FIRST RECORD OF DDT RESISTANCE IN ANOPHELES DARLINGI¹

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ABSTRACT. DDT susceptibility of Anopheles darlingi was compared from the southeastern and northwestern regions of Colombia. Mosquitoes from southeastern Colombia (Puerto Lleras) were fully susceptible to DDT but in the northwest (Quibdo) LT $_{50}$ s were 14.3 times higher. This resistance appeared to be restricted to the Quibdo area. This is the first time that the DDT resistance has been detected in this important malaria vector.

INTRODUCTION

Anopheles darlingi Root is a major malaria vector in South America and occurs in the majority of malaria endemic areas of Colombia (Fig. 1). DDT has been used for malaria control in Colombia since 1959, and routine evaluation of DDT susceptibility has been periodically conducted during the last 3 decades. Until 1980 (Organizacion Mundial de la Salud 1980), An. darlingi was considered highly susceptible to DDT throughout its range; but tests on mosquitoes from the Choco region of the northeastern part of Colombia indicated that DDT resistant individuals were present in some populations from this region (Quiñones et al. 1987). These results prompted us to conduct further susceptibility tests on mosquitoes from 2 additional widely separated geographic locations.

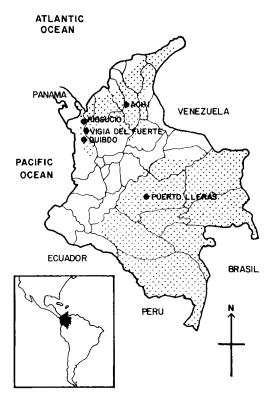
MATERIALS AND METHODS

Tests were carried out in 3 localities along the Atrato River in the municipalities of Quibdo and Riosucio, and in 2 localities of Puerto Lleras (Fig. 1). The Atrato River valley is located in northwestern Colombia (5° 41′ N, 76° 40′ W), at an elevation of 18–50 m above sea level. Annual temperatures vary from 24 to 28°C, and annual rainfall is between 4,000 and 8,000 mm, with rains occurring throughout the year. The region lies in the Very Humid Tropical Rain Forest (Ecological Holdridge Classification, IGAC 1977), where excessive humidity prevents intensive agriculture.

Puerto Lleras is located in southeastern Colombia (3° 16′ N, 73° 23′ W), 250 m above sea level. Annual rainfall is between 400 and 4,000 mm. The principal rainy season is from April to June with a second rainy period from August to November. December to March is dry. The re-

gion lies in the Humid Tropical Rain Forest (Ecological Holdridge Classification, IGAC 1977).

Susceptibility tests for adults followed techniques recommended by the World Health Organization (WHO 1981a). All mosquitoes used were field-collected females from outdoor human landing collections. After collection the mosquitoes were placed on a guinea pig to ensure full blood engorgement, a procedure which improves adult survival (WHO 1981a). Specimens



¹ Opinions and assertions contained herein are the private views of the authors and not necessarily reflect the view of the Colombian Ministry of Health.

Fig. 1. Map of Colombia showing the distribution of *Anopheles darlingi* and the location of the municipalities studied.

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Distribution of Anopheles darlingi

Municipalities studied

Table 1. Results of susceptibility tests with 4% DDT in Anopheles darlingi from 3 regions of Colombia

