RECENT OBSERVATIONS ON INSECTICIDE RESISTANCE IN *ANOPHELES STEPHENSI* IN IRAQ

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The development of physiological resistance to DDT and dieldrin in *Anopheles stephensi* is probably the most serious technical problem encountered in the malaria eradication campaigns in the Middle East. The first indications that DDT was failing to interrupt malaria transmitted by *A. stephensi* came from Saudi Arabia. Resistance, suspected in 1953 by the increase of malaria cases and the presence of *A. stephensi* resting on recently sprayed surfaces (Daggy, 1957), was confirmed in 1955 (Davidson, 1958). For 2 years DDT resistance in *A. stephensi* appeared to be confined to a small area in the northeast of Saudi Arabia but during 1957 spread to practically all the area of distribution of the species in the south of Iran[4] and Iraq (Gramiccia *et al.*, 1958).

The replacement of DDT by dieldrin initially met with success. The DDT-resistant *A. stephensi*, which in 1957 had reached high densities and had produced what amounted practically to an epidemic of malaria in the plain of Khuzistan in Iran, was drastically reduced in numbers immediately after the introduction of dieldrin. So great was the effect of this insecticide that by 1958 the species could not be found in the plains of Khuzistan[5] and Southern Iraq[6] where it has been considered the sole vector. In 1959, however, dieldrin resistance made its first appearance in the foothills of the Zagros Mountains in South-Eastern Iran.[5] By 1960 it had extended to part of the plain of Khuzistan and the following year it spread to practically all the area of distribution of *A. stephensi* in the south of Iran[5] and Iraq.[6]

The development of double resistance in one of the main vectors in Iran and Iraq posed a serious problem to the malaria eradication campaigns in the two countries. The dieldrin resistance was, as in other cases of resistance to this insecticide, of a very high order, rendering dieldrin and allied compounds operationally useless. Resistance to DDT, on the other hand, was of a low order, as observed in the field and in the laboratory (Davidson and Jackson, 1961) and it was still possible to use this insecticide for residual spraying against *A. stephensi*. This was done in Iraq in 1963, after an outbreak of malaria in Basrah city; and in view of the favourable results obtained all the Southern Region was soon put again under DDT residual spraying. When in 1965 the Central Region reverted to the attack phase due to a widespread renewal of transmission, DDT was again the insecticide chosen, thus bringing under DDT residual spraying practically all the area of distribution of *A. stephensi* in Iraq. The operational results obtained after the reintroduction of DDT have been discussed in the periodical reports of the Malaria Eradication Service of Iraq, in evaluation reports of WHO and in a recent review (WHO, 1967). The

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[5] C. M. H. Mofidi (1962). Resistance of *Anopheles (M.) stephensi* to insecticides in Iran (Unpublished paper presented to CENTO Scientific Symposium, Lahore, January 1962).

[6] T. G. Hendow (1963). Report on malaria in the Southern Region of Iraq (Unpublished report presented at the Inter-Country Malaria Eradication Co-ordination Meeting, Teheran, November 1963).

object of the present paper is only to summarize and discuss briefly recent field and laboratory investigations concerning insecticide resistance in *A. stephensi* in Iraq.

FIELD OBSERVATIONS. A comparison of the results of susceptibility tests on *A. stephensi*, carried out in Iraq from March 1958 till October 1967,[7] indicate, in broad terms, a decrease of the level of resistance from the south to the north of the country. The highest level of resistance was observed in the extreme south of the country, in the Fao area during 1966, whereas in Washash (Baghdad) in 1965 there was barely an indication of resistance.

The results of the tests carried out during the 10-year period also indicate in general terms, that, with only an important exception, to be discussed later, there was no major change in the level of resistance in each locality studied. The tests were carried out in conditions as uniform as possible, following the standard WHO method (WHO, 1963). The exposure for 1 hour to the critical 4 percent DDT concentration produced mortalities above the 20 percent level, when the mosquitoes tested had been collected in unsprayed shelters It was only in November 1966 when a significant increase of resistance was observed in Basrah Liwa.

It was noticed there that the 1-hour exposure to the 4 percent DDT concentration gave mortalities of 10.2 percent in Gezira (near Basrah city), 2.4 percent in Fadghiya (further south) and no mortality in Fao Mamlaha. In this last locality a mortality of only 2.5 percent was obtained after 4 hours' exposure to the same concentration.

These results were confirmed at the Ross Institute in London, using material sent from the field (see below); they indicate a much higher level of resistance than had previously been observed anywhere in *A. stephensi*. The increase of resistance coincided with an approximately 10-fold increase of *A. stephensi* densities in Basrah Liwa and a spread of the species beyond its normal limits of distribution in the country (specimens found as far north as Kirkuk Liwa).

