WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTÉ

WHO/Mal/68.655
WHO/VBC/68.78

ENGLISH ONLY
(avec résumé en français)

INSECTICIDE RESISTANCE IN ANOPHELES STEPHENSI IN IRAQ: AT THE END OF 1967

by

J. de Zulueta,[1] T. L. Chang,[1] J. R. Cullen[2] and G. Davidson[3]

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 two years DDT resistance in A. stephensi appeared to be confined to a small area in the north-east 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 met initially 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 had 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

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[1] WHO Malaria Eradication Evaluation Project, Beirut, Lebanon.

[2] WHO malaria eradication programme, Tunisia.

[3] Ross Institute of Tropical Hygiene, London, United Kingdom.

[4] Mofidi, C. M. H. et al. (1957) A. stephensi resistance to DDT in Iran. In: Information circular on the resistance problem, No. 11 (Mimeographed WHO document).

[5] Mofidi, C. M. H. (1962) Resistance of Anopheles (M.) stephensi to insecticides in Iran (Unpublished paper presented to CENTO Scientific Symposium, Lahore, January 1962).

[6] Hendow, T. G. (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).

other hand, was of a low order, as observed in the field and in the laboratory (Davidson & 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 object of the present paper is only to summarize and discuss briefly the field and laboratory investigations concerning insecticide resistance in A. stephensi in Iraq up to the end of 1967.

Field observations

In Table 1 are shown the results of susceptibility tests on A. stephensi in Iraq, using the standard WHO method (WHO, 1963) and the critical concentration of 4% DDT, commonly employed in these tests to determine the degree of resistance present in the tested sample. It was not used, however, in the first tests that indicated a development of DDT resistance (Gramiccia et al., 1958); so the results then obtained cannot be readily compared with those of later tests. They indicated none the less a moderate degree of resistance (LC_{50} of approximately 2%).

The results given in Table 1, based mostly on collections and tests carried out by one of us (T.L.C.) in conditions as uniform as possible, indicate, in broad terms, a decrease in 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.

In Table 1 it is indicated whether the samples tested came from sprayed or unsprayed shelters. A higher mortality could theoretically be expected with samples from the latter but this, as will be seen from the results of the table, did not always happen, probably due to the fact that the unsprayed shelters were always in areas heavily sprayed with DDT, so that a previous contact with the insecticide was highly probable before the mosquitos reached an unsprayed surface.

The variations observed from year to year in the level of resistance (as represented by the proportion surviving a standard exposure) are not of sufficient amplitude to be considered of significance, with one important exception: the increase of resistance seen in Basrah Liwa during the autumn of 1966. During the second half of November the one-hour exposure to 4% DDT gave mortalities of 10.2 in Gezira (opposite Basrah), 2.4% in Fadaghiya (further south) and no mortality in Fao Mamlaha. In this last locality a mortality of only 2.5% was obtained after four 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% 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 being 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 on resistant A. stephensi at the Ross Institute, London

The first study of the inheritance of DDT resistance in A. stephensi was made by Davidson & 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. stephensi have often a very high salinity (equal to or higher than that of sea-water) 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 Malaha 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% DDT for one hour there was a mortality of 9% (Davidson & Jackson, 1961). But in the Fao-Mamlaha population the tests in the field in 1966 produced no mortality among females exposed for one or even two hours to the same concentration. Laboratory results in London also showed no mortality to 4% DDT after one-hour exposure.

To investigate the possibility of a different genetical mechanism crosses were made in London between the Fao-Mamlaha and the Gezira colonies (after each was selected at 4% DDT for four 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% DDT for one and four hours showed that the hybrid was, if anything, more resistant than the parents, probably due to hybrid vigour (Table 2). 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% DDT for one hour indicates that the resistance is near-dominant (Table 2). Testing the offspring of the backcross of this F_1 to the susceptible on the same dosage (which kills virtually 100% 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 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 three 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.[1]

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[1] 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).

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% on one-hour exposure to 4% DDT) in areas in the south of Iran where DDT had not been used for three 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 three 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.

