

WHO/Mal/198 ✓  
12 February 1958

ORIGINAL: ENGLISH

REPORT ON A VISIT TO LIBERIA  
FOR THE TESTING OF DIELDRIN-SUSCEPTIBILITY  
IN A. GAMBIAE

18 October - 4 December 1957

by

C. D. Ramsdale<sup>a</sup>

I. INTRODUCTION

This visit coincided with that of Dr O. Mastbaum, Consultant Malariologist. It followed immediately after that of Dr H. Schoof of the US Public Health Service, who reported physiological resistance to dieldrin in A. gambiae in the area of the WHO Malaria Control Project.<sup>1</sup> The determination of the size and age composition of the house resting mosquito population, and of sporozoite rates, was done for the same places and at the same time as Dr Mastbaum's malariometrical data were obtained. The work on insecticide resistance is an extension of that started by Dr Schoof.

The geography and climate of Liberia have been described by Giglioli.<sup>2</sup>

A. gambiae, C.p. fatigans, and T. (M). africanus were the only mosquitos found resting in houses.

A map showing the places visited is appended.

II. THE WHO MALARIA CONTROL PROJECT AREA

1. Climatic conditions at Kpain, headquarters of the project, during the period of the visit were:

---

<sup>a</sup> WHO A. gambiae research and advisory team, now in Accra, Ghana.

TABLE I

Rainfall	Date of rain	Inches
	October 26	0.26
	" 28	0.11
	" 31	0.12
	November 3	trace
	" 4	0.31
	" 5	0.36
	" 6	0.66
	" 8	0.02
	" 21	0.60
	" 22	0.16
	" 27	0.20
Total 26 October - 30 November		2.80

TABLE II

Temperature	Maximum		Minimum	
	Mean	Absolute	Mean	Absolute
Screen	89.5F	93F	72F	70F
Mosquito room	80.8F	82F	77.8F	75F

TABLE III

Relative Humidity at 2 p.m.	Mean	Lowest Recorded
Screen	71%	62%
Mosquito room	86%	80%

2. Distribution and House densities of A. gambiae

TABLE IV

(a) House densities of A. gambiae as determined by pyrethrum space spraying.

Place	Last sprayed (dieldrin or BHC)	Houses Searched	A. gambiae females	
			Total	Av. per house
Bunadi	September 1957	3	6	2.0
Tiayee	" 1956	2	1	0.5
Behaye	July 1956	2	70	35.0
Vaa	September 1956	3	12	4.0
Negbaie	October 1957	4	0	0.0
Baila	November 1956	3	0	0.0
Kapita	Unsprayed	2	0	0.0
Gbarnga	November 1957	4	0	0.0
Bahn	March 1957	5	22	4.4
Graie	July 1957	3	13	4.3
Zouli	July 1957	3	3	1.0
Tapita	May 1957	6	158	26.3

(b) Towns in which day time house searches (hand catch) gave negative results.

Place	Last sprayed (dielddrin or BHC)
Kamplay	December 1956
Zuyule	February 1957
Gbobel	" 1957
Sakimpa	" 1957
Sanniquellie	" 1957
Kakata	November 1957

The distribution of A. gambiae in the project area, is shown by Table IV to be patchy. Neither distribution nor house density is related to the time that has elapsed since the last application of insecticide. This in itself, is an indication that physiological resistance in the mosquito, rather than rapid loss of residual effect of the insecticide on the walls, is responsible for the presence of A. gambiae in the houses.

House densities at the time of this investigation were well past their peak, as can be seen from the fact that at Graie, it was possible to collect by means of daytime hand catches in houses 115 female A. gambiae on 14 October and 98 females on 15 October,<sup>1</sup> whereas on 1 November, two weeks later, only 13 females were taken from three houses, even though pyrethrum space spraying was used. The houses sprayed, moreover, were ones known previously to give high catches. That no A. gambiae were found in some towns cannot be taken as an indication that resistance does not occur in these towns, the more likely explanation being that mosquitos are becoming scarcer with the drying up of breeding sites.

### 3. Insecticide resistance in A. gambiae

In all susceptibility tests either the technique of Busvine,<sup>3</sup> or the modified WHO technique employing larger tubes, was used.

Adult mosquitos for tests were obtained both by daytime hand catching in houses, and by breeding out from larvae.

