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Trends in Malaria Morbidity and Mortality in Sri Lanka

JAYARATNE PINIKAHANA* and ROBERT A. DIXON+

Trends since 1930 in malaria morbidity and mortality in Sri Lanka were analysed. The Malaria Control Programme, which began in 1945 with DDT spraying, was associated with a 100-fold reduction in morbidity and mortality over the following ten years, and gave way to the Malaria Eradication Programme in 1958. DDT spraying ceased in 1964 and a vivax malaria epidemic in 1968 returned to the island to 1952 morbidity levels, though with little mortality. After the discovery of DDT resistance in 1969, malathion spraying took over in 1973, and USAID-assisted control programme, involving case-detection and treatment, started in 1977. However, morbidity levels comparable to 1952 levels were observed in 1975 and 1986 when falciparum malaria morbidity levels were especially high. Mortality rates since 1960 have however remained lower than at any other previous time.

Key words: Malaria morbidity, Malaria mortality, Sri Lanka

INTRODUCTION

Malaria, sometimes thought to be the main cause of the downfall of the ancient Sinhalese civilisation of Sri Lanka, was probably not endemic until the twelfth century[1]. Fevers were noted by the Portuguese in the sixteenth century[2] and the Dutch in the seventeenth and eighteenth centuries[3], and by the British in the nineteenth century[4]. The first unequivocal description of malaria in Sri Lanka was made in 1821 by Marshal[5].

Quinine was used in its treatment in the second half of the nineteenth century[6], and for prophylaxis by the turn of the century. The Anti-Malaria Campaign began in 1910 with quinine distribution and environmental control.

*Department of History and Sociology, University of Ruhuna, Matara, Sri Lanka.
+Department of Public Health Medicine, University of Sheffield, U.K.

Following the report of the first malariologist in 1927[7], records of attendances of patients for malaria treatment and of deaths attributed to malaria were maintained. In this paper we summarise these records and chart the main landmarks in malaria control in Sri Lanka since then.

MATERIALS AND METHODS

We have abstracted the annual number of attendances (called 'cases') and the annual number of deaths assigned to malaria, derived from death certificates, from the annual administrative reports (1931-86) of the Anti-Malaria Campaign. Population estimates for 1931-58 were obtained from Visvalingam[8] and for 1959-86 from the 1961, 1971 and 1981 census reports of the Department of Census and Statistics, Sri Lanka. For each year the data available are the estimated total population of the country, the reported number of attendances (or re-attendances) for treatment at a health facility with symptoms of malaria (whether or not diagnosis is positively confirmed) and the number of deaths attributed to malaria. Before 1950, however, an indeterminate number of malaria deaths may instead have been assigned to pneumonia, convulsions, dysentery, diarrhoea or enteritis, according to Sivaghanasundram[9].

From these basic data the following estimated rates were computed:

Clinical attendance rate (per 10,000 population): the number of malaria-related attendances (symptoms of malaria not necessarily positively diagnosed) at a health facility divided by the total population.

Mortality rate (deaths per million population): the number of deaths attributed to malaria in the year divided by the total population.

Death/attendance ratio (deaths per 100,000 attendances): the number of deaths attributed to malaria in the year divided by the total number of malaria-related attendances during the year.

RESULTS

Fig. 1 shows the secular trend in each of these three indicators with key events in the control of malaria superimposed. A logarithmic scale is used and values below one (per 1000 population for clinical attendance rate, per million population for mortality and per 100,000 attendances for case fatality rate) are now shown. From 1957 onwards, death/attendance ratios and mortality rates are below the lower limit of the logarithmic scale, or (from 1963 ≤ 5 only) case fatality rates have not been shown because they were based on only one death.

The highest mortality rate during the period of reporting occurred during the 1934-35 epidemic. This was also the time when the clinical attendance rate was an all-time high.

The commencement of DDT (*p, p'*-dichlorodiphenyltrichloroethane) spraying in 1945, with coverage of all houses in malarial areas nationwide by the end of 1946, was evidently not sufficiently effective to prevent the 1946-47 epidemic. However, the decline in the clinical attendance and mortality rates immediately after this epidemic were so dramatic that DDT spraying was partly stopped in 1954

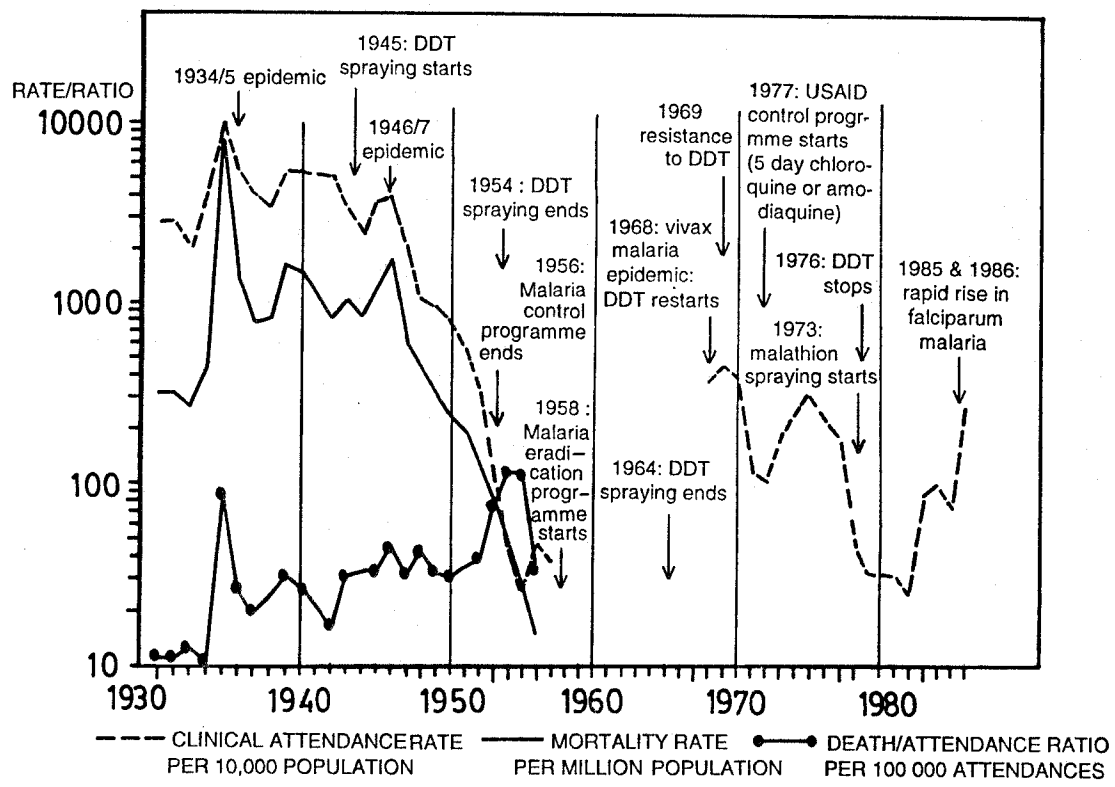

Fig. 1: Trends in clinical attendance rate, mortality rate and death/attendance ratio. Note the logarithmic scale. From 1957 onwards, the mortality and death/attendance ratios are below the minimum of the verticle scale used (see text).

and the malaria control programme as a whole was brought to an end in 1956.

In 1958 the World Health Organisation encouraged the establishment of the Malaria Eradication Programme in which DDT spraying started again on a highly selective basis until it was discontinued in 1964. During this period, surveillance, involving domiciliary visits for case detection and spraying, was highly effective in many areas but almost non-existent in some remote regions[10,11].

In 1968 an epidemic of vivax malaria, after more than ten years of low clinical attendance rates (below 1 per 1000) and mortality rates (below 1 per million population), led to the resumption of DDT spraying. The parasite reappeared in the Matale and Anuradhapura districts in the north central province[8] but by the end of 1968, epidemics of vivax malaria occurred in all the traditionally malarious areas[12]. In the following year, reports of resistance to DDT by the *Anopheles culicifacies* vector were received and the usefulness of

DDT was questioned[10]. In 1973, at the beginning of another epidemic, malathion spraying was introduced alongside DDT, which was phased out in 1976[12].

During the 1968-70 epidemic, when clinical attendance rates were comparable to those in the 1950s, the mortality rate of at most 5 deaths per million population (below the scale of the graph) was at least an order of magnitude lower than those seen during epidemics in earlier decades. Improved treatment, especially through antibiotics, presumably explains this difference. The death/attendance ratio at this time was around 1 per 10,000 population or lower (below the scale of the graph). Until the mid-1950s, the general trend in the death/fatality ratio had been upward.

The USAID-assisted malaria control programme, which began in 1977, concentrated on treatment of cases of malaria with chloroquine or amodiaquine over five days as the main method of limiting the spread of malaria and reducing mortality. The aim was to eradicate falciparum malaria and to reduce *vivax* malaria incidence. Four additional epidemiologists, two entomologists and 40,000 employees of the Anti-Malaria Campaign were involved in this operation.

By 1980 control appeared at first to have been re-established but clinical attendance rates increased rapidly from 1983 onwards with a steep rise in falciparum malaria both in 1985 and in 1986.

DISCUSSION

The recorded number of clinical attendances, on which both the clinical attendance rate and the death/attendance ratio are based, is the number of visits for treatment and not the number of malaria-positive cases diagnosed[9]. Some patients with malaria may not report for treatment at all; some patients with non-malarial fever may be included. Only if the number of attendances per true malaria case remains fairly constant over time, does the clinical attendance rate give a reasonable indication of trends in malaria incidence. If, for example, increasingly efficacious treatments over the year led to fewer revisits per case, then without any real change in incidence we would expect to see a decreasing clinical attendance rate.

Also, without any real change in true case fatality rate, our estimate, based on clinical attendances as the denominator, would show an increasing trend in death/attendance ratio. The morality rate and the death/attendance ratio will also be susceptible to changes in the completeness of registration of all deaths and in the extent of under- or over-diagnosis of malaria on death certificates.

It is not known to what extent these biases have influenced the apparent trends. The overall picture is of rapidly declining morbidity and mortality between mid-1940s and mid-1950s, with a resurgence of morbidity in the late 1960s, which has so far defied attempts to control it effectively. Mortality rates since 1960 have however remained lower than at any other previous time.

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Control of Mosquito Breeding through *Gambusia affinis* in Rice Fields

H. PRASAD*, R.N. PRASAD* and S. HAQ*

Studies on mosquito breeding and its control through *Gambusia affinis* in nursery and paddy fields after transplantation of seedlings were carried out during June to October 1991 in about 10 ha rice field area. Six anopheline species, viz. *An. culicifacies*, *An. annularis*, *An. subpictus*, *An. nigerrimus*, *An. barbirostris* and *An. aconitus*, and four culicine species, viz. *Cx. tritaeniorhynchus*, *Cx. bitaeniorhynchus*, *Cx. quinquefasciatus*, and *Aedes* sp. could be identified. These were found breeding in rice fields with fluctuations in their percentage composition, exhibiting species succession in different months. *G. affinis* survived well in submerged rice fields and provided 87.8% mosquito larval control. In rice fields which exhibited intermittent drying up leading to formation of pools, puddles etc., moderate larval control was achieved. However, in nursery rice fields, this method was not applicable. Mosquito larval control through larvivorous fish in rice fields can be achieved but the method has limitations.

