

a 63732



WHO/Vector Control/63
WHO/Mal/440
23 March 1964

ORIGINAL: ENGLISH

ECOLOGY OF INDIAN ANOPHELINE MOSQUITOS IN RELATION
TO MALARIA ERADICATION¹

by

R. Pal
Division of Environmental Health,
World Health Organization, Geneva

1. Introduction

The ecology of anopheline vectors in relation to malaria eradication has presented a very interesting study in the Indian subcontinent, since the species include a heterogeneous collection with different larval habitats and varying degrees of exophily and exophagy and endophily and endophagy. However, in spite of the diverse habits of these species malaria control to a greater or lesser degree has followed wherever premises have been sprayed efficiently with residual insecticides. The two most notorious vectors of malaria in India may be cited as examples of this: A. culicifacies is a domestic species with large numerical prevalence, predominantly zoophilic, with a poor infectivity rate, whilst A. fluviatilis is a sparse species resting mostly outdoors, predominantly anthrophilic, with high sporozoite rate. Malaria transmitted by both has been equally well controlled although it was believed at one time that it would be easier to eradicate stable malaria transmitted by A. fluviatilis than unstable malaria transmitted by A. culicifacies (Pal & Sharman, 1955). Apart from the final effect on transmission there is a wide range in the reaction of vectors to insecticidal treatment. At one extreme, species like A. minimus, A. philippinensis, A. sundaicus and A. fluviatilis (in part) have virtually disappeared from formerly prevalent areas through anti-adult measures alone. At the other extreme, species like A. jeyporiensis candidiensis and A. leucosphyrus leucosphyrus have hardly been affected.

¹ This investigation was supported by the World Health Organization and by a United States Public Health Service Research Grant (EF.194) to the World Health Organization.

During the past 10 years routine mosquito collections have been made in all 390 malaria units in India, each responsible for 1 000 000 people, and it is evident from the data collected that in some parts of the country the vector population has shown a gradual decline during this period and that in many areas densities have decreased to a level where it is often extremely difficult to find the vector species. However, the situation regarding A. culicifacies and A. fluviatilis is somewhat complicated. A. culicifacies decreased very appreciably after spraying operations had been carried out throughout the country but it has now reappeared in large numbers. Densities of A. fluviatilis also dropped considerably in South India but in the Terai area of Upper Pradesh, in spite of sustained spraying operations, the numbers remained fairly high. It has been of particular interest to study the reappearance of some of these anopheline species after the withdrawal of spraying. In the Kanara district, where only a dozen adults and a few larvae of A. fluviatilis were found in an area covering 4000 square miles, there was a gradual reappearance of A. fluviatilis; similarly in Saklespur Taluk of Mysore State both A. culicifacies and A. fluviatilis began to reappear after the cessation of spraying, although the numbers collected were low and all the specimens came from cattle sheds.

Two observations seem to be pertinent: (i) that two-three years after termination of spraying operations the vector densities have not reverted to the original level; and (ii) that the number of mosquitos in cattle sheds has considerably increased. It is possible that because of intensive spraying of all human dwellings the anthropilic proportion has gradually declined and the zoophilic proportion has risen. This is all the more significant in the case of A. fluviatilis because if the anthropilic race is eliminated the zoophilic race will not constitute a danger from the point of view of malaria transmission.

2. Susceptibility of vectors to insecticides

Large scale surveys of the susceptibility of vectors to insecticides have been carried out regularly in India, and it has been evident from the data obtained that different species have shown considerable variations in susceptibility, apart from seasonal and other fluctuations. The results of LC_{50} values for some of the important species are summarized in the following table:¹

¹ Information collected from WHO/Mal/242, WHO/Insecticides/100 and Vectors of malaria in India (1961) published by the National Society of India for Malaria and Other Mosquito-borne Diseases.

Species	LC ₅₀ (base-line data)	
	DDT	Dieldrin
<u>A. culicifacies</u> *	0.22-0.43	0.032-0.078
<u>A. fluviatilis</u>	0.35-0.64	0.24
<u>A. minimus</u>	0.18	-
<u>A. philippinensis</u>	0.28	-
<u>A. stephensi</u>	0.5	0.10

* LC₅₀ for DDT in adjoining countries: W. Pakistan (1.3), Ceylon (0.27) and Burma (0.31-0.60).

It will be evident from this table that at the one extreme A. minimus and A. philippinensis are highly susceptible to chlorinated hydrocarbons when compared, for example, with A. culicifacies and A. stephensi at the other. However, the most significant point is that in spite of DDT spraying for over 10 years most of the vector species remained susceptible to this insecticide except A. subpictus, which showed early resistance to DDT and/or dieldrin (Sharma et al., 1957), but this species apparently plays no part in malaria transmission in India. More recently, the most important developments have been the appearance of several resistant strains,¹ particularly A. stephensi and A. culicifacies.