RESUME

L'apparition d'une résistance physiologique au DDT et à la dieldrine chez Anopheles stephensi dans certaines parties de l'Arabie Saoudite, de l'Irak et de l'Iran a posé de grands problèmes techniques pour l'éradication du paludisme dans ces zones. La première indication que le DDT devenait moins efficace est parvenue en 1953 d'Arabie Saoudite : accroissement du nombre des cas de paludisme dans une zone traitée et découverte d'A. stephensi au repos sur des surfaces récemment traitées. Dès 1957, A. stephensi faisait également preuve en Irak et en Iran d'une résistance au DDT dans toutes ses zones de répartition. Le remplacement du DDT par la dieldrine en 1957 a été efficace au début, mais une résistance a été signalée à nouveau en 1959 et s'est généralisée en 1960. De plus, le degré de la résistance à la dieldrine était élevé, alors que celui de la résistance au DDT était relativement faible et qu'aux endroits où l'emploi du DDT a été repris, cet insecticide a assuré pendant environ 3 ans une protection raisonnablement satisfaisante contre le vecteur.

Toutefois, on a signalé vers la fin de 1966 une résistance au DDT d'un degré beaucoup plus élevé à Basra Liwa, en Irak, où pendant la deuxième moitié du mois de novembre 1966 l'exposition normale d'une heure au DDT à 4 % n'a entraîné aucune mortalité en un certain lieu et où une exposition de 4 heures n'a abattu que 2,5 % des insectes soumis à l'épreuve. Ces résultats ont été ultérieurement confirmés par le laboratoire du Centre international OMS de référence, du Ross Institute, Londres. D'un autre côté, de nouveaux essais effectués sur place en octobre 1967 ont fait apparaître un retour au niveau moyen antérieur de la résistance au DDT.

On savait déjà que la transmission de la résistance au DDT chez A. stephensi dépend d'un seul facteur et a un caractère presque récessif; ces résultats donnaient donc à penser qu'il pourrait exister en Irak non pas une seule forme de cette espèce, mais plusieurs, particulièrement du fait qu'à Fao les lieux de reproduction présentent un haut degré de salinité. Toutefois, en croisant, au Ross Institute, cette souche avec d'autres souches, notamment la souche sensible normale de l'Inde et la souche originellement résistante de l'Iran, on a obtenu des générations F1 et F2 viables; il n'y avait donc pas de signe de la stérilité des hybrides.

D'après les résultats de l'exposition au DDT des croisements d'hybrides de la souche résistante d'Irak avec la souche sensible de Delhi, on a considéré que la résistance observée en 1967 était presque dominante. D'autres travaux ont indiqué l'intervention d'un facteur génétique unique.

Les faits exposés dans le document conduisent aux principales conclusions suivantes :

1. Le niveau de la résistance au DDT chez A. stephensi en Irak n'a pas beaucoup varié pendant les trois premières années qui ont suivi le retour à l'emploi du DDT dans le sud du pays en 1963.

2. Un accroissement marqué de la résistance au DDT a été constaté pendant l'automne de 1966 chez A. stephensi, en coïncidence avec un accroissement de la densité des insectes de cette espèce. En 1967, on a constaté que la résistance et la densité revenaient aux niveaux qui avaient été observés avant ces pointes.

3. Après le retour à l'emploi du DDT en 1963, on a remarqué dans le sud de l'Irak une réduction importante de la transmission, mais non son interruption complète.

4. Toutes les souches d'A. stephensi qui ont été étudiées jusqu'à présent en Irak aussi bien qu'en Iran appartiennent à une espèce unique. Les changements enregistrés dans la manière dont les insectes de cette espèce résistent au DDT dans le sud de l'Irak pourraient s'expliquer par l'existence de gènes secondaires à côté de l'oligogène commun à toutes les populations étudiées. Certains de ces gènes secondaires pourraient, par un processus de sélection, être devenus presque homozygotes dans le sud de l'Irak, ce qui aurait entraîné le niveau exceptionnellement élevé de résistance qui a été constaté temporairement. Il ne semble pas, toutefois, que tous les gènes soient devenus absolument homozygotes, vu les taux accrus de mortalité qui ont été enregistrés lors des épreuves faites dans la même zone en 1967.

ACKNOWLEDGEMENTS

The authors of this paper wish to express their gratitude to all those who helped them in their investigations in Iraq, notably Dr A. Arif, Director-General of Preventive Medicine, Ministry of Health, Baghdad; Dr M. Ibrahim, Director of the Endemic Diseases Institute, Baghdad; Dr A. Aziz, Director, and Dr T. Hendow, Director, Southern Region, Malaria Eradication Organization, as well as Drs P. Singh and T. Matsushima of WHO Malaria Eradication Project, Iraq. The assistance in the preparation of this paper of Dr A. R. Zahar of WHO, Eastern Mediterranean Regional Office, is also gratefully acknowledged.