Key words: *Gambusia affinis*, Mosquito breeding, Rice fields

INTRODUCTION

A number of tropical diseases are transmitted by different species of mosquito vectors which breed in a variety of aquatic habitats. Rice fields play a very important role in building up a high adult vector density because of vast water surface areas in and around the human habitats especially during rainy season and hence incidence of malaria and other mosquito-borne diseases is associated with rice fields[1-3]. Control of mosquito breeding in rice fields is a challenge under the prevailing situations in view of the detrimental consequences of the use of insecticides. Hence the use of biological control agents has been emphasised

*Malaria Research Centre, Field Station, Khirni Bagh, Sadar Bazar, Shahjahanpur - 242 001, India.

for mosquito larval control at the genesis level, particularly in rice fields[4].

In India about 41.66 million ha land is utilised for rice cultivation[5]. In district Shahjahanpur alone about 1,48,000 ha land is under rice cultivation. Among all the available biological control agents, *Gambusia affinis* is most promising in rice fields[6-8]. However, the application of larvivorous fish in rice fields has been demonstrated only on a small scale and in a particular type of agroecosystem, whereas rice cultivation is related to varied ecological situations. Therefore, a study on mosquito breeding and its control through *G. affinis* in different stages and situations of rice cultivation has been carried out in district Shahjahanpur. The results of this study are reported in this paper.

MATERIALS AND METHODS

The experiments were designed so as to carry out the study in two phases viz. in nursery rice fields and in rice fields after transplantation of seedlings.

Survey and selection of study area

A survey of rice fields in the villages of PHC Dadraul was carried out in the months of June and July 1991. A total of 15 nursery rice fields, ranging from 600 to 800 m^2 area and 21 rice fields ranging from 1350 to 1660 m^2, area were selected in three villages, viz. Banthara, Nagaria and Dalelapur, during this period. Out of the selected rice fields, 5 from each category were held as control.

Introduction and monitoring of larvivorous fish density

Larvivorous fish, *G. affinis,* were collected from fish hatcheries and brought to the study villages. Fishes were introduced at the rate of 10 fish per m^2 water surface area in 10 experimental nursery rice fields and 17 rice fields on Day zero (D0). The fish density in the rice fields was monitored using a drum (dia. 1 m) with both sides open. The drum was placed at 5 different places in the rice fields and water from the area covered by the drum was taken out with small bowls. Fishes were captured, counted and density per square metre area was calculated. The physical condition of each rice field was also recorded weekly.

Entomological observations

Mosquito larval density was monitored in experimental and control rice fields during both the stages of rice cultivation by using a 300 ml standard dipper. Larval sampling was done at 4 different places on the periphery and one place in the centre of each field. Five dips at one place were taken and the average was recorded as per dip larval density. In nursery rice fields the larval density was monitored on different days and were denoted as D0, D1, D2, D4, D7, D21, D28 and D35, whereas in rice fields after transplantation of seedlings, it was monitored on D0, D2, D4, D6, and D8 and thereafter weekly up to Day 63. After D63 almost all the experimental and control rice fields were found dry. Hence further observations were not taken. D0 larval density was taken before the introduction of larvivorous fish in experimental plots.

Adult emergence

One hundred IV instar mosquito larvae of anophelines and culicines each were collected weekly and brought to the laboratory. The larvae were reared and the species of adult mosquitoes that emerged were counted and identified. The percentage composition of each species was recorded monthly.

Paddy growth

In each rice field, 10 paddy plants were measured randomly every week and the average height was calculated per month, to find the relation between mosquito density and species succession.

RESULTS AND DISCUSSION

Mosquito breeding in rice fields

Monthly percentage composition of anopheline and culicine mosquito species breeding in rice fields are given in Table 1. Six anophelines, viz. *An. culicifacies, An. annularis, An. subpictus, An. nigerrimus, An. aconitus* and *An. barbirostris,* and four culicine species viz. *Cx. quinquefasciatus, Cx. tritaeniorhynchus, Cx. bitaeniorhynchus* and *Aedes* species, were found breeding in rice fields. Other culicines were also recorded and are listed as unidentified species. *An. culicifacies* and *An. subpictus* were the predominant species contributing 48.8% and 61.7% in June and 51.2% and 29.0% in July respectively. In August and onwards the percentage composition of *An. culicifacies* declined drastically, varying from 3 to 0.1%. During this period, breeding of *An. culicifacies* was confined mainly to the pits situated at the periphery of rice fields. The decline in percentage composition of *An. culicifacies* was associated with paddy growth. The average height of the paddy plants varied from 5 to 22.3 cm, 38.6 to 49 cm, 50.2 to 69.8 cm, 81.1 to 93.7 cm, and 101 to 103.2 cm during the months of June, July, August, September and October respectively. The inverse relationship between paddy growth and breeding of *An. culicifacies* was reported by earlier workers[1,9].

Breeding of *An. annularis* started in late July (9.3%) and attained its peak in month of August (39.6%). In later months (Aug-Oct) other anopheline species, such as *An. nigerrimus, An. aconitus* and *An. barbirostris* were encountered. The variation in the percentage composition of different anophelines reflected a clearcut species succession during the entire course of rice cultivation. No species succession was observed in the nursery rice fields.

Among the culicines, *Cx. tritaeniorhynchus* and *Cx. bitaeniorhynchus* were found breeding throughout the rice cultivation period, whereas *Cx. quinquefasciatus* and *Aedes* species bred from June to August.

Evaluation of the control method

In the nursery rice fields the application of the fishes could not produce desirable results as there was frequent filling and flushing of water because of the usual practice of producing rice seedlings in the area. The land of the paddy nursery was maintained wet to facilitate easy germination and growth of the seedlings and as a result, pools and patches/

Table 1. Monthly percentage compositions of different mosquito species breeding in rice field

Month	Percentage compositions										
	Anopheline species					Culicine species					
	An. culicifacies	*An. subpictus*	*An. annularis*	*An. nigerrimus*	*An. aconitus*	*An. barbirostris*	*Cx. quinquefasciatus*	*Cx. tritaeniorhynchus*	*Cx. bitaeniorhynchus*	*Aedes* sp.	Other unidentified sp.
Jun	48.8	51.2	Nil	Nil	Nil	Nil	13.0	28.0	22.0	3.0	34.0
Jul	61.7	29.0	9.3	Nil	Nil	Nil	7.0	31.0	29.0	6.3	26.3
Aug	3.0	21.3	39.6	29.1	Nil	7.0	4.0	21.0	28.0	5.0	42.0
Sep	0.8	1.3	Nil	59.0	22.0	16.8	Nil	26.1	22.9	Nil	51.0
Oct	0.1	Nil	Nil	63.0	23.8	13.0	Nil	22.6	29.0	Nil	48.4

puddles were formed in and around the paddy nursery. Such a situation hinders the survival and feeding activity of the larvivorous fish and thus provides excellent opportunities for mosquito breeding[3]. A sharp decline in the fish density was noticed after D2 in most of the experimental paddy nurseries which resulted in the increase in larval density and from D7 onwards there was no difference in the larval density in experimental and control rice nurseries (Fig. 1).

The results of mosquito breeding and its control through *G. affinis* in the second phase of rice cultivation are shown through different figures depending upon the situation of the rice fields. Fig. 2 shows a remarkable reduction in the larval density in the rice fields which remained submerged during the experiment. The decline was 28.1%, 75.1% and 87.8% on D4, D21 and D35 respectively in the experimental rice fields, whereas an increase in the larval density by 8.5%, 28% and 17% on corresponding days was observed in the control rice fields. The decline was due to the successful consumption of mosquito larvae by the fish which could thrive well and could even multiply because of adequate water level (8.0-16.1 cm) in these rice fields. The mosquito fish has been used successfully in rice fields at different places. Hoy and Reed[6,10] and Hoy *et al.*[11] found that at a stocking rate of 250-300 fish/ha, *Gambusia* gave 82% control of *Cx. tarsalis* and *An. freeborni*. At a stocking rate of 2500-5000 fish/ha, it gave 96.4 -100% control of *Culex* larvae in Uzbekistan[12] and in Lauisiana, a stocking rate of 12,500-50,000 fish/ha produced 80-96% control of *Pso-*

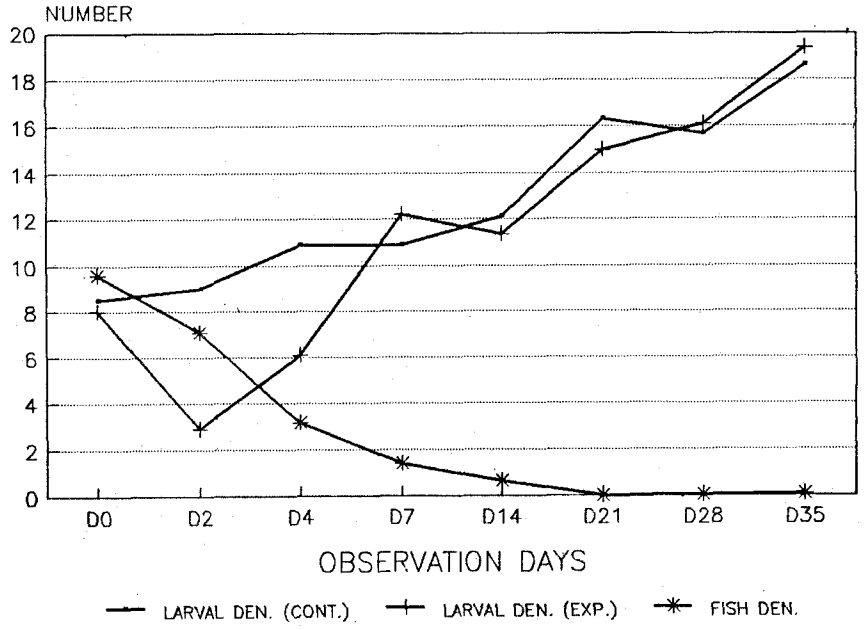

Fig. 1: **Mosquito larval control in rice nurseries through *Gambusia affinis*.**