Department			Exposure times (min).						
Municipality Locality	Date	No. of tests	3	7	15	30	60	120	240
Choco									
Quibdo									040
Barranca	Dec. 1987	6	_	1.3	1.8	3.1	19.8	72.5	94.9
				(153)*	(165)	(161)	(151)	(142)	(158)
Tagachi	Feb. 1988	6	_	1.3	2.5	5.8	20.4	75.8	86.8
8				(143)	(162)	(156)	(162)	(165)	(144)
Tagachi	Mar. 1988	6		0.6	4.3	9.4	27.5	70.4	95.
6				(168)	(164)	(170)	(171)	(159)	(162)
Barranca	Apr. 1988	6	_	1.6	2.1	5.3	17.5	54.5	87.9
2002			_	(185)	(189)	(187)	(183)	(187)	(191)
Barranca	June 1988	6	_	0	2.2	5.8	21.8	70.6	92.0
Darranca	J 44.10			(138)	(137)	(137)	(142)	(136)	(126)
Ame-Bete	July 1988	6	_	1.4	0	5.1	16.9	52.5	95.6
Time Bette	5 diy 1000	•		(140)	(131)	(137)	(142)	(139)	(137)
Choco				(- /	, ,				
Riosucio									
B. Murindo	Nov. 1988	6		17.8	41.6	79.7	97.9	100	100
D. Mulliuo	1101. 1000	Ū		(151)	(149)	(143)	(149)	(154)	(150)
Meta				(101)	()	\ >	` ,		
Puerto Lleras									
C. Caribe	Apr. 1988	4	_	73.4	91.1	90.6	100	100	100
C. Caribe	Apr. 1500	•	_	(79)	(79)	(75)	(80)	(78)	(79)
C. Rayado	May 1988	3	18.3	41.6	58.3	93.1	100	100	100
C. Mayado	Way 1900	J	(60)	(60)	(60)	(58)	(60)	(60)	(58)
C D	T 1000	4	37.9	57.5	78.7	92.3	100	100	100
C. Rayado	June 1988	4			(80)	(78)	(80)	(78)	(79)
			(79)	(80)	(80)	(78)	(80)	(10)	(19)

^{*} Percent mortality (no. mosquitoes tested).

Table 2. Results of DDT susceptibility tests (LT₅₀) for Anopheles darlingi in three regions of Colombia

Population	LT ₅₀ *	95% conf. limits*	Slope
Quibdo Riosucio	93.34 14.45	76.22-115.28 8.31-27.54	2.80 8.28
Puerto Lleras	6.51	3.44-10.77	2.80

^{*} Values expressed in min.

were kept overnight in an insulated styrofoam container and tested the next day. Temperatures ranged from 24 to 28°C, and RH was maintained between 85 and 90% by placing water-soaked cotton wool on the cups.

Batches of 15 to 20 females were exposed to 4% DDT impregnated papers for 3, 7, 15, 30, 60, 120 or 240 min. In each test, a control group was exposed to "control" paper. Treated and "control" papers were obtained from WHO. Mortality was corrected by Abbott's formula (Abbott 1925). The LT₅₀s were estimated using the SEMPLA program for IBM computers.³

Bioassays on DDT treated wood walls were conducted at monthly intervals for 5 consecutive months at Tagachi, the municipality of Quibdo. The walls were sprayed at 2 g/m²AI, the normal DDT application rate for this antimalarial program. The bioassays followed procedures recommended by WHO (1981b). Anopheline mosquitoes for bioassays were collected and handled as were those used in the susceptibility tests.

RESULTS AND DISCUSSION

Results of the susceptibility tests are presented in Table 1. Mosquitoes from the 3 locations in Quibdo had some survival during all exposure times. In contrast, the Puerto Lleras population had 100% mortality for all exposure times greater than 60 min.

The LT₅₀ in the Quibdo population was 14.3 times higher than that in Puerto Lleras (Table 2). The LT₅₀ in Riosucio was only 2.2 times higher than that in Puerto Lleras.

Susceptibility tests using the diagnostic dosage (4% DDT, 1 h exposure time) in An. darlingi from Vigia del Fuerte, the nearest municipality of Quibdo, showed similar mortalities to those found in Quibdo when exposure times were equal

³ De Geraldino, A., O. Martinez, D. Villegas, J. Valero and M. Lopez. 1988. SEMPLA: Sistema de analisis estadistico computarizado para ensayos biologicos. Resumen XV Congreso Sociedad Colombiana de Entomologia (SOCOLEN), Manizales. p 81.

