Director of CNAP, and of its professional and subordinate staff.

Within the broad objective "determination of the causes of persistence of transmission of malaria", the study comprised the following main activities:

- (a) to ascertain the local epidemiological factors of major importance;
- (b) to identify and evaluate the causes of persistence of transmission;
- (c) to develop the working methods required for the foregoing;
- (d) to train senior professional personnel in the use of these methods and in the interpretation of the results obtained.

The two representative localities, situated in the western part of the coastal plain of the country are: ATALAYA (Canton El Coyol, Municipio Acajutla, Department of Sonsonate) with 451 inhabitants initially; and FALLA (Canton Falla, Municipio Jujutal, Department of Ahuachapan) with 627 inhabitants initially. In Atalaya A. albimanus were DDT-resistant, and in Falla they were DDT-susceptible.

The houses in both localities had been sprayed with dieldrin in 1957 and 1958, and with DDT at a target dose of 2 grams/m<sup>2</sup> twice a year since 1959.

The prevalence of malaria in 1960 - as revealed by passive case detection operation - in the two cantons in which the localities are located was as follows: El Coyol - 62 cases per 1000; and Falla - 122 per 1000. The vivax/falciparum ratio was 2.8:1 in El Coyol and 1.3:1 in Falla.

The higher prevalence of malaria in Falla (with DDT-susceptible A. albimanus) than in Atalaya (with DDT-resistant A. albimanus) was suspected as being due to the more primitive type of house construction that predominated in Falla, and to the fact that the construction of the splendid new coastal highway had required the removal and rebuilding of a number of houses, and had brought a large number of temporary residents into the community.

Both localities are situated at approximately 13°40' North latitude and at 90° West longitude and at less than 50 metres above sea level. Atalaya is on the coast and Falla is about 25 km to the west and 5 km inland. In Atalaya, cotton has been cultivated since 1950, and in Falla, the first planting dates from 1961.

At the seaport of Acajutla, near Atalaya, the mean annual rainfall is 1722 mm with a recorded maximum of 2347 mm and a minimum of 1243 mm. The rainy season is from May to October with a mean maximum of 321 mm in September and a mean minimum of 1 mm in January and February. The mean annual temperature is 26.8°C with a maximum of 28°C in May and a minimum of 25.6° in January. Thus the climate is of a true "monsoon" type.

The detailed studies were carried out in Atalaya from April 1961 to April 1962, and in Falla from July 1961 to July 1962. Some special studies were continued through September 1962.

This preliminary report contains a brief description of the most important aspects of the study and some tentative conclusions based upon the still unfinished analysis of the large amount of field data that were collected.

## 2. The plan of study

2.1 Survey of inhabitants. An initial survey was made at the beginning of the study of the entire population, followed by a number of periodic surveys, some of which involved only a third of the population.

These surveys were done house to house and comprised: a census of the population; the collection of blood smears and personal interviews to obtain information relative to the malaria history of each inhabitant; the investigation of the place of origin of the human population, its habits in relation to the biting activity of A. albimanus; and the study of the movement of the human population.

2.2 Survey of habitations. An initial survey was made of all of the habitations, followed by periodic surveys during the study year. The objectives of the surveys were: to identify and locate every house in each area; to evaluate the insecticide spraying operations; to collect data that would make it possible to correlate the incidence of malaria and the type of house; measure the amount of alterations of DDT-sprayed surfaces, and of new construction between DDT sprayings. The initial survey involved the preparation of an accurate sketch map of the locality showing the location of each house, of all animal shelters, of potential mosquito-

breeding places, of fields under cultivation or in pasture, and of the principal topographic features; interviews with heads of households; the sketching of the floor plan and the outside walls of each house; and (most laborious of all) the measurement and description of all inside wall and partition surfaces.

2.3 Entomological studies. These were to cover the whole 12 months in order to obtain adequate knowledge of the local anopheline fauna, of the epidemiological importance of the species encountered, and of the behaviour of the vector (or vectors) with respect to the residues of insecticide on the walls of the houses. One group of observation was designated "routine" and another "special". These were to be executed in alternate weeks, the routine studies to be carried out for 12 months and the special studies to be done only as indicated.