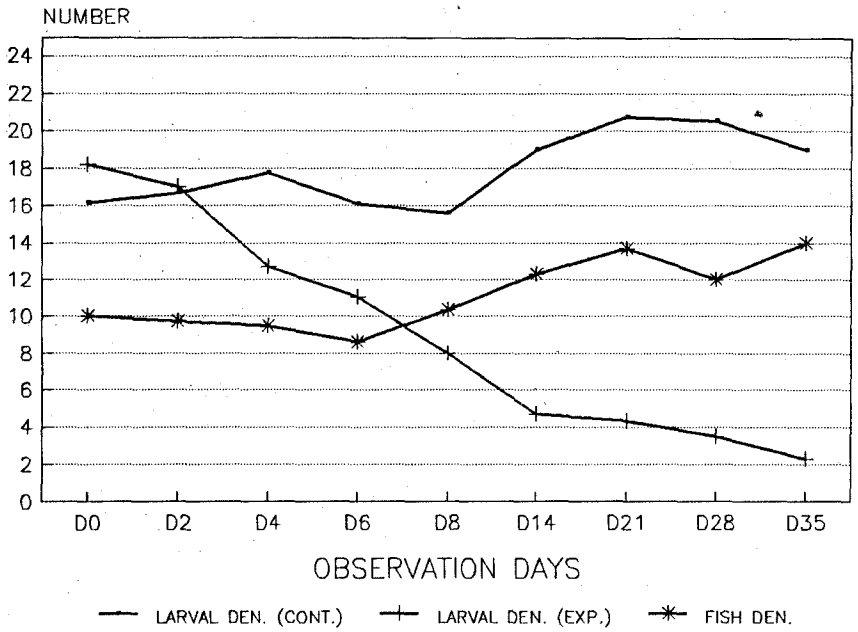

Fig. 2: **Mosquito larval control in rice field.**

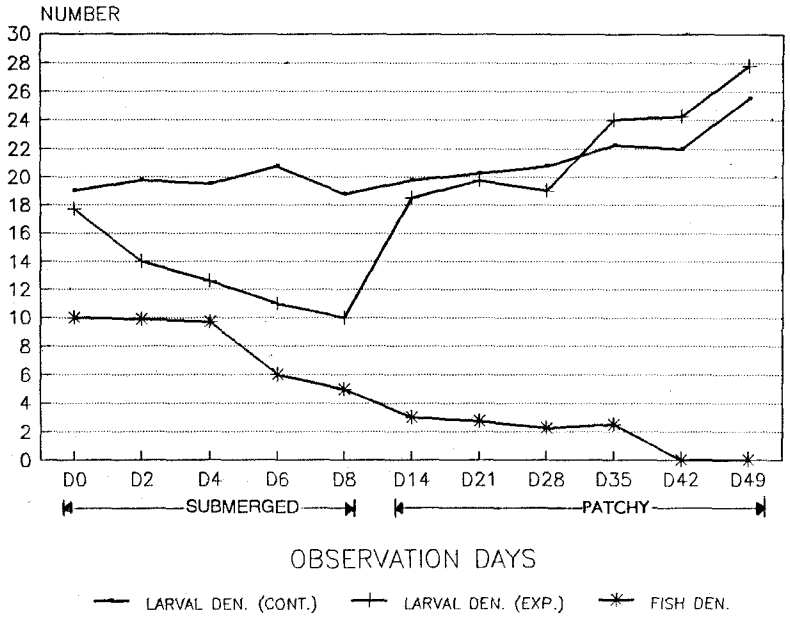

Fig. 3: **Mosquito larval control in submerged rice fields which became patchy.**

rophora confinis[13]. Kramer *et al.*[14], while evaluating *Gambusia* for mosquito control in California wild rice fields, observed that although the fish survived in the rice fields yet they did not substantially reduce mosquito populations mainly because of the large availability of alternative prey in the wild rice fields and the omnivorous feeding nature of the fish. In a large-scale field trial in California rice fields stocked with the fish at a low density produced more mosquitoes than the control rice fields, owing to decreased abundance of arthropod predators as a result of predation by the fish[15].

In our experimental rice fields the status of mosquito control could not be maintained as after D4 the number of submerged rice fields decreased gradually. The rice fields produced a heterogeneous picture of physical situations mainly in respect of water level which resulted in the formation of pools, patches and puddles. Thereafter the pattern of mosquito control in rice fields exhibited fluctuations depending upon the particular condition of the rice fields. After the formation of pools and patches the larval density in the experimental rice fields was more or less equal to, and sometimes even higher than that in the control rice fields of similar situation (Fig. 3). Such a trend was mainly because of the insufficient larvivorous fish density which gradually declined because of a considerable reduction of water level, leading to the confinement of fishes in some of the small hoof and foot prints, pools and patches. In patchy rice fields, mosquito breeding occurred in three situations, viz. pools, patches and prints/puddles.

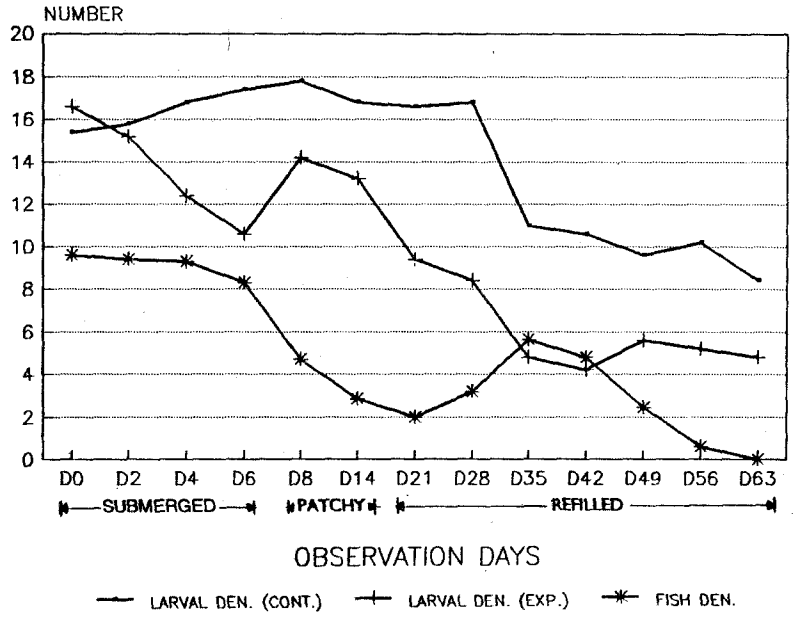

Fig. 4: **Mosquito larval control in rice fields exhibiting diversified situations.**

The pools in which sufficient numbers of fishes were trapped exhibited very low to zero mosquito density. After refilling of the patchy rice fields either by natural rainfall or through tube wells, control of mosquito breeding improved because of the increase in the fish density (Fig. 4). Reduction in density was less than in completely filled rice fields because the recruitment of the fresh stock takes its own time in building up effective fish density after the depletion. Moreover, the newly hatched *G. affinis* fish initially consume planktonic food because of their smaller size.

We conclude that *G. affinis* can control mosquito larvae in rice fields which retain enough water for their survival and feeding. This method was not successful in the case of nursery, flooded and refilled rice fields which exhibited frequent physical changes, especially in respect of water level. Such changes may compell repeated fish introduction, which is not feasible in the vast and varied rice agroecosystems. We cannot rely on a single mosquito control method in rice fields. Therefore, the exploitation of new methods should be explored. We would have to develop different methods, including the use of larvivorous fishes, bacteria (*Bacillus sphaericus* and *B. thuringiensis*) and certain environmental modifications to cope with the diversified situations in the rice fields. Nevertheless without the active co-operation of farmers it will be difficult to achieve any significant reduction in mosquito breeding in rice fields and hence mosquito-borne diseases.

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In vitro Activity of Fluoroquinolones against Chloroquine-sensitive and Chloroquine-resistant *Plasmodium falciparum*

K.D. TRIPATHI*, A.K. SHARMA+, N. VALECHA‡ and S. BISWAS‡

The *in vitro* activity of three fluoroquinolones — ciprofloxacin, norfloxacin and ofloxacin— was studied on four laboratory-adapted strains (one chloroquine-resistant) and one fresh isolate of *P. falciparum* from Delhi by the schizont maturation inhibition microtest. The IC50 concentrations (mean ± SD) were found to be as: ciprofloxacin 6.38 ± 1.34 μg/ml, norfloxacin 11.24 ± 1.27 μg/ml, and ofloxacin 22.3 ± 3.11 μg/ml, while the MIC values were 32 μg/ml, 64 μg/ml and 128 μg/ml for the three drugs in the same order. The IC50 and MIC values for chloroquine-resistant strain were not significantly different from those for the chloroquine-sensitive strains. We conclude that there is little interstrain variability in the *in vitro* susceptibility of *P. falciparum* to fluoroquinolones, and that there is no cross resistance between them and chloroquine. The reported variability in clinical response of falciparum malaria to fluoroquinolones is not likely to be due to variation in parasite sensitivity.

Key words: Ciprofloxacin, Fluoroquinolones, Norfloxacin, Ofloxacin, *P. falciparum*

INTRODUCTION

The emergence of strains of *Plasmodium falciparum* resistant to chloroquine and many other drugs in succession has stimulated efforts to identify new antimalarial agents[1]. The quinolone nalidixic acid showed weak antiplasmodial activity *in vitro*[2]. Subsequently a

*Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry - 605 006, India.
+Department of Pharmacology, Lady Hardinge Medical College, New Delhi - 110 001, India.
‡Malaria Research Centre, 22-Sham Nath Marg, Delhi - 110 054, India.

highly augmented activity of the fluoroquinolone ciprofloxacin was demonstrated by Krishna *et al.*[3] against cultured chloroquine-sensitive strain T9/96 of *P. falciparum*. These observations were extended and a number of fluoroquinolones were found to be active against a Thai multiresistant line of the parasite as well[4].

Reports on the efficacy of fluoroquinolones in curing falciparum malaria from different geographical regions are inconsistent[5-8]. The response rate has varied from 100 to 0%. We in Delhi found incomplete response with norfloxacin in a majority of cases (unpublished data). As the inconsistencies could be due to either differences in the inherent susceptibilities of the parasite or the immunological status of the host, we decided to measure the *in vitro* inhibitory activity of three fluoroquinolones on chloroquine-sensitive as well as -resistant strains of *P. falciparum* obtained from different districts of north India and on a fresh isolate from Delhi.

MATERIALS AND METHODS

Parasite culture

The following strains of *P. falciparum* grown, adopted and maintained *in vitro* at the Malaria Research Centre, Delhi, by the method of Trager and Jensen[9] were used in our study:

FSJ-A6: A chloroquine-sensitive strain obtained from Shahjahanpur.

FJB-D2: A chloroquine-sensitive strain obtained from Jabalpur.

FJB-D4: A chloroquine-resistant strain obtained from Jabalpur.

P-30: A chloroquine-sensitive strain obtained from Ghaziabad.

The cultures of these parasites in complete RPMI-1640 medium were prepared to approximately 1% parasitaemia by centrifugation and dilution with human O, Rh+ve erythrocytes at a time when about 90% of the parasites were in the early ring stage. In addition, a fresh isolate obtained from a patient at Delhi was used in the study.

Schizont maturation microtest

The sensitivity of the parasites to chloroquine and fluoroquinolones was studied by the *in vitro* schizont maturation inhibition microtest adopted by WHO[10], with the modification that instead of 50 µl, each culture well was charged with 100 µl of blood-medium mixture (containing 10 µl parasitized blood), and fresh solutions of the drugs were employed instead of predosed and dried wells. The assays were done in duplicate on flat-bottom 96-well microtitre plates which were incubated at 37°C in a candle jar for 36-40 h. The usual 24-h incubation was exceeded because it resulted in less than 10% schizont maturation in control wells.

At the end of the incubation, schizont maturation in a well was monitored in JSB-stained blood smears by counting the number of schizonts per 200 asexual parasites. The per cent inhibition of schizont maturation for each concentration of a drug was calculated

as 100-*a*, where *a* is per cent of schizonts in the test wells relative to control wells determined as $a = z/m \times 100$, where *m* and *z* are the mean numbers of schizonts per 200 asexual parasites in control and test wells respectively.

Determination of IC50 and confidence intervals

The percentage inhibition of schizont maturation (relative to control) was plotted against the concentration of the drugs on a log-probit graph and IC50 values were derived from the regression line of these plots[10]. The 95% confidence intervals (CI 95) of the IC50 values were calculated by Litchfield and Wilcoxan method as described by Swaroop *et al.*[11]

Drugs and solutions

Ciprofloxacin HCl monohydrate, norfloxacin (base), ofloxacin (base) and chloroquine diphosphate gifted by Ranbaxy Lab. Ltd. were used.

All drug solutions were prepared aseptically in sterile double-distilled water and filtered fresh before use. Norfloxacin and ofloxacin were dissolved in a minimum quantity of 0.1 *N* NaOH, at pH 8.4 for norfloxacin and 8.1 for ofloxacin solution. The concentrations of all drugs are expressed in terms of base weight.