(a) Dieldrin

The concentration of dieldrin in risella oil required to cause 100% mortality in a normally susceptible strain of A. gambiae from southern Nigeria is 0.33%,<sup>4</sup> while a dieldrin resistant strain from northern Nigeria is able to survive a one hour exposure to dieldrin 4%, a concentration which kills the hybrid. Some resistant individuals from northern Nigeria survived 18 hours exposure to dieldrin 4%.<sup>5,6</sup>

TABLE V

A. gambiae females from houses at Gbedi, exposed at Kpain to papers impregnated with a 4% solution of dieldrin in risella oil, for varying periods of time.

Dieldrin 4%	5 hours	9 hours	18 hours	Check (18 hours)
Number exposed	23	21	26	23
Number dead	5	8	24	8
Actual mortality %	21	38	92	34

Survivors to as long as 18 hours exposure to dieldrin 4% show that dieldrin resistance is present in the A. gambiae population in the WHO project area, and that the enhanced tolerance of the resistant mosquitos is of the same order as that occurring in the northern Nigerian dieldrin resistant strain.

(b) BHC

The concentration of yBHC in risella oil causing 100% mortality in a normally susceptible strain of A. gambiae from southern Nigeria is 0.025%.<sup>4</sup>

TABLE VI

A. gambiae adults (male and female) bred from wild-caught larvae and exposed to filter papers impregnated with solutions of  $\gamma$ BHC in risella oil for 1 hour, when one day old and fed on sugar only. Mortalities read 24 hours later.

BHC	0.02%	0.04%
Number exposed	20	56
Number dead	1	2
Actual mortality %	5	4

Almost complete survival to BHC 0.04% shows that, as in northern Nigeria, there is cross resistance to BHC.

(c) DDT

Physiological resistance to DDT in A. gambiae has not yet been reported. The mechanism of inheritance of DDT resistance in A. sudaicus has recently been demonstrated,<sup>7</sup> but the separating dosage used is not applicable to A. gambiae, and comparison of  $LC_{50}$ 's remains the method for detecting DDT resistance in spot checks. Survivals to high dosages, however, should always be taken as a danger sign, regardless of the level of the  $LC_{50}$ , and attempts to isolate a resistant strain by selection of survivors to high dosages might be the surest method for the detection of DDT resistance.

TABLE VII

A. gambiae females exposed for 1 hour to filter papers impregnated with solutions of DDT in risella oil. Mortalities read 24 hours later.

DDT	0.5%	1.0%	1.5%	2.0%	Check
Number exposed	80	93	103	121	71
Number dead	14	37	67	105	2
Mortality %	17	40	65	87	3
Corrected Mortality	14	38	64	87	-
$LC_{50}$ : DDT 1.1%					

The  $LC_{50}$  is higher than that obtained under laboratory conditions for both the susceptible southern Nigerian, and the dieldrin resistant northern Nigerian strains,<sup>5</sup> but is not higher than that reported from field tests in either northern Nigeria<sup>6</sup> or the Belgian Congo,<sup>8</sup> where DDT is apparently achieving control. It appears then, that susceptibility to DDT is normal, but it is suggested that exposures be done at regular intervals in the future, and that higher concentrations of DDT be also employed.

4. The extent of dieldrin resistance and the proportion of the population resistant

A. gambiae from different places in the project area were tested to discover the extent of dieldrin resistance, and the proportion of the population that was resistant. Dieldrin 0.4% and 4%, the separating dosages supplied in the WHO kit were used. It is not certain that the separating dosages applicable to A. gambiae from Nigeria will necessarily prove to be the same if, and when, resistance develops in other parts of Africa, and as Liberia is, ecologically, so different to W. Sokoto it was with some caution that they were used.

TABLE VIII

A. gambiae females caught resting in houses and exposed to papers impregnated with solutions of dieldrin in risella oil for 1 hour. Mortalities read 24 hours later.

Locality	Uncorrected mortalities		
	Dieldrin 0.4%	Dieldrin 4.0%	Check
Kpain Area	3.5 (56)	3.5 (112)	0 (39)
Bahn Area	-	0.0 (43)	0 (10)
Tapita	-	0.0 (49)	0 (10)
Gbedi	18.0 (192)	16.0 (56)	9 (66)

Figures in parentheses show numbers tested.

Mortalities for Gbedi are higher than for the rest of the area, but the check mortality is higher too. In contrast to the other areas, where tests were done on the spot, the mosquitos caught at Gbedi were exposed at Kpain, and at least some of the mortality was probably due to transport.

The lack of susceptible mosquitos found resting in houses could have been caused by a "pre-catch selection" due to the action of insecticidal wall deposits.

TABLE IX

A. gambiae adults, male and female, bred from larvae collected at Kpain and exposed, at Kpain, for 1 hour to papers impregnated with a 4% solution of dieldrin in risella oil, when 1-3 days old and fed on sugar only. Mortalities read 24 hours later.