The objective of the routine entomological studies was to measure the seasonal variation of the abundance of the vector species (which proved to be only A. albimanus) and to obtain information regarding other phenomena such as endophily, anthropophily, infectivity, longevity, DDT-susceptibility and behaviour with respect to DDT sprayed surfaces. The following procedures were used: houses captures, morning and night, on interior surfaces; captures with human bait, done simultaneously inside houses and just outside them, in the early hours of the night; captures in stables and corrals in the early hours of the night and in the morning; observations on the survival and state of feeding of the anophelines captured on the interior surfaces of the houses; dissection of the ovaries and ovarioles of anophelines; dissection of the salivary glands; and susceptibility tests to DDT at monthly intervals.

The special entomological studies were directed toward obtaining full understanding of the behaviour of the vector(s) with respect to the insecticide residues in houses and identifying the epidemiological factors of major importance to the persisting transmission of malaria. The following procedures were used: various types of captures of adult mosquitos (intra-, peri- and extradomiciliary) with human bait; searching for larvae; determination of the gonotrophic cycle of A. albimanus and of the sporogonic cycle of the plasmodial species in A. albimanus.

Among the special entomological techniques that were devised in the course of the study, and which contributed importantly to the understanding of the problem were the following: (a) night captures of anophelines resting on interior wall surfaces of houses with simultaneous captures of the anophelines which bit the people inside the house and just outside of it; (b) adult captures on inside wall surfaces, continuously or at intervals 15, 30, 60 or 120 minutes with human bait available - counting the anophelines which bit but allowing them to feed to repletion; (c) liberation of freshly engorged anophelines in sprayed and unsprayed houses which were provided with window traps; (d) captures in a pair of collapsible trap-houses - one with and one without DDT - with window traps; (e) captures in local houses, with and without DDT, in which window traps had been constructed; (f) tests of excito-repellency, using the OPS Test Box.

2.4 Malaria case detection. To determine the prevalence of malaria during the year and to ascertain variations in amount of malaria transmission, active case detection and passive case detection were used, in addition to "parasitemia surveys", done as an integral part of the surveys of inhabitants.

The active case detection consisted of the search for cases that was done by the personnel of the study who, in the course of their weekly visits to the houses, made blood smears from any resident who said he had fever or, had had it in the interval between visits.

The passive case detection comprised the work done at the "notification posts", each in charge of a lay voluntary collaborator, who made a blood smear from all persons who came to them to receive free antimalaria medication.

2.5 Investigation of malaria cases. All confirmed cases of malaria, discovered by whatever means, were investigated so that they could be classified as to: the stage of the infection; the date of the primary attack; and the probable place of infection. This classification was made on the basis of information obtained from the patient, or his parent, sometimes aided by data from files of CNAP, or as the study progressed, from its own files.

### 3. Results and discussion

3.1 Plasmodium vivax and P. falciparum were encountered in both localities with a vivax:falciparum ratio of 3.2:1 in the total number of positive blood slides examined. In Atalaya, the ratio was 1.7:1 and in Falla 3.6:1.

3.2 In the study period of 15 months the prevalence of malaria in Falla, with DDT-susceptible vector, was considerably higher than in Atalaya, with the DDT-resistant vector. In Falla, 3551 blood slides were collected, of which 527 (14.8%) were positive, 412 (78.2%) for vivax and 115 (21.8%) for falciparum. In Atalaya, 2036 samples were taken of which 126 (6.2%) were positive, 80 (63.4%) for vivax and 46 (36.6%) for falciparum.

3.3 In both localities the lowest percentage of positives was found in the samples collected in "parasitemia surveys" - 4.9% in Falla and 2.7% in Atalaya. The highest percentage of positives in Falla was obtained by the active case detection (30.6%), and in Atalaya by the passive case detection of its "notification post" (24.1%).