Larvae of A. stephensi type,² a vector of urban malaria, were reported by Raja Gopalan, et al. (1956) to be DDT resistant at Erode (Madras State). Further work on this species indicated a very high degree of resistance in the larval stage, i.e. 567 times the normal. The adults were, however, found to be only slightly tolerant, LC₅₀ = 3.2% against 1.1% for the normal strain (Mammen, 1957; Mohan & Singh, 1960, Personal communications). The adult form has now been reported by Sitaraman (1962)

¹ A. annularis, A. hyrcanus, A. splendidus and A. aconitus, which are of minor importance in transmission of malaria, have been found to be resistant to DDT or dieldrin, or both, in certain parts of the country. A. aconitus resistance to both DDT and dieldrin has been reported in Java (Muir, 1963).

² A. stephensi resistance to both DDT and dieldrin has been reported in parts of southern Iran, jeopardizing the success of the malaria eradication programme in this area (WHO/Mal/325: WHO/Insecticides/129, 1961).

to be resistant to DDT (the LC_{50} being over 4%) and tolerant to BHC and dieldrin in the Hyderabad and Visakhapattanam areas of Andhra Pradesh. Interruption of malaria transmission in areas of resistant A. stephensi has become difficult. A large number of cases were detected during May 1962 in and around Visakhapattanam, but the outbreak was rapidly brought under control by chemotherapeutic measures and a thorough spraying with BHC. A. stephensi has also posed a problem in urban areas where this species breeds mainly in wells, and the national authorities propose to carry out small scale trials with dichlorvos to try to control the situation.

During the past two years the most dramatic developments have taken place with regard to A. culicifacies. This species remained susceptible to DDT in spite of its generalized use for 10-11 years (Rao & Bhatia, 1957; Sharman et al. 1957; Bhatia et al. 1958; Pal, 1958), and this led to the belief that A. culicifacies was perhaps lacking a resistance gene.

However, resistance of A. culicifacies to dieldrin after only two to three rounds of spraying, accompanied by an outbreak of malaria, was reported in the Thana district of erstwhile Bombay State (Patel et al. 1958). An LC_{50} of 3.1% was observed for dieldrin, although the species was susceptible to DDT. Dieldrin spraying was therefore suspended and the species reverted to normal susceptibility in one season (Ramachandra Rao et al. 1960). Dieldrin resistance was also noted in the district of Udaipur in the State of Rajasthan after only two rounds of spraying (Jaipal Singh, communication to WHO). Similarly, a marked tolerance to this insecticide in A. culicifacies was reported by Subbarao (communication to WHO), apparently without any previous use of dieldrin or BHC in houses. Although no genetic experiments have been carried out, it may be inferred from the above observations that the development of dieldrin resistance in A. culicifacies fits into the general picture of its emergence as a semi-dominant or dominant gene in a few generations (Macdonald, 1959).

DDT tolerance in A. culicifacies was first noticed in 1959 in the Panch Mahal district of Gujerat State where the LC_{50} rose from 0.5% in 1947 to 2.2% in 1959 (Rahman et al. 1959). Luen & Shalaby (1962) confirmed this finding. In the period 1960-1961 a high degree of DDT resistance was found in large areas of Gujerat and Maharashtra (Patel et al. 1961), this was subsequently confirmed when resistance was also found in areas of Rajasthan, Madhya Pradesh and Uttar Pradesh (Krishnamurthy, 1962, communication to WHO). It is obvious that DDT resistance in this species has now spread to a large part of India.

In some areas of Maharashtra State, where the incidence of malaria cases was high, BHC was substituted but after only two rounds of spraying at the rate of 20 gamma isomer per square foot, A. culicifacies developed resistance to this insecticide, at a level of about two to four times. The rapid development of this resistance was considered to be due to the use of the cyclodiene group of insecticides for agricultural purposes (Sharma & Samnotra, 1962). A. culicifacies also showed an increased susceptibility to DDT.

The appearance of a DDT-resistant population in the course of two years, after a period of 9-10 years during which insecticidal pressure exerted no selective action, may be explained by incomplete coverage, poor quality of spraying and irregular intervals between spraying operations over the first 10 years. Improvement of the quality of work during the attack phase of the eradication programme has probably considerably increased selection pressure, resulting in the appearance of resistant strains. It is however interesting to note that an outbreak of malaria has not occurred in areas where spraying was satisfactory, in spite of DDT resistance in the vector species.

