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THE APPEARANCE OF DIELDRIN-RESISTANCE IN *ANOPHELES MINIMUS FLAVIROSTRIS* IN THE PHILIPPINES

by

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Introduction

The writer visited Mindanao Island, Philippines, from 15 to 24 July 1959 for the purpose of investigating the possibility of the development of dieldrin-resistance in *Anopheles minimus flavirostris*, the main vector of malaria in the Philippines. The observation of unit malariologists in 1958 had suggested that dieldrin was not producing the expected results. Preliminary susceptibility tests carried out in 1958 by Dr J. B. Mendoza, Philippine Government Chief Research Malariologist, suggested that dieldrin-susceptibility in *A. minimus flavirostris* was slightly reduced.

Mosquitos for tests were caught from two localities where water buffalo (carabao) -baited traps had been set up for some years:

(1) Malasila - a village with a population of 2067, belonging to the Municipality of Malasila, Province of Cotabato, Mindanao Island, and

(2) Kidapawan - a small town, 11 kilometres away from Malasila.

The history of insecticide spraying in these areas was as follows. For agricultural pest control, an area called Polomolok in the southern part of Cotabato (at least 100 kilometres away from Kidapawan) was sprayed from the air with BHC in 1952-1953 and 1958 against locusts. In early 1959 DDT was sprayed by power sprayer to 25 hectares of plantations in Kidapawan. For malaria control, Malasila was sprayed with DDT at 2 g/sq.m. in February 1956, and with dieldrin at 0.5 g/sq.m. in May 1957, April 1958 and February 1959. The town of Kidapawan proper was never sprayed, but the nearest area (Saguing, only half a kilometre away) was sprayed with DDT at 2 g/sq.m. in February 1956, and with dieldrin at 0.5 g/sq.m. in April 1957 and May 1958.

The following spleen and parasite rates of children (2-10 years) have been recorded in Malasila:

Date examined	Spleen rate		Parasite rate	
	Number examined	Percentage positive	Number examined	Percentage positive
March 1956 (One month after first spraying)	33	21	33	12
March 1957	32	9.3	31	3.2
March 1958	52	7.1	51	3.9
July 1959	182	7.1	182	1.1

A small number of infants was examined for malaria parasites before and after insecticide residual spraying and all were found negative. On the present visit, twelve infants were examined by the unit malariologist and also found negative.

A. minimus flavirostris is the only vector in Malasila and has its peak of density in June-July. Before residual spraying, the highest density of flavirostris in daytime collections was 0.5 per man-hour indoors and 15.4 per man-hour outdoors. Night collections revealed 16.5 mosquitos per hour per buffalo-baited trap, and 0.05 per human-baited trap.

Since the beginning of 1959 night collections have been carried out weekly in two sprayed houses in Malasila. In one of the houses 96 female flavirostris and 98 (2 male and 96 female) flavirostris were caught in single nights in March and April 1959, respectively. In the other house the highest number was 41 female flavirostris in one night in June 1959.

After learning of these high catches from a house sprayed only 1-2 months previously with dieldrin, Dr J. B. Mendoza sent a team to make investigations in Malasila and its vicinity. The team, all of whose members were from the Institute of Malariology at Tala, was sent early in June 1959. Preliminary reports by this team showed the possibility of development of dieldrin-resistance in flavirostris.

Laboratory and Field Findings

Insecticide-susceptibility tests of anopheline mosquitos were done in the unsprayed laboratory in the town of Kidapawan. During the whole period of tests, the range of room temperature was 75-87°F, and relative humidity was 70-90%. All the tests were carried out by Mr Dentis and Mr Del Rosario, both of whom were trained by the writer beforehand. Those performed during the period 15-23 July 1959 were done by the same workers, but under the direct supervision of the writer.

Adult mosquitos were caught early in the morning from buffalo-baited traps and tested after holding for 1-2 hours in mosquito cages. Only fed females were used for tests. Efforts were made to isolate the flavirostris mosquitos to be tested, but due to practical difficulties an occasional mixture of the anopheline species in the same tube could not be avoided.

The second series of tests was done during the period 15-23 July 1959, about a week after the dieldrin 4% impregnated papers had (technically speaking) expired. But check tests with laboratory-bred Aëdes aegypti (the Tala colony) exposed on some of these papers showed complete kills 24 hours after exposure on 4%, 1.6% and 0.8% papers.

The results of the tests of anopheline adults are shown in Table I. Less than 50 per cent of A. minimus flavirostris from Malasila were killed after having been exposed to dieldrin 0.8, 1.6 and 4%; 30-57% were killed by exposure to DDT 0.5%. The same species caught from Kidapawan showed mortality rates of 57% by exposure to both 1.6 and 4% dieldrin, and of 59% to DDT 0.5%. DDT at 2% gave complete kill of small numbers tested in each locality.