3.4 Although transmission of malaria occurred during the entire year, the period of intense transmission was very short, July and August being the months of greatest transmission. The application of insecticides by airplanes to the cotton fields that began in August, in the middle of the rainy season, markedly reduced the anopheline density and the number of new infections and shortened the period of high transmission. In 1961 this period would, probably, have lasted for several months in the absence of such spraying. In 1962, in Falla, the largest number of cases of malaria occurred in September, rather than in July. A delay in the onset of the rains and a modification of the application of insecticides to the cotton fields could explain this occurrence. Data of the prevalence of malaria in the two localities are shown in Tables 2 and 3.

3.5 The intensive and continuous active search for cases made by the entomological aides during routine house captures, as well as the notification of cases by the voluntary collaborators, afforded an excellent opportunity for qualitative and quantitative observations of various aspects of malaria. One of the most interesting observations was the frequency with which a person had repeated episodes

TABLE 2. DISTRIBUTION OF MALARIA CASES DISCOVERED BY ACTIVE AND PASSIVE CASE DETECTION BY MONTH IN WHICH THE BLOOD WAS FOUND POSITIVE

Year and month	ATALAYA				FALLA			
	Number of smears	Number of positive smears			Number of smears	Number of positive smears		
		Total	<u>P. vivax</u>	<u>P. falciparum</u>		Total	<u>P. vivax</u>	<u>P. falciparum</u>
1961 May	38	4	4	0	0			
June	37	4	3	1	0			
July	41	8	5	3	61	22	14	8
August	52	19	10	9	134	63	44	19
September	31	7	4	3	120	28	20	8
October	33	7	5	2	82	21	18	3
November	25	3	2	1	44	11	11	0
December	22	2	0	2	36	6	6	0
1962 January	47	4	4	0	108	18	18	0
February	15	4	4	0	28	8	8	0
March	17	4	4	0	29	6	6	0
April	12	2	2	0	34	10	10	0
May	22	1	1	0	64	14	14	0
June	25	2	2	0	77	15	15	0
July	56	7	6	1	199	57	46	11
August	58	15	14	1	241	130	99	31
September	23	6	5	1	151	81	47	34

TABLE 3. PARASITEMIA SURVEYS IN ATALAYA AND FALLA

Year and month	Number of smears examined	Percentage of positive smears	Number of positive smears		
			Total	Vivax	Falciparum
<u>ATALAYA</u>					
1961 - April-June	413	4.6	19	12	7
October*	137	3.6	5	5	0
November*	131	0.8	1	1	0
December*	131	0.8	1	0	1
1962 - March	398	2.0	8	6	2
June	406	2.5	10	7	3
<u>FALLA</u>					
1961 - July-August	607	10.7	65	41	24
October*	206	3.9	8	4	4
November*	180	2.2	4	3	1
December*	191	1.5	2	2	0
1962. - March	517	3.5	18	16	2
June	593	2.7	16	15	1

\* Random sample of one third of the population.

of patent parasitemia, generally accompanied by clinical manifestations, although, at the time of collection of each blood smear, a presumptive treatment with chloroquine (600 mg of base for an adult) was administered. In Atalaya three of 80 cases each had four separate attacks, and in Falla 36 of 309 cases had three or more such episodes, almost all being P. vivax infections. Among these 36 cases, 17 had three attacks, nine had four and 10 had five or more, during the 15 months of observation. These attacks occurred at intervals which varied from 18 days to more than one year.

3.6 The minimum duration of the sporogonic cycle of P. vivax and P. falciparum, in A. albimanus, were determined to be eight and 12 days, respectively.

3.7 Marked stability characterized the population of the localities during the study period. This is in sharp contrast to the situation in the eastern part of the coastal plain of El Salvador, where pronounced seasonal fluctuations of the population are the rule. In Atalaya the number of inhabitants encountered on six different censuses was 451, 397, 415, 396, 443 and 437. In Falla, in five censuses, the numbers were 627, 657, 622, 599 and 645.

3.8 In the initial survey of the inhabitants of Atalaya, 73.9% of the residents claimed residence of more than one year in the locality, and 54.0% in the house in which they were censused; in Falla the corresponding figures were 69.9% and 59.2%. During the period of study, in both localities, it was observed rather frequently that a family moved from one house to another in the same locality; in Falla, as a consequence of a new arrangement for the cultivation of the land, several families had to move to another part of the same locality, and build new houses, there, often using construction materials from their previous houses.