RESULTS

The control schizont maturation at 36-40 h incubation was as: strain FSJ-A6, 23.5%; strain FJB-D2, 35%; strain FJB-D4, 25%; strain P-30, 28%; fresh isolate 30%. As the difference between the duplicate wells was less than 5% with each strain, and as more than 10% schizont maturation was obtained in all control wells, the criteria for a valid test were adequately met[10].

The log-probit plots of inhibition of schizont maturation for chloroquine-sensitive strains is shown in Fig. 1, and that for chloroquine-resistant strain in Fig. 2. The regression line for chloroquine shows a marked concordance for the four sensitive strains, while for the resistant strain it is considerably shifted to the right. The regression slopes of the three fluoroquinolones are not significantly different from each other and are quite concordant for all the five strains tested. The relative position of the regression lines of the fluoroquinolones on the concentration axis is also similar for all the strains: ciprofloxacin line always lying to the left, ofloxacin being the rightmost and norfloxacin line lying in between them.

The IC50 values and their 95% confidence intervals are given in Table 1.

Chloroquine

All *P. falciparum* strains showed concentration-dependent inhibition of schizont maturation. In the case of strains FSJ-A6, FJB-D2, P-30 and the fresh isolate, nearly 50% inhibition was obtained at 2 pmol/10 μl blood and complete inhibition (MIC) at 8 pmol/10 μl blood. Thus these strains were chloroquine-sensitive as per the WHO criteria, i.e. 100% inhibition at 1.14×10^{-6} mol/l blood[10], which is equal to 11.4 pmol/10 μl blood (or concentrations lower than this). The IC50 values of these strains were very similar.

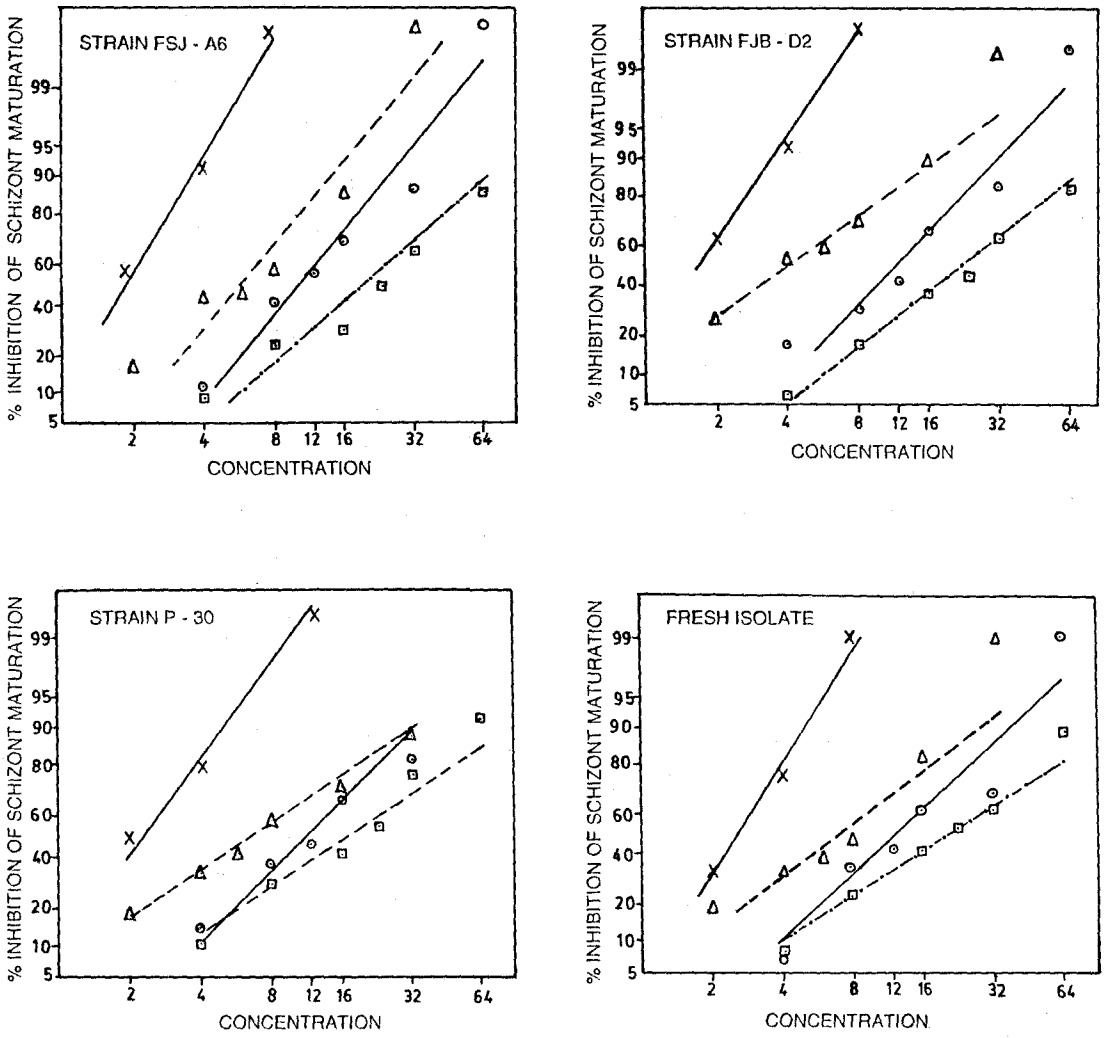

Fig. 1: **Log-probit plots of *in vitro* schizont maturation inhibition of four chloroquine-sensitive strains of *Plasmodium falciparum* [chloroquine (x ——— x), ciprofloxacin (Δ--------- Δ), norfloxacin (⊙ —— ⊙) and ofloxacin (⊡ - - - - -⊡)]. The concentration of chloroquine is expressed in pmol/10 μl of blood while that of ciprofloxacin, norfloxacin and ofloxacin in μg/ml.**

In contrast, the strain FJB-D4 showed only 8% inhibition at 2 pmol/10 μl blood and complete inhibition only at 32 pmol/10 μl blood. It was thus chloroquine-resistant. The IC50 in this case was nearly 4 times as much as in the case of the other strains.

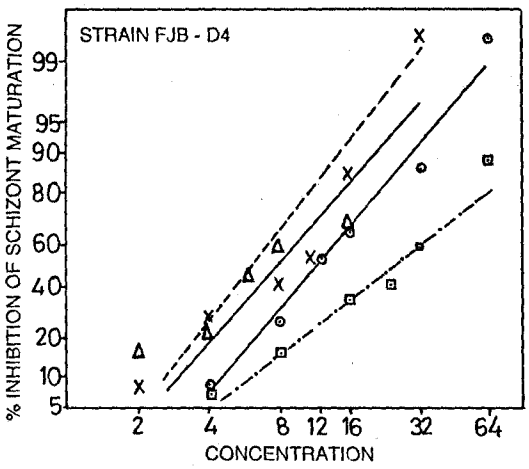

Fig. 2: Log-probit plots of *in vitro* schizont maturation inhibition of a chloroquine-resistant strain of *P. falciparum* by chloroquine and three fluoroquinolones. [Symbols and concentration scales as in Fig. 1].

Ciprofloxacin

All strains, irrespective of their choloroquine sensitivity, were inhibited in a concentration range of 2-32 μg/ml. The IC50 values for the different strains were not different from each other except for strain FJB-D2 which appeared to be somewhat more sensitive. MIC for all strains was 32 μg/ml.

Norfloxacin

A concentration-dependent inhibition was obtained between 4 and 64 μg/ml for all the strains (MIC 64 μg/ml). The fresh isolate was relatively more sensitive than the other strains.

Ofloxacin

The inhibitory concentration range was 4-128 μg/ml, as 100% inhibition was obtained only at 128 μg/ml in all cases. There was no significant difference between the IC50 values for all the five strains tested.

DISCUSSION

The three fluoroquinolones tested in the study

Table 1. Potency of three fluoroquinolones and chloroquine against four laboratory maintained strains (one chloroquine-resistant) and one fresh isolate of *P. falciparum* in the *in vitro* schizont maturation microtest

Pf strain	IC50* (95% confidence intervals)			
	Chloroquine	Ciprofloxacin	Norfloxacin	Ofloxacin
FSJ-A6	2.0 (1.8-2.2)	6.5 (5.1-8.2)	11.0 (9.6-12.5)	21.5 (18.4-25.2)
FJB-D2	1.7 (1.6-1.9)	4.1 (3.6-4.7)	12.8 (11.5-14.2)	24.5 (21.5-27.9)
P-30	2.3 (2.0-2.6)	6.9 (6.0-8.0)	11.5 (10.0-13.1)	17.5 (14.4-21.2)
Fresh isolate	2.1 (1.9-2.3)	7.6 (6.6-8.7)	9.3 (8.1-10.7)	22.5 (18.8-26.9)
FJB-D4	8.5 (7.7-9.4)	6.8 (6.1-7.6)	11.7 (10.4-13.1)	25.5 (21.8-29.8)
Mean ± SD	2.03** ± 0.25	6.38 ± 1.34	11.24 ± 1.27	22.3 ± 3.11

*IC50 - chloroquine in pmol/10 μl blood, of fluoroquinolones in μg/ml; ** Mean of chloroquine-sensitive strains FSJ-A6, FJB-D2, P-30 and the fresh isolate.

inhibited *P. falciparum* whether or not the parasite strain was chloroquine-sensitive or -resistant. There was little difference in the sensitivity among the five strains to the fluoroquinolones. Ciprofloxacin was about twice as active as norfloxacin and about 4 times as active as ofloxacin.

The IC50 values obtained in other available reports are given in Table 2. A remarkable concordance between the studies from different geographical regions as well as between chloroquine-sensitive and -resistant strains is evident. This shows that there is no cross resistance between chloroquine and fluoroquinolones. Our values obtained in the schizont maturation microtest are very close to those obtained by Midgley *et al.*[4] in the 48 h [^{3}H] hypoxanthine incorporation test, with the same rank order of potency, viz. ciprofloxacin > norfloxacin > ofloxacin.

It is to be noted that the conventional oral doses of these fluoroquinolones produce peak plasma concentrations (ciprofloxacin 500 mg — 1.9 to 2.8 μg/ml; norfloxacin 400 mg — 1.5 μg/ml; ofloxacin 600 mg — 11.0 μg/ml)[12] that are considerably lower than their IC50 concentrations against *P. falciparum*. Hence they appear to need forceful supplementation by host factors to achieve clinical response. It is therefore not surprising that cure rates from 100[5] to 0%[7,8] as well as intermediate degree of responses[6] have been reported.

The patient from whom the fresh isolate was obtained for the study was treated with norfloxacin, 800 mg twice daily for 3 days, which achieved a radical cure despite the fact that the *in vitro* IC50 was 9.3 μg/ml. Moreover, we have obtained complete response in 20% and 80% patients respectively with 400 mg and 800 mg twice daily of norfloxacin

Table 2. Comparison of the reported *in vitro* activity of the fluoroquinolones against chloroquine-sensitive and -resistant strains of *P. falciparum* from different parts of the world

Reference	*Pf* strain used	Chloroquine sensitivity	Test model	IC50 (μg/ml)		
				Ciprofloxacin	Norfloxacin	Ofloxacin
Krishna[3]	T9/96	Sensitive	[^{3}H] Hypox	8.5	—	—
Midgley[4]	K1(Thai)	Resistant	[^{3}H] Hypox	5.2	13.3	22.5
Watt[7]	Thai isolates	Resistant	[^{3}H] Hypox	6.6	11.6	—
Stromberg and Bjorkman[8]	Sierra Leone isolate	Resistant	Schizont maturation	3.0	—	—
Present study	4 Strains from north India	3 Sensitive 1 Resistant	Schizont maturation	6.38 ± 1.34*	11.24 ± 1.27*	22.3 ± 3.11*
	Delhi isolate	Sensitive				

[^{3}H] Hypox = 48 h; [^{3}H] Hypoxanthine incorporation test; * ± SD.

in falciparum malaria, and incomplete response in the remaining patients (unpublished data). Vivax malaria has also responded similarly[13].