Dieldrin 4%	Males	Females	Total
Number Exposed	147	75	222
Number Dead	7	1	8
Actual Mortality %	4	1	4

Almost complete survival of the bred out material in Table IX rules out the possibility of "pre-catch selection" being responsible for the low mortalities recorded in Table VIII. The use of larvae collected at Kpain also precluded the possibility of any selective mortality due to transport of the larvae over long distances.

TABLE X

A. gambiae adults, male and female, the  $F_1$  offspring of wild-caught resistant females, bred in the laboratory and exposed for 1 hour to papers impregnated with a 4% solution of dieldrin in risella oil when 1-3 days old and fed on sugar only. Mortalities read 24 hours later:

Dieldrin 4%	Males	Females	Total
Number Exposed	120	143	263
Number Dead	5	2	7
Actual Mortality %	4	1	3

That the  $F_1$  offspring of wild-caught females surviving exposure to dieldrin 4% bred true for resistance is further proof that the population is homozygous for the dieldrin-resistant gene.

Some of the parents of the mosquitos exposed in Table X originated from Gbedi, which indicates that the A. gambiae population at Gbedi is, like that in the rest of the area, almost wholly homozygous resistant, and that the mortalities recorded in Table VIII are due to causes other than exposure to dieldrin.

#### 5. Resistance and totality of insecticide coverage

Tables VIII, IX and X show the A. gambiae population to be composed almost entirely of homozygous dieldrin resistant individuals.

It is generally assumed that the gene conferring resistance is at a disadvantage in the absence of insecticides.<sup>9,10</sup> If this assumption is accepted, then the insecticide coverage throughout the project area must be adequate, and the shelters at present unsprayed be unimportant. This it is difficult to accept. Indeed, at Gbedi, all the houses were replastered immediately after the first application of insecticides there and they have not been sprayed since.<sup>2</sup> It is in effect an untreated village supporting a sizeable mosquito population. Even if the evidence of Table X is disregarded and the corrected mortality recorded in Table VIII is taken as being entirely due to susceptibility, some 90% of the A. gambiae population at Gbedi is homozygous for the resistant gene; and this in the absence of the selective pressure exerted by insecticides.

This state of affairs supports the hypothesis that the gene conferring resistance is in fact advantageous, even in the absence of insecticides and that once it is established in sufficient numbers in a population it could, without further selection by insecticides, naturally replace the susceptible gene.<sup>11</sup>

#### 6. Age composition of the A. gambiae population and sporozoite rates

Not all gorged females developed eggs. In East Africa, it has been demonstrated that newly emerged females require two blood meals to complete the first gonotrophic cycle, and that the great majority of gorged females failing to develop eggs are newly emerged. This phenomenon, together with the sporozoite rate, has been used to estimate the age composition of mosquito populations.<sup>12</sup> An attempt to age the A. gambiae population in the project area, using the same technique, was attempted.

Gorged females were caught both by hand catch and by pyrethrum space spray methods. Dissection of those caught by the hand catch method was delayed until the following day, by which time they fell clearly into two groups, those with ovaries still at Christopher's Stage II, and not developing eggs, and those half gravid. Those caught by means of the pyrethrum spray method were dissected the same day and, using Macan's fine classification, divided into those with ovaries not developed beyond Stage mid II and which would not have developed eggs, and those with ovaries that had developed to Stage late II and beyond and which would have become gravid.

No observations have yet been made in Liberia to determine how many blood meals are necessary for completion of the first gonotrophic cycle. If, as is possible, some females require only one blood meal for maturation of the first eggs, the pregravid rate recorded in Table XI will be lower than the actual percentage of nullipares present in the population. This does not seem to have been the source of any considerable error, however, and the results obtained fit well into the pattern to be expected in a population which is declining as a result of the onset of the dry season.

TABLE XI

Age composition of the A. gambiae population and sporozoite rates.

Place	Number		Pregravid Rate	Salivary Glands		Sporozoite Rate
	Gravid	Pregravid		Total	Sporozoite Positive	
Bahn area	82	10	11	82	17	20
Graie area	11	2	15	13	1	8
Tapita	133	8	6	116	12	10
Behaye	69	10	12	73	5	7
Total	295	30	9	284	35	12
Oct/Nov 1956 <sup>2</sup>				67	1	1.5
Oct/Nov 1955 <sup>2</sup>				188	2	1.06
Sept 1955 <sup>2</sup>				47	6	12.76

Where control is being achieved, the proportion of young nulliparous females in the mosquito population will be high, and the numbers living long enough to become infective, extremely low. Table XI shows that at the time of this investigation only about 10% of the population (subject to the source of error mentioned above) were newly emerged, some 90% had lived long enough to take two blood meals, and that at least 12% were old enough to be infective.