3.9 Among the habits of the people related to anopheline activity, the most significant was that of their presence outside of their houses during the early hours of the night, where on the porch or veranda or other unprotected environs of the house the majority of the people are highly exposed to the bite of the anopheline vectors.

3.10 The great majority of the residents of both localities rarely spent the night away from their localities. Nevertheless, within each locality during the early hours of the night, there is considerable movement of the inhabitants who visit each other, usually staying outside of their houses. In view of the known exophily and exophagy of A. albimanus, such visiting must contribute to the dissemination of courses of infection within the locality.

3.11 In the localities studied (more so in Falla than in Atalaya) there was a predominance of houses of a primitive type with many openings and discontinuities in the outer walls. In the initial survey of houses in Atalaya the average percentage of openings in the walls was found to be 20%, and in Falla 45%. Stated in another way, the average house in Atalaya can be said to lack almost one entire wall, and in Falla almost two.

3.12 In Atalaya and Falla 57% and 18%, respectively, of the houses had verandas or porches, with roofs, where the family commonly spends the major part of its time, when "at home" either cooking, eating, resting or visiting.

3.13 The initial survey of houses - done in Atalaya and in Falla four to five months after the last DDT spraying - revealed, in Atalaya 4% of the houses not sprayed and Falla 27% - because they had been constructed after the spraying round. The total of houses not sprayed, for whatever reason was, respectively to 6% and 28%. During the period of study, a very small number of new houses was built in Atalaya but in Falla, for reasons stated above, this number was large, principally in the first semester of 1962.

Some data from the initial survey of houses in Atalaya and Falla are tabulated below.

<u>Details</u>	<u>Atalaya</u>		<u>Falla</u>	
Number of houses studied, including "annexes"	99		146	
Number of new houses constructed since the last spraying - and percentage	4	4%	39	27%
Total number of houses unsprayed at the time of the survey - and percentage	6	6%	41	28%
Total number of inhabitants in the houses studied (at the time of the initial survey of houses)	430		582	
Average number of inhabitants per occupied house	4.6		5.1	
Total area of outside wall surfaces	4 539 m <sup>2</sup>		5 625 m <sup>2</sup>	

<u>Details</u>	<u>Atalaya</u>	<u>Falla</u>
Open area of outside wall surfaces	833 m <sup>2</sup>	2 550 m <sup>2</sup>
Ratio of open area to total area of outside wall surfaces	20%	45%
Total sprayable inside wall surface area	7 820 m <sup>2</sup>	10 148 m <sup>2</sup>
Total sprayed inside wall surface area	7 057 m <sup>2</sup>	6 696 m <sup>2</sup>
Ratio of sprayed to sprayable inside wall surface	90%	66%

3.14 Only three species of Anopheles were found in the localities studied: Anopheles (Nyssorhynchus) albimanus, A. (Anopheles) pseudopunctipennis, and A. (Arribalzagia) punctimacula, with the first by far the most abundant. In Atalaya, for example, A. albimanus comprised 98.8% of the total of 33 859 specimens of anophelines taken during the routine captures during the entire year of study; A. pseudopunctipennis followed with 1.0% and A. punctimacula with 0.2%. Similar distribution was found in Falla.

3.15 The only species found with sporozoites was A. albimanus, with two specimens positive among the several thousand dissected. One positive was taken in Falla, and the other at Hacienda Catarina, a place contiguous to Falla where occasional mosquito captures were made. The positive from Falla was captured with human bait outside of a house in the early hours of the night; the other resting on the inside wall surface of an unsprayed house during the morning.

3.16 From the observations of the behaviour of the three species, it appears that A. albimanus was the only one which could be epidemiologically incriminated as responsible for the transmission of malaria, in both localities, even though it did not reveal marked endophily or anthropophily. It entered the human habitations in large numbers and at all hours of the night, but it did the same in animal shelters, principally stables, where they were, as a rule, encountered in greater density than in the houses, even when the latter were not sprayed with DDT. It attacked man, during the entire night, inside houses as well as just

outside on the veranda, in the outside kitchen or even in the open air; but it also attacked the cattle, both in the stables and in the corrals under the open sky. Its density could be described as high during the entire year, especially so during the rainy season. (The high density observed in the dry season was due to the irrigation of pastures and forage crops where ideal breeding places developed.) The application of insecticides by aircraft to the cotton fields caused a considerable reduction of the anopheline density in the first three months of the crop spraying period. This was followed, however, by a rise during the last three months, in spite of continued spraying of the crops.