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TABLE 1. SUMMARY OF ADULT SUSCEPTIBILITY TESTS CARRIED OUT IN IRAQ ON A. STEPHENSI SINCE THE DETECTION OF DDT RESISTANCE

Observer	Locality	Liwa	Date	Percentage mortality*	
				4% DDT (1 hour exposure)	Control
Petridis & Caprari**	Hartha	Basrah	March-April 1958	6.3 (80)	1.2 (84)
Chang	Gezira, Shatt-al Arab	Basrah	October 1961	64.7 (68)	1.0 (100)
Sacca	Gezira, Shatt-al Arab	Basrah	May 1962	76.5 (98)	0.0 (100)
Chang	Gezira, Shatt-al Arab	Basrah	October 1962	53.6 (56)	0.0 (35)
Thymakis**	Gezira, Shatt-al Arab	Basrah	August 1963	20.6 (63)	0.0 (99)
Moosami	Hartha	Basrah	July 1964	40.0 (177)	5.3 (57)
Chang	Gezira, Shatt-al Arab	Basrah	September 1964	60.7 (61)	0.0 (60)
Chang & Cullen	Sugash Shyukli	Nassiriyah	April 1965	26.7 (116)	0.0 (61)
Chang & Cullen	Gezira, Shatt-al Arab	Basrah	May 1965	34.3 (108)	0.0 (64)
Chang & Cullen	Washash	Baghdad	November 1965	85.0 (80)	0.0 (39)
Chang & Cullen	Gezira, Shatt-al Arab	Basrah	November 1965	54.7 (86)	0.0 (39)
Chang & Cullen	Fao	Basrah	November 1965	76.3 (38)	0.0 (31)
Chang & Cullen	Gezira, Shatt-al Arab	Basrah	May 1966	65.7 (99)	0.0 (77)
Chang & Cullen**	Gezira, Shatt-al Arab	Basrah	May 1966	54.5 (112)	0.0 (88)
Chang & Cullen**	Fao	Basrah	May 1966	37.4 (24)	

* The figures in brackets after the percentage mortalities indicate the number of mosquitos tested.

** Samples from sprayed shelters.

TABLE 1. SUMMARY OF ADULT SUSCEPTIBILITY TESTS CARRIED OUT IN IRAQ ON A. STEPHENSI SINCE THE DETECTION OF DDT RESISTANCE (continued)

Observer	Locality	Liwa	Date	Percentage mortality*	
				4% DDT (1 hour exposure)	Control
Chang & Cullen***	Gezira, Shatt-al Arab	Basrah	November 1966	10.2 (235)	0.0 (119)
Chang & Cullen***	Fadaghiya	Basrah	November 1966	2.4 (82)	0.0 (23)
Chang & Cullen***	Fao	Basrah	November 1966	1.0 (200)	1.0 (100)
Tikriti	Gezira, Shatt-al Arab	Basrah	November 1966	58.7 (145)	0.0 (25)
Chang	Gezira, Shatt-al Arab	Basrah	December 1966	57.1 (492)	0.0 (100)
Chang	Fao	Basrah	December 1966	19.0 (84)	
Chang**	Fao	Basrah	December 1966	0.0 (20)	
Chang	Fadaghiya	Basrah	December 1966	29.2 (48)	
Chang	Gezira, Shatt-al Arab	Basrah	May 1967	29.6 (220)	0.0 (112)
Chang**	Gezira, Shatt-al Arab	Basrah	May 1967	4.0 (75)	0.0 (25)
Chang	Swaib	Basrah	May 1967	31.1 (180)	1.1 (90)
Chang***	Gezira, Shatt-al Arab	Basrah	October 1967	21.3 (80)	0.0 (19)
Chang**	Gezira, Shatt-al Arab	Basrah	October 1967	43.0 (100)	2.0 (49)
Chang**	Fadaghiya	Basrah	October 1967	16.7 (18)	0.0 (8)
Chang***	Fao	Basrah	October 1967	36.9 (65)	0.0 (22)
Chang**	Moawiya	Basrah	October 1967	30.8 (13)	0.0 (18)
Chang**	Swaib	Basrah	October 1967	24.0 (50)	0.0 (18)

* The figures in brackets after the percentage mortalities indicate the number of mosquitos tested.

** Samples from sprayed shelters.

*** Mixed samples from sprayed and unsprayed shelters (where no separate records exist).

TABLE 2. 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 brackets after the percentage mortalities indicate the numbers exposed.

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