A high sporozoite rate at the time that the population is declining, and contains a predominance of old females is what can be expected in an area where man is almost exclusively the source of the blood meal.

### III. HARBEL AREA

Houses in labour camps on the Firestone Rubber Estate were searched for adult mosquitoes. Camps number 16, 24, 25, 26 and 31, having the longest history of dieldrin treatment, and being situated closest to Kakata in the WHO project area, were visited. No anopheles were found. The Firestone camps are particularly well sited, but in the unsprayed area in the vicinity of the LITM mosquitos were also scarce. House searches on three days, and evening and early night biting catches on human bait, both in and out of doors, on three nights, yielded only nine female A. gambiae, none of which survived a one hour exposure to a filter paper impregnated with an 0.4% solution of dieldrin in risella oil.

The presence of dieldrin resistance in this area was not detected.

### IV. ICA MALARIA CONTROL PROJECT AREA

Adult A. gambiae in the ICA project area, which is centred on Monrovia, are extremely scarce. In five days searching 10 females, all from the Paynesville-Johnsonville area, were found resting in houses. Of these, only one survived long enough to be tested for dieldrin resistance.

This female was exposed for one hour, to a filter paper impregnated with a 0.4% solution of dieldrin in risella oil, and survived.

While it may be argued that the survival of one mosquito to dieldrin 0.4% may be due to "vigour tolerance", the possibility, or, especially in view of events elsewhere in Liberia, the probability of physiological resistance being involved, must be admitted.

It was hoped that the offspring of this female would provide definite information on the presence, or not, of physiological resistance, and after exposure it survived transport to Accra, but owing to the unfavourable conditions under which it had to be kept there, it died a week after arrival, without having laid. This was disappointing as it had been hoped to demonstrate that detection of insecticide resistance, on the evidence provided by a single wild-caught female, was possible.

#### V. VOINJAMA AREA

Dieldrin resistance in A. gambiae has previously been reported from northern Nigeria and Upper Volta Province of French West Africa, both of which are in the dry savannah belt to the south of the Sahara. Liberia lies almost entirely within the upper Guinean Forest region,<sup>13</sup> and it is only in the northern tip, in the Voinjama district, where it was thought that ecological conditions comparable with those occurring in northern Nigeria or the Upper Volta, could conceivably occur, though it was realized that even there it was unlikely.

The WHO project area extends beyond Zorzor, in the direction of Voinjama, as far as Konya. A visit was made to the Voinjama district with the object of testing for resistance both mosquitos from an unsprayed area adjacent to the WHO project, and if possible, mosquitos from savannah country. Houses were searched in Luyema, Duogomai, Botossu, and Kollimai. Voinjama town was not visited, but Kollimai is only two hours walk to the south, and from the high ground at Duogomai there were good views to the north and west. The path from Konya to Kollimai and beyond is almost entirely through high forest. While much of the forest in the Voinjama-Bolahun region may have been cleared for agricultural purposes, it was quite evident, even from the admittedly sketchy impressions received, that dry savannah conditions, where much of the A. gambiae breeding is in permanent, or semi-permanent water, in association with A. pharoensis and where climatic conditions are severe for the adult, as in western Sokoto, do not occur in Liberia. An annual rainfall of 94" at Voinjama, spread over most of the year,<sup>2</sup> supports this view.

No mosquitos were found in Luyema, Duogomai or Botossu, and only two female A. gambiae were found in houses at Kollimai. Both these were susceptible.

VI. CULEX P. FATIGANS

Wharton,<sup>14</sup> using the Busvine-Nash technique in Malaya, reports a  $LC_{50}$  of dieldrin 0.4% for C.p. fatigans in unsprayed areas and 100% survival to dieldrin 4% in a sprayed area where resistance has occurred.

Adult C.p. fatigans collected in houses at Kakata, were tested at Kpain immediately after the journey. Of 51 adults exposed to dieldrin 4%, 30 survived. Subsequently C.p. fatigans adults caught resting in houses at widely separated places in the treated areas, were tested at central points nearer to the place of capture.

TABLE XII

C.p. fatigans adults, male and female, caught resting in houses, exposed for 1 hour to papers treated with a 4% solution of dieldrin in risella oil.

Mortalities read 24 hours later.