In the dry season, the longevity of A. albimanus appeared to be relatively short, since specimens with evidence of more than two ovipositions were rarely encountered. In the rainy season, specimens with a greater number of ovipositions were captured with greater frequency - eight being the largest number of ovipositions counted in one individual. The two specimens which were sporozoite-positive showed five ovipositions.

The low density of A. pseudopunctipennis and of A. punctimacula did not permit significant observations with respect to their behaviour, but they appear to be species with zoophilic and exophilic tendencies, and the former, when it enters houses, possibly does so more in search of shelter than of human blood.

3.17 In spite of the fact that A. albimanus does attack man in great numbers inside houses - about 1800 specimens were once counted while biting two men inside a house during three hours of exposure in the early hours of one night - the number of A. albimanus found resting on interior wall surfaces, either at night or in the morning, was always very small. In Atalaya where A. albimanus is DDT-resistant, in 3447 night house inspections an average density of only 76 albimanus per 1000 houses inspected was observed during the entire year of study. In 4777 morning inspections the comparable figure was 71 per 1000 house inspections. In unsprayed houses this density was consistently greater than in sprayed houses, and, in the latter, the greater part of the resting anophelines were captured on unsprayed surfaces, such as walls or roofs renewed or altered, clothes and hanging objects, and new furniture. In Atalaya the density index of A. albimanus per capture in

sprayed houses was 0.054 at night and 0.059 in the morning, whereas in unsprayed houses it was 0.976 and 0.553, respectively - or 18 and nine times greater. Of the specimens captured in the sprayed houses, 33% were resting on unsprayed surfaces.

3.18 An analysis, still incomplete, of the study data does not reveal any significant differences between the intradomiciliary activity (endophagy) of A. albimanus in sprayed houses compared with unsprayed houses. In other words there is as yet no evidence of a repellent action of DDT, as a result of which the number of A. albimanus entering houses would be reduced.

3.19 Neither the DDT-susceptible or the DDT-resistant populations of A. albimanus rested very long on sprayed surfaces. Because of the phenomenon of excito-repellency, A. albimanus avoids prolonged contact with the insecticide, soon leaving the sprayed houses either before and after feeding. A large percentage of the specimens which behave in this manner do not rest for a period sufficient to receive a lethal dose of insecticide, and survive 24 hours after their brief contact. This conclusion is based on the following data.

(a) The very low density of specimens found resting on sprayed wall surfaces, coupled with a high percentage of survival of the mosquitos captured.

(b) In night captures of two to three hours' duration inside a house combined with continuous search of the inside wall surfaces, a smaller number of A. albimanus was taken resting on these walls than was taken biting the residents. Under these conditions, the captures in sprayed houses in Atalaya gave a ratio of three A. albimanus biting to one resting. In unsprayed houses the ratio was 1:1.

(c) In night captures on inside wall surfaces, with human bait exposed to the anopheline activity inside the house and counting the A. albimanus which bit but not capturing them, the number of engorged specimen captured on the walls was consistently and markedly less than the number of those which bit regardless of whether the wall captures were made continuously or every 15, 30 or 60 minutes. The greater the interval between the captures on the walls, the smaller was the percentage of specimens captured in relation to the number which bit.