We therefore conclude that fluoroquinolones are equally effective against chloroquine-sensitive and -resistant *P. falciparum* in culture, and that there is little inter-strain variation in susceptibility. The previously reported *in vitro* rank order of potency among three fluroquinolones is confirmed. Our study indicates that the inconsistency in clinical response of falciparum malaria to fluoroquinolones in different studies is not likely to be due to differences in parasite susceptibility, but most probably is due to differences in host factors. In view of the IC50 values being higher than the clinically attained concentrations of the fluoroquinolones, this study suggests only an adjuvant role of these drugs in falciparum malaria.

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Insecticide Susceptibility Status of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* in Panaji, Goa

D. THAVASELVAM*, ASHWANI KUMAR* and P.K. SUMODAN*

Adult and larval insecticidal tests conducted in Panaji, Goa, revealed that adults of *An. stephensi* were resistant to DDT (4.0 %), dieldrin (0.4 %) and malathion (5.0 %). The larvae of *An. stephensi* were also resistant to DDT (2.5 mg/l) and malathion (3.125 mg/l). *Cx. quinquefasciatus* adults were resistant to DDT, dieldrin, malathion and fenitrothion (1.0%), and larvae were highly resistant to DDT but showed low resistance to malathion and fenitrothion (0.125 mg/l). Adults of *Ae. aegypti* were resistant to DDT and dieldrin and the larvae showed resistance to DDT but good susceptibility to fenitrothion and complete susceptibility to malathion. The triple resistance of *An. stephensi* to DDT, dieldrin and malathion is intriguing as malathion has never been used in the health sector in Goa. A very limited use of malathion was done in the agricultural sector in Chandor area, which is 50 km away from the study area.

Key words: DDT, Dieldrin, Fenitrothion, Malathion, Resistance/Susceptibility, Temephos

INTRODUCTION

Panaji, the capital city of Goa, has witnessed rising trends of malaria since 1986[1] and the incidence has increased substantially the following years. The main vector control tools utilised by the local health authorities to control transmission of malaria were DDT spraying, space spraying with pyrethrum extract as imagocides and temephos (Abate, 1 ppm) as larvicide. However, these insecticides did not produce the desired results as is evident from the increase in the malaria incidence from 352 cases in 1986 to 4407 in 1987 and to 5666 in 1988 (Source: NMEP, Goa). In the absence of any previous report on insecticide suscepti-

*Malaria Research Centre, Field Station, Directorate of Health Services Building, Panaji - 403 001, India.

Table 1. Adult and larval susceptibility status of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*

Species	Insecticide	Dosage % (against adults) mg/l (against larvae)	Exposure time (h)	Sample size x replicates	% mortality			
					Experiment		Control	
					Field Pop.	Lab Pop.	Field Pop.	Lab Pop.
An. stephensi								
Adult	DDT	4.0	1	20 x 5	10	13	1	0
	Dieldrin	0.4	1	20 x 5	18	22	3	3
	Malathion	5.0	1	20 x 5	26	31	0	1
	Fenitrothion	1.0	2	10 x 5	82	90	4	2
Larva	DDT	2.5	24	25 x 4	4	11	—	—
	Malathion	3.125	24	25 x 4	52	61	—	—
	Fenitrothion	0.125	24	25 x 4	94	84	—	—
	Temephos	0.625	24	25 x 4	100	100	—	—
	Control	Ethanol	24	25 x 4	0	0	—	—
Cx. quinquefasciatus								
Adult	DDT	4.0	4	20 x 5	18	26	2	3
	Dieldrin	0.4	1	20 x 5	12	14	0	0
	Malathion	5.0	1	20 x 5	41	44	0	1
	Fenitrothion	1.0	2	10 x 5	72	82	0	0
Larva	DDT	2.5	24	25 x 8	7.5	14	—	—
	Malathion	3.125	24	25 x 8	76	84	—	—
	Fenitrothion	0.125	24	25 x 8	91	97	—	—
	Temephos	0.625	24	25 x 8	100	100	—	—
	Control	Ethanol	24	25 x 8	0.5	0	—	—
Aedes aegypti								
Adult	DDT	4.0	1	20 x 5	22	30	0	0
	Dieldrin	0.4	1	20 x 5	34	46	2	0
	Malathion	5.0	1	20 x 5	93	95	1	1
	Fenitrothion	1.0	1	20 x 5	87	93	0	0
Larva	DDT	2.5	24	25 x 8	64	72	—	—
	Malathion	3.125	24	25 x 8	100	100	—	—
	Fenitrothion	0.125	24	25 x 8	93.5	98.5	—	—
	Temephos	0.625	24	25 x 8	100	100	—	—
	Control	Ethanol	24	25 x 4	0.5	0	—	—

Pop. - Population.

bility of malaria vector *An. stephensi*, it was thought necessary to determine susceptibility in this species as well as in the vectors of filariasis *Cx. quinquefasciatus* and *Ae. aegypti*, the vector of DHF virus, against DDT, dieldrin, malathion, fenitrothion and temephos (only against larvae at 1 ppm dosage).

MATERIALS AND METHODS

Laboratory tests were conducted between August 1991 and February 1992 with the insecticide-impregnated papers and insecticide solutions supplied by WHO; and recommended procedures were followed for conducting susceptibility tests[2,3]. Fully fed females of test species were collected from unsprayed areas of Panaji and surroundings situated at 15° 31'N latitude and 73° 52'E longitude. Also, fully-fed F_1 generation adult females were exposed to the test insecticides for comparison of their mortality with adults from the field. Five replicates of 20 females each were exposed to each insecticide and mortality was recorded after 24 h holding time in experimental and control tubes. Similarly, to ascertain the larval susceptibility of the test species, healthy IV instars belonging to F_1 generation and maintained on dog biscuits and yeast extract at a ratio of 3:1, were also exposed to the test insecticides. For this purpose, 4 replicates each with 25 larvae of *An. stephensi* and 8 replicates each with 25 larvae of *Cx. quinquefasciatus* and *Ae. aegypti* were tested at 25°C±1°C and 80±2% RH.

RESULTS AND DISCUSSION

The results of susceptibility tests of adults and larvae are given in Table 1.

An. stephensi: Both the field and F_1 adults of *An. stephensi* were found resistant to DDT, dieldrin and malathion but they were susceptible to fenitrothion to a good degree. The susceptibility in F_1 adults was however higher than in the field populations. Similarly, *An. stephensi* larvae showed high resistance to DDT and fenitrothion and comparatively less resistance to malathion, although they were totally susceptible to temephos.

Culex quinquefasciatus: The adults of this species were found resistant to all the test insecticides to a variable degree, being highly resistant to dieldrin and DDT and moderately resistant to malathion. *Cx. quinquefasciatus* adults showed a low resistance to fenitrothion. On the other hand, larvae showed high resistance to DDT as in the case of adults but a low grade resistance to malathion and fenitrothion. Like *An. stephensi*, the larvae of this species were fully susceptible to temephos.

Aedes aegypti: The adults of this species showed a high degree of resistance against dieldrin. They were, however, highly susceptible to malathion and fenitrothion. The larvae of *Ae. aegypti* on the other hand were fairly susceptible to DDT, but there was 100 per cent mortality when exposed to malathion, fenitrothion and temephos.

The adults of malaria vector *An. stephensi* in Panaji and its surroundings were resistant to DDT, dieldrin and malathion, in accord with the earlier reports from other parts of India[3-5]. As far as fenitrothion is concerned, both the adults and larvae of *An. stephensi* showed a high degree of susceptibility. On the other hand, there was a clear indication of

development of resistance against malathion in this species. The larvae of *An. stephensi* and *Ae. aegypti* in this area are still fully susceptible to temephos.

The development of resistance in *An. stephensi* to DDT may perhaps be attributed to its continuous use by the NMEP from 1964 onwards. The DDT spray was intensified after the outbreak of malaria in 1986. Although dieldrin has never been used in Goa, the low mortalities in the test species could be due to development of cross resitance to this insecticide due to DDT resistance. However, the good degree of resistance to malathion in *An. stephensi* was interesting, given the fact that malathion has never been used in the health sector for mosquito control prior to these tests. In the agriculture sector too, a very limited use of malathion was made against rice borer from 1986 to 1988 in Chandor area of Goa (Personal communication from Department of Agriculture, Goa). As Chandor is at an aerial distance of 50 km from Panaji and our larval surveys have never yielded *An. stephensi* breeding from the rice fields, the possibility of exposure of this species to malathion in rice fields is ruled out. On the other hand, the common use of Baygon (mixtue of pyrethrum and malathion) in the households in urban areas against mosquitoes and cockroaches could have exerted selection pressure on *An. stephensi* and *Cx. quinquefasciatus* for malathion resistance in this area.

The adult and larval surveys have revealed that *An. stephensi* in Goa is presently distributed in a radius of 15 km around Panaji, near the coast (MRC, IDVC Report, 1992). We have never encountered this species in upper reaches. Contrary to these observations, Borcar *et al.*[6] had reported the distribution of *An. stephensi* in Goa between 1000 and 2000 ft above sea level and not in the coastal and subcoastal areas below 1000 ft. It is therefore necessary to ascertain whether the triple resistant *An. stephensi* population now distributed in and around Panaji has migrated from neighbouring Maharashtra or Karnataka states. This could be done by comparing cytogenetically the *An. stephensi* population of Goa with those from neighbouring states and also their susceptibility to various insecticides. Further studies on these lines are recommended.

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Laboratory and Field Evaluation of Spherix, a Formulation of *Bacillus sphaericus* (B-101), to Control Breeding of *Anopheles stephensi* and *Culex quinquefasciatus*

P.K. MITTAL*, T. ADAK*, C.P. BATRA* and V.P. SHARMA+

Spherix, a powder formulation of *Bacillus sphaericus* strain B-101, serotype H5a 5b, was evaluated against larvae of *Anopheles stephensi* and *Culex quinquefasciatus* in both the laboratory and field. In laboratory tests the formulation @ 0.1 g/sq m produced 100% mortality against larvae of both mosquito species at room temperature (28-32°C). The larvicidal activity of Spherix against *An. stephensi* @ 0.5 g/sq m persisted for over 12 weeks under laboratory conditions. Field evaluation of Spherix @ 0.25-2.0 g/sq m produced 95-100% reduction in the larval density of both *An. stephensi* and *Culex quinquefasciatus* within 48 h in different habitats, and the larvicidal activity persisted for 2-4 weeks in water habitats.