TABLE I. INSECTICIDE-SUSCEPTIBILITY TESTS OF ANOPHELINE ADULTS

Species	Locality	Date	Controls		Dieldrin %						DDT %					
					0.8		1.6		4.0		0.5		1.0		2.0	
			No.t.	% m.	No.t.	% m.	No.t.	% m.	No.t.	% m.	No.t.	% m.	No.t.	% m.	No.t.	% m.
A. minimus flavirostris	Malasila	19-30/6/59	90	1	106	25	119	44	-	-	88	57	34	80	22	100
		16-23/7/59	37	5	99	25	80	35	65	36	36	30	-	-	-	-
	Kidapawan	16-23/7/59	18	0	42	40	21	57	21	57	22	59	13	100	10	100
A. vagus limosus	Malasila	16-23/7/59	-	-	27	59	33	49	11	82	42	50	34	85	12	92
	Kidapawan	16-23/7/59	21	5	56	36	52	65	44	90	63	44	15	80	17	94
A. maculatus	Malasila	16-23/7/59	24	4	75	99	50	100	33	100	36	58	12	83	-	-

No.t. = number of mosquitos tested

% m. = percentage of observed mortality

In A. vagus limosus from both localities only 49-65% were killed by dieldrin 1.6%; DDT at 2% gave kills of 92-94%. A. maculatus were almost all killed by dieldrin 0.8%, and all killed by 1.6 and 4%.

In addition, a few other anopheline species were also tested but the numbers were too small for reaching firm conclusions. However, for preliminary information we may mention that all of A. kochi and A. tessellatus were killed by dieldrin 0.8, 1.6 and 4%; 71% and 93% of A. ludlowi were killed by dieldrin 0.8 and 1.6% respectively, and 75% and 93% of A. subpictus indefinitus were killed by these two concentrations.

Only 5 out of 9 flavirostris were killed by exposure to 4% dieldrin papers for two hours instead of the usual exposure for one hour.

Special tests were made on two groups of flavirostris bred out from larvae which survived after having been exposed for 24 hours to dieldrin solutions of 0.02, 0.1 and 0.5 p.p.m., or to BHC solutions of 0.02 and 0.1 p.p.m. The results were as follows:

TABLE II. SERIAL EXPOSURES OF ADULTS BRED OUT FROM SURVIVORS OF LARVAL TESTS

Date and exposure to impregnated papers	Surviving A. m. flavirostris bred out after tests with:							
	Dieldrin 0.02, 0.1, 0.5 p.p.m.				BHC 0.02 and 0.1 p.p.m.			
	♀	♀	♂	♂	♀	♀	♂	♂
	No.t.	Dead	No.t.	Dead	No.t.	Dead	No.t.	Dead
(1) 20/7/59 to dieldrin 1.6%	6	0	7	0	3	0	5	0
(2) 21/7/59 the same specimens to dieldrin 4%	6	0	7	0	3	0	5	0
(3) 22/7/59 the same specimens to DDT 0.5%	6	1	7	1	3	0	5	2
(4) 23/7/59 those again surviving, to DDT 1%	5	3	6	4	3	3	3	2

The above results suggest that a more or less pure resistant strain of flavirostris was selected after exposure of larvae to insecticide solutions of dieldrin and BHC. All adults resulting from these larvae survived after being exposed to dieldrin 1.6% and again to dieldrin 4%.

For tests of anopheline larvae, fourth instar larvae of flavirostris were collected from a stream near Malasila and tested by the WHO method. The insecticide solutions were checked with laboratory-bred aegypti larvae and found satisfactory. The results are shown in Table III.

TABLE III. LARVAL TESTS WITH A. M. FLAVIROSTRIS AND AEDES AEGYPTI

Insecticide		flavirostris		aegypti	
		Number tested	Percentage mortality	Number tested	Percentage mortality
Dieldrin p.p.m.	0.02	119	10	40	100
	0.1	125	31	45	100
	0.5	133	70	-	-
Gamma-BHC p.p.m.	0.02	106	7	19	100
	0.1	132	60	25	100
	0.5	64	100	-	-
DDT p.p.m.	0.004	127	43	22	0
	0.02	132	90	40	75
	0.1	61	100	45	100
Controls		147	3	38	0

In the tests on flavirostris, made on 16-21 July 1959, there were seven replicates (each with 20-25 larvae) except for 0.5 p.p.m. BHC and 0.1 p.p.m. DDT when there were only three replicates; in the aegypti tests, done on 20-21 July 1959, there were only one to two replicates.

Table III shows that a concentration as high as 0.1 p.p.m. dieldrin could kill only 31% of flavirostris and 0.1 p.p.m. gamma-BHC killed 60%, while 0.1 p.p.m. DDT gave 100% mortality.

The nulliparity rate in four species of anophelines was studied by examination of the tracheoles of the ovaries, according to the method of Detinova (1945). This was done by Miss Cagampang under the direct supervision of the writer. All the anophelines dissected were caught from Malasila and Poblacion. The results of the dissections are summarized in Table IV.