A significant example of this are the results of the captures summarized below:

House	Wall surface	Wall capture interval (in minutes)	Ratio: number of mosquitos biting to number captured
Atalaya "A"	Sprayed with DDT	15	10:1
		30	25:1
" "A"	Whitewash over DDT	15	4:1
		30	7:1
" "A"	Resprayed with DDT	15	11:1
" "B"	Sprayed with DDT	Continuous	13:1
		15	15:1
		30	16:1
		60	26:1
Falla	Sprayed with DDT	15	4:1
		30	9:1
		60	15:1

(d) In each of two one-room houses that had two window traps and no other openings, engorged A. albimanus were liberated shortly after having been collected in a cattle shed, with the following results:

Details	Percentage of mosquitos:	
	that left	that stayed
DDT-resistant area (Atalaya)		
A - Unsprayed house		
3-hour observation	8.7%	91.3%
B - DDT-sprayed house		
1.5 hours observation	90.3%, with 64.2% alive at 24 hours	9.7% (6.1% died)
DDT-susceptible area (Falla)		
C - DDT-sprayed house		
1.5 hours observation	89.1%, with 62.8% alive at 24 hours	10.9% (8.3% died)

(e) In a one-room house in Atalaya, that had been sprayed with DDT at a target dose of  $2 \text{ g/m}^2$ , and that had no openings in the walls or ceiling except one door and two windows, the door was provided with louvres so placed that mosquitos could enter but not leave. Removable window traps were placed on the outside of the two windows and a series of captures made, some of two or three hours and others of 12 hours. Human bait was available to the mosquitos, all A. albimanus, which entered spontaneously, but the mosquitos were not captured when they bit.

The results of several captures are summarized as follows:

Details of captures	Total number captured	Number alive	Number dead
In the window traps	209* (80.1%)	110	99 after 24 hours
In the room, at the end of the observation period	52 (19.9%)	8	44 picked up dead
Total	261 (100%)	118	143

\* Of these 209 females, only 73% were engorged, the remaining 56, or 27% being without blood.

(f) For a better understanding of the behaviour of A. albimanus in sprayed and unsprayed houses, a pair of experimental trap-houses, collapsible and portable, was constructed using angle irons and plywood. The houses measure 3.2 metres long by 2.4 metres wide, and 2.4 metres high. They have two louvres which, by their inclination, permit mosquitos to enter but not to leave, and two window traps. Each entrance louver measures 80 x 60 cm and each window trap aperture 40 x 30 cm. The interior surfaces were entirely lined with paper, which was sprayed with insecticide before being stapled to the plywood. One house was treated with DDT at  $2 \text{ g/m}^2$  and the other was the untreated control. The houses were set up a short distance from each other, and in the same orientation with respect to a known breeding place of A. albimanus. The two houses were used simultaneously.

In a series of captures made in Falla, of 12 hours' duration each (from 6.0 p.m. to 6.0 a.m.), changing the window traps each half hour, and with two men sleeping in each house the following results were obtained:

Details of captures	In the DDT-sprayed trap-house		In the unsprayed trap-house	
	Total number of mosquitos	No. alive at 24 hrs	Total number of mosquitos	No. alive at 24 hrs
In the window traps	256* (91%)	81 (32%)	107** (67%)	97 (91%)
In the house, at the end of the observation period	24 (9%)	-	52 (33%)	-
Total	280 (100%)	-	159 (100%)	-

\* Of these 256 females, 68% were engorged, and 83, or 32% were without blood.

\*\* Of these 107 females, 90% were engorged, and only 11, or 10% were without blood.

(g) Tests done with the Excito-Repellency Test Box, Model OPS,<sup>1</sup> confirmed the tendency of A. albimanus to depart rapidly from surfaces treated with DDT. In a series of tests done with the DDT-resistant A. albimanus from Atalaya the following results were obtained:

	DDT-sprayed box	Unsprayed control box
Percentage that left the box	95% of 875 females	2% of 399 females
Percentage that survived 24 hours	52% " " "	97% " " "

<sup>1</sup> A description of the Excito-Repellency Test Box by Rachou, Moura-Lima, Duret and Kerr will be given in one of the next issues of the WHO/Mal series.  
/Editor's remark/

3.20 DDT-resistance contributed to the survival of a large proportion of the anophelines that enter houses, but remain only a short time after being irritated as a result of contact with the insecticide residues on the walls of the houses.