Key words: *Anopheles stephensi, Bacillus sphaericus, Culex quinquefasciatus*, Efficacy, Spherix, Vector control

INTRODUCTION

Anopheles stephensi, a major urban vector of malaria, and *Culex quinquefasciatus*, a vector of bancroftian filariasis and a nuisance pest mosquito, are the two major mosquito species which breed profusely in urban areas. The present strategy for controlling of malaria and mosquitoes in urban areas involves the use of chemical larvicides which are applied at weekly intervals. The continued use of chemical insecticides results in the gradual building up of resistance in mosquitoes as well as in environmental pollution. Chemical insecti-

*Malaria Research Centre, 2-Nanak Enclave, Delhi - 110 092, India.
+Malaria Research Centre, 20-Madhuvan, Delhi - 110 092, India.

cides are toxic to the non-target natural predators of mosquitoes which co-inhabit with the mosquito larvae. Alternatively biological control agents, such as larvivorous fish, have been successfully used under bioenvironmental methods for the persistent control of mosquito breeding[1]. However, larvivorous fishes cannot be used in all types of habitats such as temporary pits, polluted drains, thickly vegetated ponds, sullage pools, septic tanks, curing water, etc. Certain strains of *Bacillus sphaericus* have been reported to be highly toxic to larvae of *Culex* and some *Anopheles* species[2-4] and are safe to other non-target organisms. In addition, *B. sphaericus* preparations have been reported to recycle and persist for several weeks in certain habitats[5,6,7]. However, the activity and persistence of *B. sphaericus* preparations depend on the type of strain, its fermentation and formulation methods and also on environmental factors[8,9]. This paper reports the results of laboratory and field evaluation of Spherix, a new formulation of *B. sphaericus* B-101, against larvae of *An. stephensi* and *Cx. quinquefasciatus*.

MATERIALS AND METHODS

Spherix, a powder formulation of *B. sphaericus* strain B-101, serotype H5a 5b, produced in Russia by the Ministry of Medical Industry, was supplied by M/s. Chemicals International Ltd.

Laboratory evaluation

The efficacy of Spherix was determined against laboratory-colonized larvae of *Anopheles stephensi* and *Culex quinquefasciatus* at two different room temperatures: 8-22°C in January and 28-32°C in May. The tests were conducted in enamel trays (15 x 20 cm), containing 500 ml stored tap water in which 25 III instar larvae of the particular species were added along with 100 mg larval food (dog biscuit + yeast). Different dosages of the bioinsecticides, viz. 0.004, 0.02, 0.1 and 0.5 g/sq m, were applied on the surface of water in the form of a suspension, after making necessary dilutions. The experiment was repeated at least three times with concurrent control.

The toxicity of the bioinsecticide against larvae of *An. stephensi, Cx. quinquefasciatus* and certain non-target natural predators of mosquitoes, viz. larvivorous fish *Gambusia affinis* and *Poecilia reticulata*, frog tadpoles, notonectid bugs and *Mesocyclops* (copepods) was determined by exposing these organisms at different, serially diluted concentrations of the bioinsecticide preparation in separate experiments. Initially some preliminary tests were performed to test the range of these concentrations. Based on the tests the different concentrations used against mosquito larvae ranged between 0.00625 and 0.8 mg/l, while against non-target organisms other than notonectid bugs, the concentrations ranged between 125 and 1000 mg/l. For notonectid bugs the different concentrations used was in the range of 12.5-100 mg/l. A concurrent control was run for each test. The mortality among the different organisms, exposed to different concentrations of the bioinsecticide and controls, was recorded daily up to one week of observation. At least three replicates were used for each concentration.

The persistence of larvicidal activity of Spherix against *An. stephensi* was determined

in the laboratory in 5-litre beakers. The tests were performed in the months of January to March 1992 at room temperature (18-26°C). Each beaker contained four litres of water. The bioinsecticide suspension was applied on the surface of water to get a dose equivalent of 0.5 g/sq m. Four replicates of the test and two replicates of control were used. For each test, 25 III instar larvae were exposed in both treated and control beakers, and the mortality was recorded after 72 h of exposure to account for any delayed response. The dead larvae in the treated and the control containers were allowed to settle down, while the living larvae were removed after 72 h. The test was repeated at weekly intervals up to 12 weeks.

Field studies

Evaluation of Spherix against *An. stephensi* and *Cx. quinquefasciatus* was carried out in small borrow-pits, cesspools and blocked cement drains in and around Delhi. The trials were carried out during May to November 1991. The pits were located in a low-lying land and the area of these pits measured 1.5-5 sq m. The water in these pits was clear and had an acidic pH (5.5-6.5). The pools were made of waste-water collections and had an alkaline pH (7.0-9.0). The drains were cemented and blocked intermittently (length, 20-100 m; width, 0.5-1.0 m) and had a pH varying from 7 to 9. Heavy breeding of *An. stephensi* was observed in the newly constructed drains and in fresh rain-water collections. In some of the drains the blockage was of transient nature and therefore the water did not remain stagnant. The level of organic pollution in different habitats was categorised as low (fresh rain-water collection), moderate (standing water collection) and high (sewage water collection).

Spherix was applied as a diluted suspension with the help of a compression pump. The required dosages of the bioinsecticide (viz. 0.25-2.0 g/sq m) were obtained by spraying 0.25-2% suspension over a precalculated surface area @ 100 ml/sq m. Some of the identical habitats were left untreated to serve as control. A dipper was used to estimate immature densities by taking 5 dips in small pits (up to 10 sq m) and 10 dips in pools and drains. The immature density was determined before and after the treatment in control and experimental (treated) habitats at an interval of 24 h up to 2 days, then after 7 days and then at weekly intervals. The per cent reduction in the density of larvae was calculated by using Mullas's formula[10], which is given below:

$$\% \text{ reduction} = 100 - \frac{C_1}{T_1} \times \frac{T_2}{C_2} \times 100$$

where C_1 is number of larvae in untreated (control) habitat before treatment; C_2, number of larvae in control habitat after treatment; T_1, number of larvae in treated habitat before treatment; and T_2, number of larvae in treated habitat after treatment.

RESULTS AND DISCUSSION

Laboratory studies

Results of laboratory evaluation of Spherix against larvae of *An. stephensi* and *Cx. quinquefasciatus* are given in Table 1. The efficacy of the *B. sphaericus* preparation varied against different species and at different

Table 1. Laboratory evaluation of Spherix against larvae of *Anopheles stephensi* and *Culex quinquefasciatus*

Dosage of Spherix g/sq m	% mortality			
	An. stephensi		*Culex quinquefasciatus*	
	24 h	48 h	24 h	48 h
	(Temperature 18-22°C)			
0.5	1.8	76.4	68.6	98.6
0.1	1.3	37.0	57.4	90
0.02	0.5	8.0	57.6	81
0.004	—	—	46	88
Control	0.6	0.6	3.0	6.0
	(Temperature 28-32°C)			
0.5	98	100	100	100
0.1	93	100	100	100
0.02	40	80	99	99
0.004	—	—	95	96
Control	0	0	2.0	3.0

temperatures. At a room temperature of 18-22°C, the bioinsecticide @ 0.1 g/sq m produced only 37% and 90% mortality against *An. stephensi* and *Cx. quinquefasciatus*, III instar larvae, after 48 h. However, 100% mortality was obtained against both the species at the same dosage when the tests were repeated at a room temperature of 28-32°C. Though varying results were obtained at different temperatures in the laboratory tests, the bioinsecticide produced high mortalities against both mosquito species, particularly at a temperature which is highly conducive for the proliferation of these mosquito species in nature.

Table 2 shows the comparative toxicity of Spherix against larvae of *An. stephensi*, *Cx. quinquefasciatus* and various non-target organisms including the larvivorous fish *Gambusia* and *Poecilia*, frog tadpoles, notonectid bugs and *Mesocyclops*. The LC_{50} value of Spherix against larvivorous fish *Gambusia* and *Poecilia* and also frog tadpoles and *Mesocyclops* were greater than 1 g/l, which is approximately 5000 and 20,000 times more than required against larvae of *An. stephensi* and *Cx. quinquefasciatus* respectively (Table 2). Similarly the LC_{50} values against notonectid bugs *E. indica* and *Anisops* species were respectively greater than 100 mg/l and 50 mg/l, which are also approximately 500 and 250 times higher than in *An. stephensi* larvae. Various strains of *B. sphaericus* have been reported to be highly specific against larvae of *Culex* and *Anopheles* mosquitoes and almost non-toxic to non-target organ-

Table 2. Comparative toxicity of Spherix, against larvae of *An. stephensi*, *Cx. quinquefasciatus* and certain natural predators of mosquito larvae

Organism	Species	LC_{50} after 48 h mg/l	
Mosquito larvae	*An. stephensi*	0.2	—
	Cx. quinquefasciatus	0.05	
Larvivorous fish	*Gambusia affinis*	>1000	(>125*)
	Poecilia reticulata	>1000	(>125*)
Amphibians	(Frog tadpoles)	>1000	(>125*)
Notonectid bugs	*Enithares indica*	>100	(>25*)
	Anisops sardea	>50	(>12.5*)
Copepods	*Mesocyclops*	>1000	(>125*)

*No mortality was observed up to one week exposure period at these concentrations.

isms[11]. The formulated products may however produce some toxic effects. The results of our study confirm the safety of this formulation against various non-target organisms at dosages required against mosquito larvae. The larvicidal activity of Spherix against *An. stephensi* under laboratory conditions persisted for 12 weeks, when tested in containers treated with Spherix @ 0.5 g/sq m (Fig. 1). During this period the per cent mortality against *An. stephensi* declined from 88-34% after 5 weeks, but later, much higher per cent mortalities were obtained up to 12 weeks. Silapanuntaku[12] reported prolonged larvicidal activity of *B. sphaericus* up to 9 months in tap water in shallow jars. Davidson *et al.*[2] reported recycling and amplification of *B. sphaericus* 1593 in the larval cadavers. The persistence of larvicidal activity in our study up to 12 weeks after an early decline just after 5 weeks, may also probably be due to recycling and amplification of spores in the cadavers of dead larvae.

Field evaluation

The efficacy and persistence of larvicidal effect of Spherix against *An. stephensi* and *Cx. quinquefasciatus* in different habitats are given in Tables 3 and 4. The bioinsecticide formulation @ 0.25 g to 2.0 g/sq m produced over 95% reduction in the density of III and IV instar larvae of both the mosquito species in most habitats, within two days of its application. However, the larvicidal activity persisted for different durations in different habitats probably depending upon pH and organic pollution besides other environmental factors.

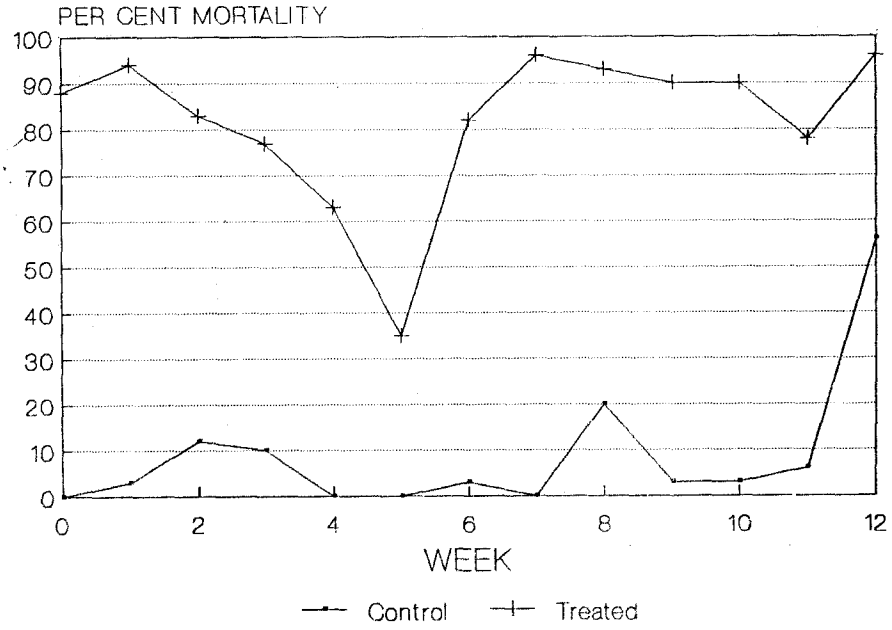

Fig. 1: Efficacy and persistance of larvicidal activity of 'Spherix' against larvae of *An. stephensi* under laboratory conditions.