3. Probable ecological changes in Anophelines

The probable ecological changes in A. culicifacies that explain the development of insecticide resistance without occurrence of malaria have been investigated by a number of workers (Luen & Shalaby, 1962; Shalaby, 1962; Bhatia & Deobhankar, 1962). These may be summarized as follows:

- (i) Although A. culicifacies builds up very high densities in these areas, an increasing proportion of mosquitos rests outdoors.
- (ii) The resistant strain of A. culicifacies is short-lived when compared with the susceptible strain.
- (iii) DDT had an irritant effect on the anopheline population, although the latter was resistant to DDT.
- (iv) Contact between man and A. culicifacies has been very low.

These points are discussed below in more detail since they have an important bearing on the problem.

(1) Outdoor resting

Das & Mammen (1959) observed a number of A. culicifacies resting outdoors. In areas of Madhya Pradesh a large majority of A. culicifacies were also collected from outdoor shelters. A WHO team confirmed these findings by using artificial pit shelters to study the outdoor resting of mosquitos in some of these areas. Theoretically, this type of behaviour is incidental because with spraying of premises the endophilic element of the population is presumably destroyed and the exophilic element builds up. However, to evaluate this situation precisely it is essential to ascertain the extent to which the species is exophilic prior to the start of spraying. It is unfortunate that in the past no such emphasis has been laid on this aspect as it was not considered to constitute any danger from the viewpoint of malaria transmission.

(ii) Short life span

A further ecological consequence of DDT resistance in A. culicifacies was that the resistant strain was short-lived (Shalaby, 1962). Age determination was carried out by Polovodova's method. The author stated however that sampling was restricted to human habitations or mixed dwellings and therefore, with the development of resistance to DDT on the part of A. culicifacies, the older females might show an increasing avoidance of indoor resting places, and thus have been excluded from the sampling.

(iii) Irritability

A. culicifacies is well known to be irritated by DDT (Vishwanathan, 1955; Pal & Sharman, 1955) but as judged by mortality in exit traps of experimental huts it remains in contact with treated surfaces long enough to pick up a lethal dose of the insecticide. DDT selection pressure over several years does not appear to have changed the irritability level of the species even during the phase of the development of resistance to DDT (Shalaby, 1962). Tests carried out using the WHO method indicate that the irritability of DDT resistant and susceptible A. culicifacies was approximately the same. These observations do not seem to agree with the findings of Bhatia & Deobhankar (1962) who reported decreased irritability in DDT-resistant A. culicifacies in the Dhulia district as compared with the irritability of susceptible

A. culicifacies from the Poona and Thana districts of Maharashtra State. Shalaby (1962), in the Dhulia district found a reduction in the number of DDT-resistant A. culicifacies entering treated experimental huts and an absence of resting on DDT-treated walls. This, as Davidson (1962, communication to WHO) has pointed out, is not in agreement with other field observations. Large numbers of A. sundaicus were seen resting on DDT-treated walls in the Semarang area of Java in 1955, even on the day after spraying when white deposits of the insecticide were very much in evidence. A. stephensi was also found in DDT-treated houses in Saudi Arabia in the day time in the same year. This is in marked contrast to DDT-sprayed houses in areas where the mosquito is susceptible to the insecticide. According to Davidson, the implication of these observations is that resistant mosquitos are less irritated by DDT than susceptible ones. It could be added that there does not seem to be a simple correlation between irritability and resistance, that complete lack of susceptibility is not always associated with complete lack of irritability, though in most cases a decrease in susceptibility is associated with a decrease in irritability. In the field resistant A. sundaicus and A. stephensi have been found resting on DDT deposits, but experimental laboratory work (Davidson) failed to indicate any marked difference in the irritability of susceptible and resistant anopheline strains. To explain this behaviour it will be necessary to postulate that in a DDT-resistant population there are individuals in which the detoxification mechanism comes into play before the irritant effect of the insecticide has manifested itself.

No quantitative estimation of the level of irritability of A. culicifacies prior to the initiation of spraying is available. However no change in the status of irritability of this species to DDT is likely to have occurred during the course of spraying, and in fact the delayed appearance of resistance may also have been due to the irritability factor. With the appearance of this resistance there has been no evidence of an increased tendency of A. culicifacies to rest on surfaces that have been freshly sprayed with DDT. Moreover, the continued successful control in areas where this species is resistant strengthens the evidence that irritability may not be associated with resistance in A. culicifacies.