3.21 A very interesting and important finding was that of marked variation from month to month in the degree of physiological resistance to DDT of the population of A. albimanus at Atalaya. This variation appears to be related to the changes in abundance of the species. The findings are summarized in the following tabulation:

Year and month	Percentage survival in WHO Susceptibility Tests*	Index of abundance**	Season	Air spraying of cotton
1961 - March	75	-	Dry	None
April	79	-	"	"
May	38	129	"	"
June	30	184	Rainy	"
July	16	447	"	"
August	39	184	"	Yes
September	63	40	"	"
October	73	11	"	"
November	58	106	Dry	"
December	48	316	"	"
1962 - January	42	500	"	"
February	55	496	"	None
March	70	500	"	"
April	45	471	"	"

\* Using 4% DDT papers with an exposure period of one hour (WHO technique).

\*\* Mean number of A. albimanus females per stable capture, done by three men during three hours, 6-9 p.m. (two captures per week).

The tabulation shows that in the months of the lowest abundance A. albimanus showed its highest degree of resistance, and vice versa. It may be mentioned that in localities near Atalaya, but without any cotton cultivation, A. albimanus is DDT-susceptible.

3.22 In view of the observed habit of A. albimanus to rest only short periods on surfaces with DDT residues, it seems evident that the numerous openings in the walls of the primitive houses would greatly facilitate the departure of the mosquitos from such houses.

3.23 Marked exophagy of A. albimanus was observed in both localities. When intra- and peridomiciliary captures with human bait were made simultaneously at the same house a larger number of A. albimanus was usually taken outside the house than inside it. In Atalaya, for example, in 150 such captures, 102 (68%) were positive inside the house and 132 (88%) outside, 1104 mosquitos being captured inside and 2109 outside. In the whole year of study an ID:PD ratio of 1:2 was observed. In Falla, as in Atalaya, peridomiciliary captures with human bait yielded large numbers of A. albimanus.

3.24 The peridomiciliary activity of A. albimanus is greatly fostered by the habit of the people to spend much time outside their homes, during the early hours of the night.

3.25 The finding of sporozoites in the salivary glands of one A. albimanus taken biting outside a house, shows that transmission can occur at that site. Incidentally, the entomological aide who captured the infected A. albimanus biting him, developed a primary attack of vivax malaria with patent parasitemia 14 days after the biting incident.

3.26 The transmission of malaria by A. albimanus is importantly affected by a number of different facets of its behaviour, namely: its lack of a well-defined preference for human over animal blood; its habit of resting either in animal shelters or in houses, often in very large numbers; its willingness to bite man either inside or outside of human habitations. Also of importance are its physiological resistance to DDT and its irritability to DDT, at least in the study area. In both Atalaya and Falla, the periods of high transmission were the periods of greatest abundance of A. albimanus, and of the highest relative humidity of the atmosphere. In the dry season A. albimanus remained abundant in spite of its short average length of life but malaria transmission fell to very low levels. The application of agricultural insecticides by aircraft to the cotton fields in the middle of the rainy season reduced the abundance of A. albimanus and, also the amount of malaria transmission.

4. Conclusions

It is our final conclusion that the persisting transmission of malaria in the localities studied is due to the following interrelated causes:

(a) Principal causes

(i) The behaviour of A. albimanus inside houses, where it rests on DDT-sprayed surfaces for only short periods either before or after taking a human blood meal, and then, irritated by contact with the DDT, leaves the DDT-treated surfaces and even the houses, very often without picking up a lethal amount of insecticide.

(ii) The peridomiciliary activity of A. albimanus.

(b) Secondary causes

(i) The large numbers of houses of a primitive type of construction, the outside walls of which have an excessive number of openings.

(ii) The habit of the human population to be outside its houses during the hours when A. albimanus is active.

(iii) In Falla, the large number of unsprayed houses, either newly built or remodelled, during the interval of six months between DDT sprayings.

(iv) In Atalaya, the physiological resistance of A. albimanus to DDT.

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- (a) to acquaint WHO staff, national institutes and individual research or public health workers with the changing trends of malaria research and the progress of malaria eradication by means of summaries of some relevant problems;
- (b) to distribute to the groups mentioned above those field reports and other communications which are of particular interest but which would not normally be printed in any WHO publications;
- (c) to make available to interested readers some papers which will eventually appear in print but which, on account of their immediate interest or importance, deserve to be known without undue delay.

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