Table 3. Field evaluation of Spherix against larvae of *An. stephensi* in different habitats

Habitat	Date of treatment	Water conditions organic (pH) pollution	Dosage g/ sq m	No. of replicates	Average larval density per dip (III + IV instars) pre-treatment	Per cent reduction in larval density (III + IV instars only) post-treatment (Days)					
						1	2	7	14	21	28
Borrow-pits	7.5.91	Moderate (5.5-6.5)	0.5	3	5.83	100	100	98.1	100	48.2	35.2
			1.0	3	13.65	98.3	98.9	89.3	100	100	100
			2.0	3	10.1	98.6	100	100	—	—	—
Cesspools	19.6.91	Moderate (8.5)	0.5	1	11.6	100	100	12.3	—	—	—
			1.0	1	5.8	100	100	83.3	9.2	—	—
Blocked	19.6.91	Low (7.5)	1.0	1	4.7	100	100	75.4	73.3	50	—
cement drains	19.8.91	Low (7)	0.5	4	18.5	98.6	100	99.1	97.8	94.6	46.22
	20.8.91	Moderate (9.0)	0.5	2	29.2	100	100	0	—	—	—
	3.9.91	Low (7.5)	0.5	3	17.5	100	100	96.9	89.4	82.9	—
		Moderate (8.0)	0.5	3	11.1	100	100	18.6	—	—	—
	18.9.91	Low (7)	0.25	2	46.3	86.1	96.3	91	100	—	—

Table 4. Field evaluation of Spherix against *Culex quinquefasciatus* in different larval habitats

Habitat	Date of treatment	Water conditions organic (pH) pollution	Dosage g/sq m	No. of replicates	Average larval density per dip (III + IV instars) pre-treatment	Per cent reduction in larval density (III + IV instars only) post-treatment (Days)				
						1	2	7	14	21
Cesspools	8.5.91	High (7.5-8)	1.0	1	30	98.3	99.5	94.7	76.3	97.3
			2.0	1	1000	99.0	100	99.9	99.7	99.9
Cesspits	19.6.91	High (9.5)	0.5	2	12	100	98.3	4.7	dried	
Drains	19.6.91	Moderate (8.5)	1.0	1	95.6	100	100	62.4	79.6	95.1
	20.8.91	Moderate (8.5)	0.5	2	278	99.9	99.9	98.8	94.8	83.5
	3.9.91	Low rain-water collection (7.0)	0.5	3	16.46	92.1	100	74.7	52.3	—
	10.9.91	Moderate (8.0)	0.5	2	49.1	97.6	98.25	97.6	88.5	71.4
			1.0	1	17.4	91.5	100	98.9	98.3	91.6
	13.11.91	High (8.0)	0.5	2	999	82.5	85.9	85.5	85.7	62.6

The larvicidal activity of Spherix against *An. stephensi* persisted for 2 to 4 weeks at a dosage of 0.5 and 1 g/sq m in small borrow-pits with slightly acidic pH (5.5-6.5) and moderate organic pollution, while in pools with alkaline pH (8-9) and higher organic pollution the bioinsecticide activity declined after 3 days. In blocked cement drains with rain water collection (pH, 7-9) the bioinsecticide activity persisted from 2 days to 3 weeks (Table 3). An important factor which probably affected the persistence of the bioinsecticide activity in drains is the nature of blockage. Since in some drains the blockage was transient in nature, the water did not remain stagnant for a long time, thus affecting the persistence of larvicidal activity. The larvicidal activity of Spherix against *Culex* persisted from 2 days to 3 weeks in different habitats (Table 4). In pools with high organic pollution and a pH of 7-8 the bioinsecticide @1 and 2 g/sq m produced over 95% reduction in the density of III and IV instar larvae up to 3 weeks of observations. However, in cess pits with high pollution and at a pH of 9-10, the duration of larvicidal activity lasted only 2 days. In blocked drains with moderate to high organic pollution and (pH 7.5-9), Spherix produced 86-100% reduction in the larval density within 2 days and the efficacy lasted about 3 weeks (Table 4).

From the field trials it is evident that Spherix is highly effective against larvae of *An. stephensi* and *Cx. quinquefasciatus* at various dosages, viz. 0.25 to 2.0 g/sq m, in different types of breeding habitat, but the persistence of its larvicidal activity against both the species is adversly affected at higher pH of the water in the habitat. In addition to pH, higher organic pollution in the habitats also affects the persistence of *B. sphaericus* formulation particularly at low dosages. Mulla *et al.*[13] reported an inverse relationship between the efficacy of *B. sphaericus* 2362 against mosquito larvae and level of pollution in water. In our study, high initial activity of Spherix at low doses, viz. 0.25 to 0.5 g/sq m, even in the polluted habitat indicates its usefulness in the control of mosquito breeding.

The results also indicate that higher doses of Spherix produce a longer duration of control against larvae of both the species. Similar results showing longer durations of larvicidal effect at higher doses with other *B. sphaericus* preparations, have been reported earlier[7,14]. The persistence of larvicidal activity of *B. sphaericus* formulations may be due to recycling and multiplication of spores in larval cadavers and certain aquatic situations[2,15] or may be simply due to long-term persistence of sufficient and accessible toxin or a combination of both. Though the results of our study do not ascertain the recycling potential of Spherix under field conditions, they do indicate the persistence of its larvicidal effect for about 3 weeks in most of the stagnant water habitats. Hence, further trials are contemplated to study the impact of Spherix on the control of vector of malaria and filariasis in different endemic areas of the country.

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Interspecific Associations among Anophelines in Different Breeding Habitats of Kheda District Gujarat: Part II — Non-Canal Area

R.N. BHATT*, R.C. SHARMA*, H.C. SRIVASTAVA*, A.S. GAUTAM* and D.K. GUPTA*

A total of 25,858 anophelines comprising 15 species emerged from 1104 samples of immatures from 9 breeding habitats surveyed for one year in six villages representing the non-canal area of Kheda district. Species diversity and homogeneity were maximum in seepage drains. *An. annularis*, *An. barbirostris*, *An. culicifacies*, *An. stephensi* and *An. subpictus* were ubiquitous. Interspecific associations occurred more commonly in habitats representing the stable ecosystem. Community groups of anophelines in different habitats were identified.

Key words : Anophelines, Diversity, Habitats, Interspecific associations

INTRODUCTION

Neither organisms nor species populations exist by themseleves in nature but are always part of an assemblege living together as a community in the same area or habitat and interact in predation and competition for food and space. Several characteristics of communities that can be measured and studied include species diversity and interspecific associations to decide (i) what species should be grouped as a community, and (ii) where community boundaries should occur[1].

Continuous use of insecticides in agriculture and public health programmes has been responsible for initiating species replacement in mosquitoes. Insecticides can also cause a change in breeding habitats[2]. This study was aimed at identifying the community structures and species diversity in different breeding habitats and at delineating the range of

*Malaria Research Centre, Field Station, Civil Hospital, Nadiad - 387 001, India.

conditions under which anophelines breed. The objective was of special relevance to malaria vectors as it is the species such as *An. culicifacies* and *An. stephensi* that have shown a tendency to escape the ordinary confines of their natural breeding habitats to invade niches established by man (intradomestic breeding sources) in the rural environs and thus to provide easy links for disease transmission[3].

In India, anophelines breeding in various habitats have been studied[4-8]. Investigations on interspecific associations among mosquito larvae to determine the community characteristics have also been carried out [9-11]. Interspecific associations and species abundance in different breeding habitats of canal-irrigated areas in Kheda district, Gujarat, have also studied[12]. Our study was undertaken in different larval habitats of the non-canal area of Kheda district where the availability of breeding sources varies with the season as compared to relatively stable ecosystem represented by the canal-irrigated area.

MATERIALS AND METHODS

Studies were carried out from April 1990 to March 1991 in six villages representing plain (2), riverine (2), hilly (1) and coastal (1) areas. No canal irrigation system existed in these villages. Except for hilly and coastal areas, all the physiographic areas have been described earlier[3]. The small hilly region is represented by a few villages on the northernmost part of Balasinor and Kapadwanj talukas. The coastal area is represented by 5 villages as identified by the Government of Gujarat[13]. Larval and pupal collections were made from all the breeding sources every fortnight in each village and larval abundance was estimated by counting the number of larvae per dip with a standard enamel dipper. The larvae were brought to laboratory and reared, and the individuals emerging from each sample were identified using Christophers' key[14].

To facilitate statistical analysis the habitats surveyed have been classified into nine categories. Except for the habitats defined below, all have been described in earlier studies[3].

Swamps : Seepage of subsurface water at the bottom of ravine.

Seepage drain : Narrow, slow-flowing water due to seepage from the hill.

Borrow-pit : Small depression formed due to earth excavation for household and other purposes.

The first two habitats were encountered in hilly and riverine areas only. The Shannon-Wiener diversity index (H') was calculated from species abundance data for each habitat. A community with a few evenly represented species can have the same diversity index as the one with many, unevenly represented species. To keep distinct these two ingredients of diversity, equitability or homogeneity (J') was measured[15]. Homogeneity (range 0-1) is maximum when species are evenly distributed and minimum when the fauna is dominated by a large number of a few species. 'Species means' among different habitats were compared using one-way analysis of variance, and among means, differences were detected using Tukey multiple comparison

test[16]. Sampling efforts varied from month to month and the monthly collections do not represent a complete survey of all the breeding habitats due to seasonality. Therefore, the species that emerged were pooled for each habitat and associations among the anopheline larvae in different habitats were quantified using C_8 index of Hurlbert[17]. Statistical significance was assessed with the corrected χ^2 formula of Pielou[18]. When any expected cell total was less than or equal to five, an exact test was applied.