Table 3. Results of DDT susceptibility tests using the diagnostic dosage* in 3 populations of Anopheles darlingi from Colombia

Department Municipality Locality	Collection date	No. of tests	% mortality (no. tested)
Antioquia			
Vigia del Fuerte			
Buchado	May 1988	2	15.9 (119)
Buchado	Feb. 1989	$\frac{1}{2}$	16.0 (212)
Vigia cabecera	Feb. 1989	$\overline{2}$	31.5 (149)
Choco		_	01.0 (143)
Riosucio			
Murindo	Sept. 1988	1	97.1 (71)
Bolivar	•	.~	01.1 (11)
Achi			
Puerto Betania	July 1988	11	99.2 (1198)

^{*} Diagnostic dosage = exposure to 4% DDT for 1 h.

Table 4. Bioassay mortality of Anopheles darlingi exposed for 30 min on wood walls sprayed with DDT (2 g/m²AI), Tagachi, Quibdo, Colombia, in 1988

Months after	Month of spray			
spraying	February	July		
1	_	96.6 (59)*		
2	_	95.0 (80)		
3	87.0 (178)	93.4 (61)		
4	70.9 (173)	88.4 (95)		
5	92.5 (54)			

^{*} Percent mortality (no. mosquitoes tested).

(Table 3). In the municipality of Achi (Fig. 1), located approximately 300 km from Quibdo, mortality was over 99%, indicating DDT susceptibility similar to that of *An. darlingi* in Puerto Lleras.

Bioassay results obtained on wood walls in the Quibdo area are shown in Table 4. Mortality for the first 4 months ranged from 70.9 to 96.6% and was 92.5% in the test carried out 5 months after treatment. Thus, although the susceptibility tests reflected DDT resistance in this population, greater than 70% mortality was observed in anophelines placed in contact with DDT deposited on the walls following routine field control procedures.

This is the first time that DDT resistance has been documented for An. darlingi. Based on results from these studies, An. darlingi resistance to DDT in Colombia is presently restricted to the Quibdo area. Evaluation of mosquitoes from nearby areas and future monitoring of An. darlingi from this area is recommended. The use of alternative insecticides, for at least 1 or 2 spray cycles to determine efficacy, should be investigated.

ACKNOWLEDGMENTS

The cooperation of the Campanas Directas. Ministry of Public Health, Colombia, is gratefully acknowledged, special recognition goes to A. Rodriguez and J. Forero. The useful suggestions of G. P. Georghiou, University of California, are gratefully acknowledged. We thank all the field personnel of the malaria control program who assisted, with special thanks to A. Hernandez for data collection from the Bolivar region. We are deeply grateful to R. Londoño, SOCOLEN, for allowing us the use of the SEM-PLA software program. We also thank G. A. Fleming, PAHO entomologist in Bogota, M. J. Nelson, PAHO entomologist in Panama and P. Reiter and G. G. Clark, CDC entomologists in Puerto Rico, for their comments and constructive review of this manuscript.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267.

IGAC. 1977. Instituto Geografico Agustin Codazzi. Zonas de vida y formaciones vegetales de Colombia, 238 p.

Organizacion Mundial de la Salud. 1980. Resistencia de los vectores de enfermedades a los plaguicidas. Serie de informes tecnicos 655, 92 p.

Quiñones, M. L., M. F. Suarez and G. A. Fleming. 1987. Estado de la susceptibilidad al DDT de los principales vectores de malaria en Colombia y su implicacion epidemiologica. Biomedica 7:81-86.

World Health Organization. 1981a. Instructions for determining the susceptibility or resistance of adult mosquitoes to insecticides. Unpublished document. WHO/VBC 81.806.

World Health Organization. 1981b. Instructions for the bio-assay of insecticidal deposits on wall surfaces. Unpublished document. WHO/VBC 81.5.