TABLE IV. DETERMINATION OF NULLIPARITY RATES BY THE TRACHEOLES OF THE OVARIES

Species	Number dissected	Percentage parous	"p" (probability of mosquito survival through one day)
A. m. flavirostris	164	91	0.96
A. vagus limosus	126	79	0.89
A. maculatus	59	81	0.90
A. ludlowi	26	73	0.86

In other words, the daily mortality rate was 4% for flavirostris, 10% for maculatus, 11% for limosus and 14% for ludlowi.

Discussion

1. Insecticide-resistance in anopheline adults

No baseline value of LC_{50} for flavirostris from unsprayed areas was available. However, the LC_{50} data for the same species caught in the same locality (Kidapawan) where the writer carried out tests during his present visit were reported by the WHO ATME No. 4 in October-November 1957 as 0.03% dieldrin. This value was obtained in the area after spraying with one round of DDT and one round of dieldrin.

The present findings show that the LC_{50} of flavirostris in Malasila was over 4% dieldrin. This means that a degree of dieldrin-resistance of at least

100 times had developed in flavirostris, if 0.03% dieldrin is taken as an LC_{50} value for a normal strain. Flavirostris from Kidapawan has also developed resistance to dieldrin at least 50 times higher than this value.

It was rather peculiar to get the same mortality of flavirostris by exposure to dieldrin 1.6% and 4%. One explanation may be that the flavirostris mosquitos tested were a heterogeneous population of which the susceptible individuals (and also the hybrids?) were killed by either dieldrin 1.6% or 4%, while the resistant individuals could not be killed by either dieldrin 1.6% or 4%. If this is so the homozygous resistant mosquitos now constitute about 64% of the Malasila population and about 43% of the Kidapawan population.

The LC_{50} of DDT to dieldrin-resistant flavirostris was about 0.5%. It seems that this mosquito also probably has an increased tolerance to DDT of about five times, compared with the LC_{50} of 0.09% DDT to a normal strain of its closely-related subspecies, A. minimus minimus, in Taiwan. This increased tolerance to DDT is probably a collateral effect of dieldrin resistance. In Java the writer found that dieldrin-resistant A. subpictus subpictus also had an increased tolerance to DDT of four times.

A. vagus limosus also showed resistance to dieldrin, probably fifty times higher than normal susceptibility if we use the baseline value for A. vagus vagus in Java (LC_{50} of dieldrin 0.035%). A. vagus limosus was, however, still susceptible to DDT.

It is also interesting to note that, after larval selection with insecticide, unfed young flavirostris could all survive 1.6% and again 4% dieldrin. After exposure to DDT 0.5% the mosquitos were carried from Kidapawan to Davao by car for three hours and the next morning from Davao to Manila by plane for two-and-a-half hours, but most of them still survived.

2. Insecticide-resistance in A. m. flavirostris larvae

Generally, resistance should be suspected if an LC_{50} of the larvae is above 0.1 p.p.m. DDT. For dieldrin this figure should be much lower.

In the present tests the LC_{50}'s to flavirostris larvae were about 0.2 p.p.m. dieldrin and a little below 0.1 p.p.m. gamma-BHC. This shows that flavirostris larvae have developed high resistance to dieldrin and show cross-resistance to BHC. Unfortunately, again no baseline value for these larvae was available for comparison.

3. Longer life of dieldrin-resistant flavirostris

In the 1957 report of the WHO ATME No. 4, Macdonald estimated the probability of survival through one day of a susceptible strain of flavirostris in the Philippines as 0.91, based on the sporozoite and total infection rates reported by Manalang. The present estimate of the probability of survival through one day of dieldrin-resistant flavirostris was 0.96, based on the USSR technique of using the pattern of ovarian tracheoles for separating nulliparous and parous mosquitos. This shows a daily mortality rate of only 4% in the dieldrin-resistant strain, as against 9% for the susceptible strain.

4. Dieldrin-resistant flavirostris in relation to the malaria eradication programme in the Philippines

At the beginning of the Philippine malaria control programme DDT was used. After 1 to 2 rounds of DDT spraying, a change was made to dieldrin, for reasons unconnected with anopheline susceptibility to insecticides. Since late 1958 certain parts of the Philippines have been switched back to DDT, but other parts still use dieldrin in order to finish the stock of dieldrin on hand.

After the finding of dieldrin-resistant flavirostris, the decision was made to stop immediately the spraying of dieldrin in the whole country and to use DDT instead. In order to ascertain the extent of distribution of the dieldrin-resistant strain of flavirostris in the Philippines, a national team will be sent out soon to make tests in certain other parts of the country, such as Palawan, Mindoro and Northern Luzon. Later on, the team will be sent to Leyte, an unsprayed area, to obtain baseline values of insecticide-susceptibility of the local anophelines.

Conclusion

Anopheles minimus flavirostris, the main malaria vector in the Philippines, has developed in the two investigated localities on Mindanao a high degree of resistance to dieldrin, a certain degree of cross-resistance to BHC, and probably an increased tolerance to DDT. This may be a cause of the failure hitherto to stop malaria transmission in certain areas of the Philippines.

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REFERENCE

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