INSECTICIDE SUSCEPTIBILITY STUDIES OF THREE CRYPTIC SPECIES OF THE *ANOPHELES BALABACENSIS* COMPLEX

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INTRODUCTION

An important pre-requisite in vector control practice is the baseline determination of the insecticide susceptibility status of the target species before the appropriate chemical measures are applied. In practical terms this requirement depends on the need to know as accurately as possible the specific status of the vector concerned. It has been shown that individuals of different sibling species of the *An. gambiae* complex differ in their respective biologies such as the ability to transmit malaria, man-biting behaviour and relative susceptibilities to insecticides. Often these characteristics appear to be species-specific in *Anopheles*. The fact that '*Anopheles balabacensis*' is not a single species but a group of genetically distinct but morphologically extremely similar species (Hii, 1982) requires an alternative interpretation for the data collected by Davidson (1975). He reported an overall 59% mortality of '*An. balabacensis*' males and females when exposed for one hour to 1% fenitrothion. This population had no previous exposure to this insecticide and its taxonomic status was also not ascertained then. In the light of Hii's (1982) biosystematic studies, it is expected that different species may require different strategies for control. From the public health viewpoint adoption of the appropriate strategems may lead to an improvement and better understanding in the control of the associated disease.

The members of the *An. balabacensis* complex comprise major vectors of malaria in Sabah, Thailand and neighbouring Asian countries. In Sabah, DDT at 2g/m^2 (2 cycles per year) has been used for malaria control since 1958 (Chow, 1970; Hii, 1979); in Thailand and the Arunachal Pradesh state of India, residual sprays of DDT have been used for a number of years (Scanlon and Sandhinand, 1965, Ismail and Phinichpongse, 1980; Pattanayak *et al*, 1980). DDT resistance has so far not been encountered for any member of the complex yet although varying degrees of success in malaria control have been documented. Poor results mainly occur when DDT spraying on a poor coverage or low dosage basis fails to interrupt malaria transmission in areas where *An.* '*balabacensis*' is the major vector. The assumption that '*balabacensis*' is the only vector may account for the lack of success in malaria control. However, as a proof, firm correlations between sibling species of the *balabacensis* complex and characters which are relevant to epidemiology and control must be established first so that reliable predictions can be made. For example, if sibling species A is found to be more tolerant than species B in certain regions and one is prepared to assume that in some other regions house spraying will be effective where only species B is found but not if species A is present. If such correlation is reliable enough to make such predictions, it would be essential to devote effort and

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resources to identifying sibling species in relation to vector control.

Since there are no recent baseline susceptibility test data of the population from chromosomally-identified taxa, this study was undertaken to screen the three crytic population against three groups of insecticides (chlorinated hydrocarbons, organophosphates and carbamates) and to provide baseline toxicity values which could be used to determine whether or not resistance has developed.

MATERIALS AND METHODS

The following colonies were used:

(1) Species A: Bangkok/A - derived from a subcolony held at the National Institute of Health Laboratory, Bethesda, Maryland, U.S.A. and provided by Dr. R.W. Gwadz. It was originally called the Bangkok colony collected in 1964 at Khao Mai Khaeo, Chon Buri Province, Thailand (Esah and Scanlon, 1966).

(2) Species B: Perlis/B - from a colony held at the Institute for Medical Research, Kuala Lumpur, Malaysia and originating from wild females collected in North Perlis in 1967.

(3) *An. balabacensis*: Sabah/C - founded in London from eggs collected from Lingan Papar district on June-July, 1979.

The details of insectary conditions and maintenance procedures are given by Bryan (1973). Standard tests for susceptibility to the three groups of insecticide were made on the adult progeny (less than 24 hr old) of the three chromosomally-identified colonies using the method recommended by WHO (1975). All the tests were performed under insectary temperature and humidity conditions. Mosquitoes were examined after 24 hr and mortality percentages were corrected by Abbott's formula.