Tests carried out in December 1966 and May 1967 indicated a diminution of resistance, although at Gezira only 4 percent mortality was obtained in May following the usual critical exposure Searches for *A. stephensi* in Fao during that month failed to secure sufficient specimens for testing, most of the breeding places having been flushed by floods. For this reason the level of resistance remained unknown in Fao during the spring of 1967, but by October it appeared to be close to the level found before the peak of resistance of the autumn of 1966. This also seemed to be the case in other localities of Basrah Liwa, where resistance declined and at the same time densities of *A. stephensi* diminished.

LABORATORY INVESTIGATIONS. The first study of the inheritance of DDT resistance in *A. stephensi* was made by Davidson and Jackson (1961) using a resistant strain from Moawiya (Basrah) supplied by Gramiccia in 1957 and a susceptible one from India, kept at the Ross Institute for a long period of time. The work demonstrated the monofactorial type of inheritance of DDT resistance in *A. stephensi* and its near-recessive character. It showed also that although the two strains studied came from widely separated areas, they belonged to a single species *(A. stephensi mysorensis)*, as the fertile offspring obtained from all crosses indicated.

The possibility that more than one form of this species existed in Iraq had not, however, been ruled out. Of particular interest was the Fao area where, in contrast with other parts of Southern Iraq, transmission had persisted since the reintroduction of DDT in 1964. The fact that in Fao the breeding places of *A.*

[7] Detailed information on these tests can be found in the following unpublished WHO document:

J. de Zulueta, T. L. Chang, J. R. Cullen, and G. Davidson (1968). Insecticide resistance in *Anopheles stephensi* in Iraq at the end of 1967. WHO/Mal/68.655 and WHO/VBC/68.78.

stephensi often have a very high salinity (equal to or higher than that of seawater) suggested the possible existence of a salt-water-breeding *A. stephensi* biologically distinct from the freshwater form.

To elucidate this problem, material from various localities in Iraq was sent to the Ross Institute in London (usually newly-laid eggs placed in a sealed plastic envelope, sent in an airmail letter). Altogether three different strains from Iraq, namely from Gezira (Basrah), Mamlaha (Fao) and Washash (Baghdad) were crossed with two other strains of *A. stephensi* maintained at the Ross Institute: the original strain from Delhi (India) with which the first investigations on DDT resistance (Davidson & Jackson, 1961) had been made and a strain from Kazerun (Iran) supplied by the Institute of Public Health Research. The following crosses were performed between the five strains:

Washash male x Mamlaha female
Gazalia male x Mamlaha female
Mamlaha male x Washash female
Gazalia male x Washash female
Mamlaha male x Delhi female
Washash male x Delhi female
Kazerun male x Washash female

All crosses produced viable F_1 generations, the males of which had normal testes containing normal spermatozoa. When left in 1 ft^3 cages F_1 males and females mated and the latter laid viable F_2 eggs.

There is thus no evidence of hybrid sterility between the five populations of *A. stephensi* from India, Iraq and Iran. This, of course, does not rule out altogether the possible existence of a species complex, members of which could conceivably be interfertile in the laboratory but prevented from interbreeding in the field by behavioural differences. But this is unlikely, as such differences preventing interbreeding over a considerable period of time should lead to the development of genetic differences producing intersterility.

If the existence of a single species of *A. stephensi* seemed to be established despite the above reservations, the increase of DDT resistance beyond what had been considered hitherto the limits of homozygous resistance raised the question of whether more than one type of DDT resistance existed in the south of Iraq. Early investigations had shown that among homozygous resistant females exposed to 4 percent DDT for 1 hour there was a mortality of 9 percent (Davidson and Jackson, 1961). But in the Fao-Mamlaha population the tests in the field in 1966 produced no mortality among females exposed for 1 or even 2 hours to the same concentration. Laboratory results in London also showed no mortality to 4 percent DDT after 1-hour exposure.

To investigate the possibility of a different genetic mechanism, crosses were made in London between the Fao-Mamlaha and the Gezira colonies (after each was selected at 4 percent DDT for 4 hours). As in other crosses already discussed, there was again no question of hybrid sterility and the F_2 generation was readily produced. Testing both the F_1 and F_2 generations on 4 percent DDT for 1 and 4 hours showed that the hybrid was, if anything, more resistant than the parents, probably due to hybrid vigour (Table 1). The indication of these results is that both resistances are genetically identical.

Crosses were also made between the Fao-Mamlaha strain and the Delhi susceptible strain. The mortality in the F_1 exposed to 4 percent DDT for 1 hour indicates that the resistance is near-dominant (Table 1). Testing the offspring of the backcross of this F_1 to the susceptible on the same dosage (which kills virtually 100 percent of susceptibles), indicates a single genetic factor as being involved. The indication of these crosses was, therefore, that the same oligogene was always involved and that an explanation of the enhanced resistance seen in a strain like the one of Fao-Mamlaha might

TABLE 1.—Results of crosses with two resistant strains (Gezira and Mamlaha) and one susceptible strain (Delhi) of *A. stephensi*.