(iv) Low level of contact, and consequent lack of malaria

Insecticide resistance in the vector without occurrence of malaria, is a curious phenomenon as pointed out by Hamon & Garrett-Jones (1963), but a close scrutiny of the situation reveals that malaria transmission did not occur in these areas, because continuous indoor residual spraying over many years had depleted the natural reservoir of infection and this had created a situation of anophelism sine malaria. Although high densities of A. culicifacies were built up, individuals with malaria parasites were scarce, particularly when surveillance operations were well carried out. Furthermore, man/mosquito contact was reduced by the irritant effect of DDT and by the decreased longevity of the resistant strain of A. culicifacies. The national administration proceeded with the withdrawal of spraying in these areas as soon as the necessary criteria were met. From the viewpoint of resistance it will be interesting to watch the situation, since there are already indications that following this withdrawal there is an increase in susceptibility.

4. Conclusion

Experience in India¹ and other parts of the world has brought to light a number of new technical problems, some of which are of very great operational significance. Apart from physiological resistance to insecticides mosquito irritability also appears to play an important role, either in interrupting malaria transmission, as in A. culicifacies in India, or by being responsible for the failure of control operations, as in A. albimanus in Mexico and El Salvador and A. pseudopunctipennis in Mexico.

¹ In Assam, malaria transmission has been going on in some areas despite DDT spraying and the primary vector A. minimus is highly susceptible to this insecticide. It would seem that in the absence of A. minimus other species, which are not efficient vectors because of their slight contact with man, assume importance and are responsible for a low degree of transmission. In this particular case A. leucosphyrus leucosphyrus has been suspected to be the secondary vector and, as indicated earlier, this species seems to be refractory to DDT spraying. Some other method of control may therefore have to be instituted to meet the situation.

The ability of the vectors to enter sprayed houses at night, to feed on the inhabitants and to escape after little or no contact with the sprayed walls seems to be the most important factor in the persistent transmission in this area. Similarly, in El Salvador it has been shown that physiological resistance is not correlated with malaria transmission and that the behaviour of the vector is responsible. There are nevertheless some well-known problem areas, such as those with A. stephensi in India and Iran, where the vector has developed physiological resistance to both the DDT and dieldrin groups of chlorinated hydrocarbons. With increasing attention being focused on problem areas it is evident that when DDT is the insecticide concerned the behaviour aspect may be the principal factor involved, and the study of behaviour reactions of vectors should be given high priority.

REFERENCES

- Bhatia, S. C., Deobhankar, R. B. & Vittal, M. (1958) Indian J. Malar. 12, 371-376
- Bhatia, S. C. & Deobhankar, R. B. (1962) Indian J. Entom. 24, 36-52
- Das, M. & Mammen, M. (1959) Bull. nat. Soc. India Malar. 7, 157-168
- Hamon, J. & Garrett-Jones, C. (1963) Bull. Wld Hlth Org. 28, 1-24
- Luen, S. C., & Shalaby, A. M. (1962) Bull. Wld Hlth Org. 26, 128-134
- MacDonald, G. (1959) Riv. Parassit. 20, 305-315
- Muir, D. A. (1963) WHO/Mal/415; WHO/Vector Control/54
- Pal, R. & Sharma, M. I. D. (1955) Indian J. Malar. 9, 385-401
- Pal, R. (1958) Indian J. Malar. 12, 383-399
- Patel, T. B. et al. (1958) Indian J. Malar. 12, 367-370
- Patel, T. B., Ramachandra Rao, T. & Bhatia, S. C. (1961) Indian J. Malar. 15, 57-70
- Rahman, J., Roy, M. L. & Kuldipsing, H. (1959) Indian J. Malar. 13, 125-130
- Raja Gopalan, N., Vedamanikkam, J. C. & Ramoo, H. (1956) Bull. nat. Soc. India Malar. 4, 126-128
- Ramachandra Rao, T. & Bhatia, S. C. (1957) Indian J. Malar. 11, 261-270
- Ramachandra Rao, T., Bhatia, S. C. & Deobhankar, R. B. (1960) WHO/Mal/270;
WHO/Insecticides/114
- Shalaby, A. M. (1962) WHO/Mal/348
- Sharma, M. I. D. & Krishnamurthy, B. S. (1957) Indian J. Malar. 11, 231-237
- Sharma, M. I. D., Krishnamurthy, B. S. & Singh, N. N. (1957) Indian J. Malar. 11, 404-414
- Sharma, M. I. D. & Samotra, K. G. (1962) Bull. nat. Soc. India Malar. 10, 151-154
- Sitaraman, N. C. (1962) As quoted by B. S. Krishnamurthy, 1962 (Personal communication to Director, Central Institute for Communicable Diseases, India)
- Vishwanathan, D. K., Bhatia, S. C. & Halgeri, A. V. (1955) Indian J. Malar. 9, 51-70