Baseline susceptibility tests were made using standard WHO insecticide adult testing kits at diagnostic concentrations (4.0% DDT, 0.4% dieldrin, 5.0% malathion, 1.0% fenitrothion and 0.1% propoxur) for 1, $1\frac{1}{2}$ and 2 hr exposure and a 24 hr holding period. Impregnated papers were either supplied directly by WHO or prepared in the department from stock solutions supplied by WHO.

The selection of DDT and fenitrothion colonies from the Perlis/B and Sabah/C stocks was carried out with a view to establishing a highly resistant population. 4% DDT and 1% fenitrothion papers were used in the adult test kits, the latter being changed monthly. At each generation the survivours of the highest exposure period which give less than 30% survival were transferred into cages and allowed to mate among themselves or hand-mated as in the Sabah/C colony using the modified forced mating technique of Ow Yang *et al.*, (1963.) A blood meal was offered 2 days after the treatment and subsequently twice a week in order to obtain eggs.

The susceptibility of field populations of *An. balabacensis* to insecticides in Sabah were also carried out.

During the last 25 years, the bionomics of *An. balabacensis*, the primary malaria vector in Sabah has been studied. Among this is a series of insecticide susceptibility tests carried out before and after the launching of the Malaria Eradication Programme (1961-1970) which was reverted into a Control programme from 1971 to the present. After an initial malaria control pilot project (1955-1960) conducted in the interior residency of Sabah (in Keningau and Tenom districts), DDT proved to be an effective insecticide although dieldrin showed considerable promise and was also used for several years in other coastal districts in 1961-1962. This review is mainly based on unpublished reports by national and WHO staff of the malaria programme.

RESULTS

The percentage mortalities shown by adult populations for the three colonies are shown in Table 1. All three are suscetible to malathion,dieldrin,propoxur and pirimiphos-methyl. However, *An. dirus* species B appears to be tolerant to fenitrothion whereas species A is very susceptible. Species B and *An. balabacensis* also appear to be tolerant to 4% DDT.

Table 2 summarises an attempt to isolate a DDT and a fenitrothion resistant colony by selection. There are no indications of any response towards complete resistance after the seventh generation in the two selection lines (1 and 1½ hr exposures). Increasing the exposure time to 2 hr did not produce an increase of the mortality figures. On the other hand a response to DDT selection was

Table 1

Percentage mortalities of *An. dirus* species A, species B and *An. balabacensis* for susceptibility tests.

Species	Insecticide and Concentration	Exposure time (mins)	Percent in mortality
An. dirus	4% DDT	60	98.9 (99)*
Species A	1% fenitrothion	60	98.6 (147)
	,,	120	100.0 (115)
	0.1% propoxur	60	100.0 (86)
	0.4% dieldrin	60	100.0 (89)
	5% malathion	60	98.5 (204)
An. dirus	4% DDT	60	63.3 (983)
Species B	,,	90	75.6 (180)
	,,	120	98.5 (115)
	1% fenitrothion	60	15.0 (201)
	,,	90	79.5 (595)
	,,	120	98.5 (212)
	0.1% propoxur	60	100.0 (78)
	0.4% dieldrin	60	100.0 (62)
	5% malathion	60	96.1 (799)
	,,	90	100.0 (166)
An. balabacensis	4% DDT	60	79.9 (756)
	,,	90	91.9 (99)
	1% fenitrothion	60	80.9 (586)
	,,	120	100.0 (105)
	0.1% propoxur	60	100.0 (50)
	0.4% dieldrin	60	100.0 (55)
	5% malathion	60	100.0 (102)

* In parentheses are total numbers of one-day old males and females exposed.

Table 2

Results of selection pressure of 4% DDT and 1% fenitrothion for 1, 1½ and 2 hours on *An. dirus* species B (Perlis/B).