Captured at Tested at	Firestone			
	Bahn Bahn	Kakata LITM	Camp 25 LITM	Arthington Monrovia
Number exposed	110	59	5	12
Number dead	5	2	0	5
Actual mortality %	5	4	0	41

The high survival rate to dieldrin 4% shows dieldrin resistance in C.p. fatigans to be widespread in the treated areas of Liberia.

Resistance of the C.p. fatigans larvae to dieldrin, BHC, or allied compounds, has previously been reported from Malaya and India,<sup>10</sup> but this appears to be the first record from Africa.

Summary

1. A. gambiae, C.p. fatigans and a few T. (M). africanus were the only mosquitos caught resting in houses.

2. House densities at the time of this visit, as determined by pyrethrum space spraying (and in some cases where no mosquitos were found, by hand catching) are given for a number of towns in the WHO project area.
3. Patchy distribution and varying house densities are shown to be due to factors other than (a) the time that has elapsed since the last application of insecticide or (b) the presence of resistance.
4. Reported resistance to dieldrin and normal susceptibility to DDT in A. gambiae in the WHO project area is confirmed and cross resistance to BHC is reported.
5. The occurrence of a highly dieldrin resistant A. gambiae population is shown to be widespread in the WHO project area.
6. The implications of the occurrence of a homozygous insecticide resistant mosquito population in an area where insecticide coverage of all available resting places has not been achieved, is discussed.
7. The approximate age composition of the A. gambiae population in the WHO project area, and the sporozoite rates at the time of the visit are reported.
8. Owing to the dearth of A. gambiae in both sprayed and unsprayed areas at Harbel, no conclusion about the presence or not of "potential resistance" could be reached. There was no sign of dieldrin resistance in the A. gambiae found.
9. On the evidence of one mosquito tested, the possibility of the development of a dieldrin resistant strain of A. gambiae in the ICA project area is revealed.
10. It is concluded, after visiting the Voinjama district, that ecological conditions comparable with those obtaining in western Sokoto, do not occur in Liberia.
11. It was not possible to obtain enough mosquitos for testing from the unsprayed area between Zorzor and Voinjama.
12. Dieldrin resistance in C.p. fatigans is shown to be widespread in all the treated areas in Liberia.

Acknowledgements

Dr Miller of the LITM, Dr Lyon of the Firestone Rubber Estate and Dr Cook and Mr Karam of the ICA project based on Monrovia provided staff and laboratory accommodation and gave permission to work in their respective areas.

Mr McChristie took part in all work carried out in the WHO project area, and it is largely due to his efforts that it was possible to cover so much ground. Mr Mason, responsible for the insectory and the entomological staff often worked long hours under all sorts of conditions. Mr Pedersen and his staff promptly met all demands made on their resources.

Thanks are due to all.

REFERENCES

1. Schoof, H. (1957) Report to WHO on susceptibility tests in Liberia
2. Giglioli, M. (1957) Report of the entomologist WHO/UNICEF RL Malaria Project, 1955-1957
3. Busvine, J. (1954) Wld Hlth Org. techn. Rep. Ser. 80
4. Davidson, G. (1956) Insecticide resistance in A. gambiae Giles. A case of simple Mendelian Inheritance. Nature, 178
5. Davidson, G. (1956) A further note on insecticide resistance in A. gambiae Giles. Nature, 178
6. Armstrong et al. (1956) Report on a field investigation into the extent and degree of insecticide resistance in A. gambiae in the area of the western Sokoto Malaria Project, WHO/Insecticides/52
7. Davidson, G. (1957) Insecticide resistance in A. sudaicus. Nature (in press)
8. Holstein, M. (1957) WHO/Mal/189
9. Milant, R. (1954) WHO First Symposium on Control of Insect Vectors of Disease, page 253
10. Busvine, J. (1957) Insecticide resistant strains of insects of public health importance. Trans. roy. Soc. trop. Med. Hyg. 51
11. Davidson, G. (1957) Discussion on a paper "Insecticide resistant strains of insects of public health importance" read by J. Busvine. Trans. roy. Soc. trop. Med. Hyg. 51
12. Gillies, M. T. (1954) Recognition of age-groups within populations of A. gambiae by the pregravid rate and the sporozoite rate. Ann. trop. Med. Parasit. 48
13. Chapin, J. P. (1932) Birds of the Belgian Congo. Part I. Bull. Amer. Museum of Natural History, 65 (quoted by Giglioli)
14. Wharton, R. H. (1957) Dieldrin-resistance in Culex pipiens fatigans. Bull. Wld Hlth Org. 17 (in press)

SKETCH MAP SHOWING PLACES VISITED