Strain	Exposure time	Percentage mortalities *
Mamlaha	1 hour	0 (90)
	4 hours	15 (450)
Gezira	4 hours	11 (211)
Mamlaha male x Gezira female, F_1	1 hour	2 (138)
	4 hours	5 (74)
Gezira male x Mamlaha female, F_1	1 hour	1 (71)
	4 hours	0 (24)
Mamlaha male x Gezira female, F_2	1 hour	0 (45)
	4 hours	0 (166)
Gezira male x Mamlaha female, F_2	1 hour	3 (135)
	4 hours	0 (29)
Mamlaha male x Delhi female, F_1	1 hour	7 (313)
Backcross to Delhi female	1 hour	55 (788)

* The figures in parentheses after the percentage mortalities indicate the numbers exposed.

be sought in the possible effect of ancillary genes.

DISCUSSION. From the information summarized in this paper it is clear that the level of resistance of *A. stephensi* in Iraq did not suffer any significant change until the autumn of 1966. For 3 years since its reintroduction in 1963, DDT had been used in the south of the country without this selective pressure bringing any visible stepping-up of resistance. When an increase finally took place in the autumn of 1966 it coincided with a great increase of *A. stephensi* densities in the south of the country and a spread of the species towards the north, beyond its normal range of distribution.

From the results of tests in the field and the laboratory in November 1966, it appears that the populations of *A. stephensi* in Fao and in other areas of the Basrah Liwa were then approaching near-homozygosity for the oligogene and the ancillary genes responsible for DDT resistance. Such a degree of resistance combined with the high densities had, as would be expected, a marked effect on the epidemiological situation. Transmission, which had been much reduced in Basrah Liwa since the reintroduction of DDT in 1963 (WHO, 1967), increased in the area despite good spraying coverage and satisfactory surveillance operations. This was particularly clear in the locality of Fao where, despite much thoroughness in the operations and supplementary measures such as a third annual spraying cycle and two mass radical chemotherapy treatments, there was a marked increase of transmission during the second half of 1966.

The situation regarding the DDT resistance of *A. stephensi* in Iran was similar to that observed in Iraq, as expected in view of the similarity of topographical and ecological conditions in the two countries. Extensive field investigations carried out in the south of Iran since DDT resistance was first detected in 1957 have shown that the level of resistance did not suffer any marked change in this vast territory until the end of 1965.[8] Tests carried out that year in Iran by two of us (J. R. C. and J. de Z.) confirmed previous findings including the persistence of high levels of resistance (mortalities of 33–64 percent on 1-hour exposure to 4 percent DDT) in

[8] Institute of Public Health Research. Progress report on investigations and researches on malaria epidemiology and malaria eradication in Iran, 1964–1965. (Unpublished report presented to the Sixth Iran-Iraq Border Meeting on Malaria Eradication, Basrah, December 1965).

areas in the south of Iran where DDT had not been used for 3 years, an indication that complete reversion to susceptibility in a field population may be a long process.

The year 1966 saw a stepping-up of resistance in *A. stephensi* in the plain of Khuzistan, parallel to that seen on the Iraqi side, though no resistance levels as high as those observed in the Fao area have been reported from Iran. It seems probable, therefore, that the thorough DDT spraying coverage in Basrah Liwa, particularly in Fao, and the use in this locality of three instead of two spraying cycles in 1966, may have contributed to the development that year of an exceptionally resistant population.

CONCLUSIONS. From the information presented in this paper, the following main conclusions can be drawn:

1. The level of DDT resistance in *A. stephensi* in Iraq did not suffer any significant change during the first 3 years following the reintroduction of DDT in the south of the country in 1963.
2. A marked increase of DDT resistance was seen during the autumn of 1966 in *A. stephensi* coinciding with an increase of density in this species. A reversion towards the resistance and density levels observed before this peak was noticed in 1967.
3. Since the reintroduction of DDT in 1963 a substantial reduction but not a complete interruption of transmission has been observed in the south of Iraq.
4. A single species of *A. stephensi* is represented by all the strains so far studied from Iraq as well as Iran. The changes in the pattern of resistance to DDT in this species in the southern part of Iraq might be explained by the existence of genes ancillary to the oligogene common to all the populations studied. Some of these ancillary genes may have been selected to near-homozygosity in Southern Iraq leading to the exceptionally high temporary level of resistance observed. Absolute homozygosity for all the genes does not appear to have been achieved however, in the light of the increased mortalities recorded in tests in the same area in 1967.

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