Generation Number	Hours of Exposure	Percentage mortality	
		4%DDT	1% fenitrothion
0	1	64.3 (983)*	34.0 (94)
	1½	75.6 (180)	86.9 (346)
1	1	27.3 (114)	38.2 (265)
	1½	59.4 (1308)	82.9 (2011)
2	1	46.2 (450)	43.8 (429)
	1½	60.8 (1707)	75.3 (2325)
3	1	78.7 (1798)	53.6 (823)
	1½	84.3 (413)	74.3 (1504)
4	1	68.7 (562)	79.8 (2732)
	1½	89.6 (77)	96.5 (657)
5	1	76.8 (371)	64.4 (871)
	1½	84.5 (84)	85.7 (180)
6	1	70.6 (934)	59.5 (186)
	1½	73.8 (408)	78.5 (93)
7	1½	68.7 (773)	84.1 (214)
	2	67.4 (344)	77.5 (765)
	2½	–	85.7 (143)
8	1½	50.6 (156)	78.8 (212)
	2	61.3 (142)	82.8 (122)
9	1½	48.8 (41)	83.6 (67)
	2	–	61.9 (42)
10	1½	70.6 (184)	95.2 (126)
	2	–	78.4 (51)

* In parentheses are total numbers of one-day old males and females exposed.

observed in *An. balabacensis* over four generations but results are inconclusive as the selection had to be discontinued (Table 3).

Insecticide susceptibility tests on adult anophelines were carried out extensively in several districts during 1959 to 1970 using serial concentrations of DDT-impreganted papers to establish the baseline values of LC_{50} and LC_{100}. The results are presented in Table 4. Since then, periodical checks at 6 to 12 monthly intervals were carried out with diagnostic exposure where only one insecticide concentration (4% DDT) is used for one hour. The results are pictorially shown in Fig. 1.

An. balabacensis was first found to show vigour tolerance to DDT in Sabah (Lingan, district of Papar) in 1970 after 27 cycles of DDT spraying. Susceptibility tests have shown a steady increase in the level of DDT tolerance for the parst 15 years (Table 5). Before 1970, 8 of the 9 localities (89%) tested in Sabah showed over 95% mortality when exposed to 4% DDT paper for one hour.

Table 3

Results of selection pressure of 4% DDT (1 and 1½ hour exposures) on *An. balabacensis* (Sabah/C).

Generation	Percentage mortality		
	1 hour	1½ hour	2 hours
Parent	84.7 (216)	94.9* (59)	100 (80)
1	74.5 (251)	91.8 (49)	–
2	59.4 (490)	65.9 (414)	87.5 (56)**
3	21.0 (76)	48.8 (41)	–
4	40.3 (119)	45.1 (275)	–
5	44.3 (61)	–	–

* In parentheses are total numbers of one-day old males and females exposed.

** Mortality figure was from adults taken from the 1½ hour selection line, i.e. the F_2 generation.

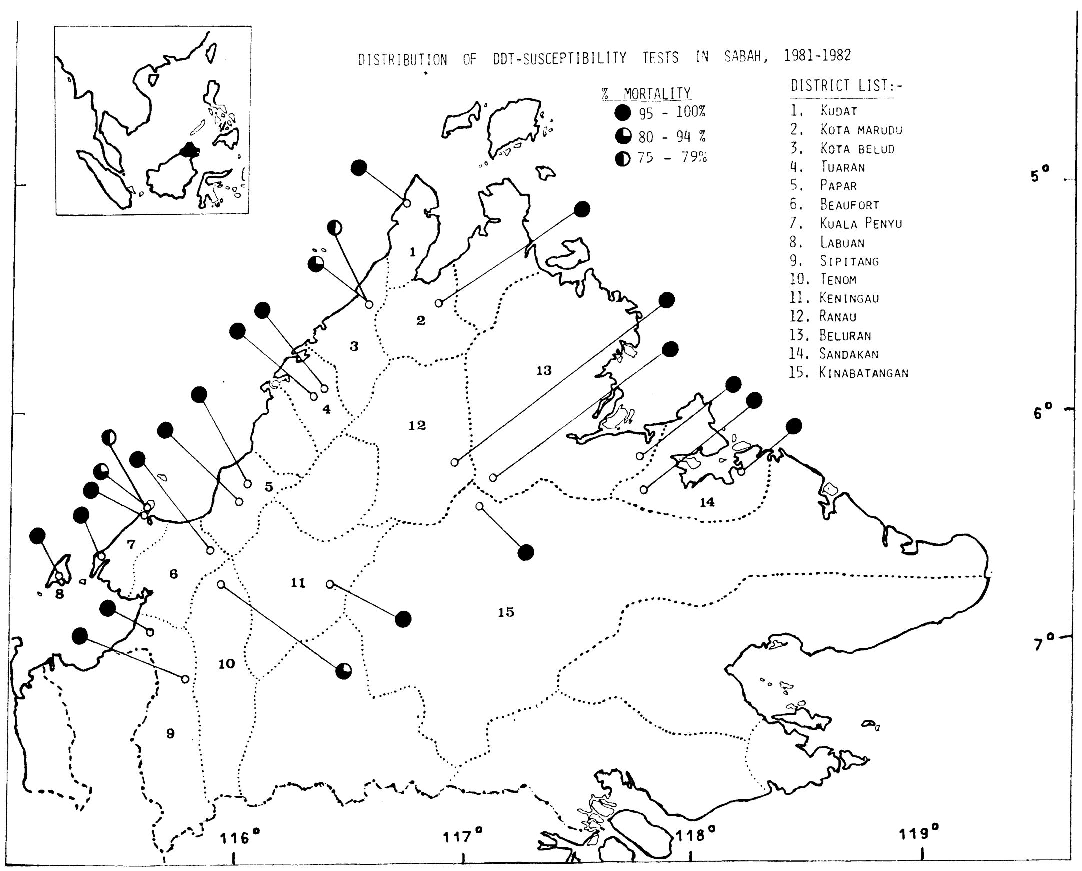

Fig. 1—Distribution of DDT susceptibility tests in Sabah, 1982.

Table 4

Susceptibility of wild populations of *An. balabacensis* females to DDT in Sabah, Malaysia.

Locality	Date of test	Spraying status	DDT LC_{50}	DDT LC_{100}
North	Feb/March, 1957	Unsprayed	0.37*	2.0*
Keningau	Oct/Nov., 1957	Unsprayed	0.44*	2.0*
	November, 1959	2 cycles dieldrin	0.70	–
		2 cycles DDT	0.70	4.0
	October, 1960	4 cycles DDT	1.30	4.0
	Sept., 1962	8 cycles DDT	1.50	–
	January, 1963	9 cycles DDT	1.70	4.0
	November, 1963	10 cycles DDT	–	4.0
	June, 1967	12 cycles DDT	1.50	4.0
	August, 1968	16 cycles	0.85	2.0
South Keningau	Feb/March, 1957	2 cycles DDT	0.72*	2.0*
Tenom	Oct/Nov., 1957	3 cycles DDT	0.65*	2.0*
	Feb/March, 1958	4 cycles DDT	0.65	2.0
Semporna	August, 1965	10 cycles DDT	–	2.0
	May, 1976	33 cycles DDT	1.35	3.0
Kuala Penyu	April, 1970	15 cycles DDT	1.18	2.2
	November, 1970	15 cycles DDT	0.68	1.6
Papar	January, 1970	5 cycles DDT	1.30	2.0

* Data collected from 1957 were based on the Busvine and Nash (1953) technique.

Table 5

Changes in levels of DDT susceptibility of *Anopheles balabacensis* in 16 districts of Sabah, Malaysia from 1967 to 1982.

Years tested	Number of areas tested	% of total areas showing % mortality of		
		75 - 79	80 - 94	95 - 100
1967-1970	9	–	11	89
1971-1976	11	–	18.2	81.8
1976-1982	80	2.5	18.8	78.8

This percentage of localities declined gradually to 78.8% for the last seven years. On the other hand, areas showing 80-94% mortality did not change much at all betwen 1971-1975 and 1976-1982.

DISCUSSION

It appears that there is some degree of tolerance to DDT and fenitrothion in *An. dirus* species B the nature of which is not fully understood. It is certain that this was the same species tested by Davidson (1975) who obtained a low mortality (59% kill) when 787 males and females of '*An. balabacensis*' were exposed for 1 hr to 1% fenitrothion. This was verified by checking the X chromosomes of fourth instar larvae salivary glands from the same colony that then existed in the Ross Insectaries (Hii, 1982). The term 'vigour tolerance' is used in the way Ismail and Phinichpongse (1980) defined as indicating merely a condition of reduced susceptibility resulting from the continued selection of a population that does not have specific genes for resistance to the particular insecticide. There is also some evidence to show that tolerance to DDT may be due to seasonal variations in morphophysiological characteristics of *An. balabacensis* populations in Sabah. This could probably explain the seasonal variations in the susceptibility of this species to 4% DDT.

It is of interest to note that the LC_{50} (= 0.3%) of '*An. balabacensis*' (= *An. dirus* species A of Peyton & Harrison) from Khao Mai Keao, Thailand correspond closely to the normal range of *An. balabacensis* from Sabah. The present study indicates that at the diagnostic concentration, *An. dirus* species A is susceptible to DDT. However, Ismail and Phinichpongse (1980) reported '*balabacensis*' to be susceptible to DDT in 2 cantons of Thailand but appeared tolerant in the Bandong canton. In the latter area, 4 h exposures were required to produce 100% mortality in 2 tests (in Sabah, 2 h exposures gave 100% mortality in 18 out of 20 tests). They concluded that the insecticide status of the vector species was indeterminate and suggested further tests to distinguish betweeen tolerant and truly resistant individuals. It may also be possible that they were dealing with a mixture of sympatric species when more than one cryptic species of the taxon '*An. balabacensis*' was sampled in the area.

The results suggest that DDT, fenitrothion, propoxur and malathion are effective against some members of the complex with the exception of *An. dirus* sepcies B and *An. balabacensis* which show a considerable degree of tolerance to DDT and fenitrothion. Both species will readily feed on cattle. The vast expanse of the country and breeding places available compared to human dwellings would mean that a large proportion of the vector population, by virtue of their resting habits do not remain long enough inside human habitations and therefore has a greater survival rate. It is reasonable to presume that, taking the overall vector population, the insecticide pressure on it by residual spraying is very little. Any selection towards a resistant gene is bound to be lost in the general population and would have little chance to establish itself. Field selection for resistance can also be reduced by minimizing mortality, not by dropping the dose, which may encourage survival of heterozygotes but by leaving more surfaces unsprayed (Wood and Mani, 1981, Curtis and Davidson, 1981). In the field, selection pressure of DDT resulting from house spraying may not be great enough to markedly accelerate the development of DDT resistance in *An. balabacensis* due to such factors as : the uniformity of the residue; the decay of the residue between one spraying and the next; the average duration of house resting of the mosquitoes and its variability; inadequate spraying coverage of the houses (Curtis and Davidson, 1981).

SUMMARY

Susceptibilities of two colonies of the taxon *An. dirus* (one from Perlis and from Thailand) and one colony of *An. balabacensis* from Sabah to DDT, dieldrin, malathion, fenitrothion and propoxur were determined. DDT and fenitrothion tolerance was found in *An. dirus* species B and *An. balabacensis*. No resistant strain was isolated as the two

colonies were not either homozygous or nearly so for resistance.

Field testing of the susceptibility of the adults of *An. balabacensis* to DDT was carried out between 1957 to 1976. The results indicated a progressive rise in the LC_{50} levels greater than 1% in almost all instance. The variation in the number of sprays applied in some districts have resulted in varying sensitivities. Association between the changes in levels of DDT susceptibility and exophilic habit of *An. balabacensis* has been observed but needs further confirmation. The significance of these findings and the difficulties in distinguishing tolerant from truly resistant individuals are discussed in relation to accurate species identification.

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