RESULTS AND DISCUSSION

A total of 1104 samples of immatures were collected from nine breeding habitats and 25,858 anophelines representing 15 species were identified (Table 1a and 1b). Habitatwise data from different areas were pooled due to

Table 1a. Diversity and abundance of anophelines in different larval habitats

	Pond	Well	Rice field	River	River-bed pool
No. of samples	54	317	36	35	29
Mean species (±SE)	1.88±0.16	1.87±0.06	2.15±0.27	2.11±0.15	2.32±0.20
No. significance associations	4	5	4	0	0
Mean pH (range)	7.86 (6-8)	7.5 (6-9)	7.25 (7-8)	7.1 (7-8)	7.1 (7-8)
Diversity index (N')	0.922	1.114	0.660	0.890	0.918
Mean density (±SE)	3.68±1.16	2.13±0.29	4.89±2.39	1.91±0.03	3.38±1.40
% frequency of species					
An. aconitus	0.37	0.31	0.34	0.0	0.27
An. annularis	4.47^{bc}	2.82^{bc}	3.20^{bc}	4.51^{bc}	0.54^{bc}
An. barbirostris	7.13^{ab}	9.38^{a}	2.58^{ab}	0.39^{b}	0.27^{b}
An. culicifacies	14.27^{bc}	8.60^{bc}	3.61^{bd}	62.47^{a}	42.03^{ab}
An. fluviatilis	0.45^{b}	0.63^{ab}	0.43^{b}	0.0	0.0
An. jamesii	0.0	0.03	0.0	0.0	0.0
An. nigerrimus	0.45	0.79	1.20	0.39	0.0
An. pallidus	0.0	0.03	0.08	0.0	0.0
An. splendidus	0.0	0.13	0.94	0.0	0.27
An. stephensi	0.45^{bc}	11.23^{a}	0.68^{bc}	1.57^{bc}	4.01^{bc}
An. subpictus	72.28^{ab}	66.82^{ab}	86.23^{ab}	30.64^{ab}	52.07^{ab}
An. tessellatus	0.07	0.0	0.43	0.0	0.0
An. theobaldi	0.0	0.03	0.43	0.0	0.40
An. vagus	0.0	0.0	0.0	0.0	0.13
An. varuna	0.0	0.06	0.0	0.0	0.0
Adults emerged	1317	3159	1162	509	747

Table 1b. Diversity and abundance of anophelines in different larval habitats

	Seepage drain	Swamps	Borrow-pit	Hoof/Tyre print	Anova*
No. of samples	94	109	482	28	
Mean species (±SE)	2.71±0.15	2.66±0.13	1.23±0.04	1.82±0.17	
No. significance associations	10	15	5	0	
Mean pH (range)	7.09 (6.5-8)	7.18 (7-8.5)	7.36 (6-9)	7.2 (6-8.5)	
Diversity index (N')	1.672	1.617	0.141	0.395	
Mean density (±SE)	3.02±0.54	3.23±0.72	14.11±3.08	16.45±5.22	
% frequency of species					
An. aconitus	2.27	0.76	0.05	0.19	ns
An. annularis	32.23^{a}	12.92^{ac}	0.17^{bc}	0.09^{bc}	***
An. barbirostris	1.57^{ab}	2.05^{ab}	1.07^{ab}	0.09^{b}	**
An. culicifacies	31.54^{acd}	35.47^{ac}	1.12^{b}	11.98^{bc}	***
An. fluviatilis	6.81^{a}	2.22^{ab}	0.01^{b}	0.0	**
An. jamesii	0.75	0.53	0.0	0.0	ns
An. nigerrimus	0.69	0.71	0.08	0.0	ns
An. pallidus	0.25	0.44	0.0	0.0	ns
An. splendidus	3.91	2.13	0.01	0.0	ns
An. stephensi	1.20^{bc}	6.24^{ac}	0.05^{b}	2.18^{bc}	***
An. subpictus	17.28^{b}	34.58^{ab}	97.35^{a}	85.25^{ab}	**
An. tesselatus	0.0	0.49	0.05	0.0	ns
An. theobaldi	1.19	0.85	0.0	0.0	ns
An. vagus	0.0	0.0	0.01	0.0	ns
An. varuna	0.25	0.58	0.0	0.19	ns
	1585	2244	14884	1051	

*One way analysis of variance, ns = (P>0.05); ** P<0.01; ***P <0.001, frequencies followed by same letter in a row were not significantly different (P>0.05) using Tukey multiple comparison test.

their insignificant difference and analysed. The systematic account of the number of species observed to breed, their abundance, homogeneity (J') and larval associations are given under each breeding habitat. Larval associations include only those species showing significant positive or negative C_8 values; non-significant and zero associations have been excluded for brevity's sake.

Ponds

Species : 9
Homogeneity : 0.42

Larval associations :

Positive : An. nigerrimus with *An. fluviatilis* and *An. barbirostris.*

Negative : An. subpictus with *An. annularis* and *An. fluviatilis* (Table 2,a).

An. subpictus, An. culicifacies, An. barbirostris and *An. annularis* constituted 98% of the total adults that emerged (Table 2,a). *An. fluviatilis* was not recorded in the previous studies carried out in this area[3,12] but there are reports of its breeding in ponds[9,19].

Wells

Species : 13
Homogeneity : 0.43
Larval associations :

Positive : An. annularis with *An. culicifacies* and *An. barbirostris; An. subpictus* with *An. stephensi; An. barbirostris* with *An. fluviatilis* and *An. nigerrimus* (Table 2,b).

Predominance of *An. subpictus* is similar to the earlier observations[12]. *An. varuna* showed little breeding preference, while the presence of *An. subpictus*, *An. culicifacies* and *An. stephensi* is supported by earlier observations[7,9] (Table 1a).

Rice fields

Species : 12
Homogeneity : 0.27
Larval associations :

Positive : An. splendidus with *An. stephensi* and *An. culicifacies; An. annularis* with *An. barbirostris;* and *An. culicifacies* with *An. stephensi* (Table 2,c).

An. subpictus was the predominant species. *An. annularis, An. barbirostris, An. culicifacies* and *An. nigerrimus* accounted for 10% and the rest of the 7 species for only 4%. Occurrence of *An. fluviatilis, An. splendidus* and *An. theobaldi* is in agreement with the observations made in Orissa (Table 1a).

River

Species : 6
Homogeneity : 0.5
Larval associations : Nil

Only six species were found breeding and *An. subpictus* and *An. culicifacies* were dominant accounting for 93% of the adults that emerged, which is in agreement with the previous observation[10] (Table 1a).

River-bed pools

Species : 9
Homogeneity : 0.42
Larval associations : Nil

In addition to the species reported to breed[3], five species, viz. *An. aconitus, An. barbirostris, An. splendidus, An. theobaldi* and *An. vagus,* were encountered. The fauna was dominated by *An. subpictus* and *An. culicifacies* (94%) followed by *An. stephensi.*

Seepage drains

Species : 13

Homogeneity : 0.65

Larval associations :

Positive : *An. stephensi* with *An. theobaldi* and *An. culicifaces*; *An. fluviatilis* with *An. aconitus* and *An. splendidus*; *An. barbirostris* with *An. annularis, An. nigerrimus* and *An. jamesii;* and *An. varuna* with *An. splendidus*

Negative : *An. subpictus* with *An. annularis* and *An. fluviatilis* (Table 2, d).

An. annularis and *An. culicifacies* were predominant species owing to their preference

Table 2. Coefficient of association (C_8) of anophelines in different habitats

(a) **Ponds**

	aconitus						
annularis (an)	0.0	an					
barbirositris (ba)	0.571	0.274	ba				
culicifacies (cu)	0.0	0.100	0.084	cu			
fluviatilis (fl)	0.0	0.0	0.0	0.059	fl		
nigerrimus (ni)	0.0	0.0	0.338**	0.0	0.420	ni	
stephensi(st)	0.0	0.0	0.0	0.051	0.0	0.0	st
subpictus	0.0	-0.613**	0.0	-0.253	-0.610*	0.0	0.0

(b) **Wells**

	annularis						
barbirostris (ba)	0.389**	ba					
culicifacies (cu)	0.103***	0.036	cu				
fluviatilis (fl)	0.044	0.378*	-0.356	fl			
nigerrimus (ni)	0.253	0.109***	0.018	0.0	ni		
splendidus (sp)	0.0	0.020	0.0	0.0	0.0	sp	
stephensi (st)	0.0	-0.295	0.037	-0.559	0.0	0.0	st
subpictus	0.209	-0.102	0.407	-0.0	0.020	-0.559	0.341**

(c) **Rice fields**

	annularis						
barbirostris (ba)	0.699***	ba					
culicifacies (cu)	0.0	0.0	cu				
nigerrimus (ni)	0.188	0.188	-1.0	ni			
splendidus (sp)	0.204	0.204	0.336*	0.0	sp		
stephensi (st)	0.0	0.0	0.226*	0.0	1.0*	st	
subpictus	0.0	-0.358	1.0	-0.518	0.0	0.0	

contd...

Table 2. Coefficient of association (C_8) of anophelines in different habitats (*contd.*)

(d) Seepage drains

	aconitus										
annularis (an)	0.038	an									
barbirostris (ba)	-0.510	0.087*	ba								
culicifacies (cu)	-0.269	-0.235	-0.310	cu							
fluviatilis (fl)	0.591***	0.077	0.0	-0.249	fl						
jamesii (ja)	0.0	0.026	0.300**	-0.569	0.058	ja					
nigerrimus (ni)	0.0	0.0	0.279**	-0.526	0.0	0.0	ni				
splendidus (sp)	0.0	0.0	0.0	0.0	0.186*	0.0	0.0	sp			
stephensi (st)	0.0	-0.559	-1.6	0.546*	0.0	0.0	0.0	0.282	st		
subpictus (su)	-0.248	-0.374**	0.0	0.035	-0.588**	0.0	0.0	-0.221	-0.536	su	
theobaldi (th)	0.0	0.0	0.0	0.030	0.064	0.0	0.0	0.120	0.242*	-0.344	th
varuna	0.0	0.0	0.0	0.0	0.078	0.0	0.0	0.154*	0.0	-1.0	0.0

(e) Swamps

	aconitus											
annularis (an)	0.0	an										
barbirostris (ba)	0.0	0.119	ba									
culicifacies (cu)	0.0	-0.259*	-0.746	cu								
fluviatilis (fl)	0.394*	0.169	0.107	0.0	fl							
jamesii (ja)	0.0	0.082	0.0	-1.0*	0.0	ja						
nigerrimus (ni)	0.0	0.063	0.0	-0.721*	0.0	0.231	ni					
pallidus (pa)	0.0	0.043*	0.062	0.0	0.0	0.0	0.0	pa				
splendidus (sp)	-1.0	0.109	0.078	-0.128	0.0	0.0	0.0	0.0	sp			
stephensi (st)	0.0	-0.280	-0.253	0.053	0.0	-1.0	0.0	0.0	0.162*	st		
subpictus (su)	0.0	-0.376	-0.564	0.360	-0.188	-1.0	-1.0	0.0	-0.188	0.213	su	
theobaldi (th)	0.0	0.0	-1.0	0.047*	0.0	0.0	0.0	0.0	0.193**	0.096*	0.0	th
varuna	0.197*	0.030	0.0	0.0	0.136*	0.0	0.0	0.0	0.073	-1.0	-1.0*	5.0

(f) Borrow-pits

	annularis					
barbirostris (ba)	0.239*	ba				
culicifacies (cu)	0.083**	0.037	cu			
nigerrimus (ni)	0.160***	0.102**	0.0	ni		
stephensi (st)	0.232*	0.0	0.0	0.0	st	
subpictus (su)	-0.101	-0.087	-0.097	0.0	0.0	su
tesellatus	0.0	0.0	0.028	0.0	0.0	0.0

*levels of significance for $\chi^2_{(1)}$: *P < 0.05; **P<0.01; ***P<0.001.

for aquatic weed-infested and fresh slow-flowing water. Highest per cent of positive occurrence was observed for *An. fluviatilis*, *An. jamesii*, *An. splendidus* and *An. theobaldi*. Maximum number of species per sample were collected and the fauna exhibited maximum diversity and homogeneous distribution (Table 1b).

Swamps

Species : 14
Homogeneity : 0.61
Larval associations :
Positive : An. theobaldi with *An. culicifacies* and *An. stephensi; An. annularis* with *An. pallidus; An. fluviatilis* with *An. aconitus* and *An. varuna; An. nigerrimus* with *An. jamesii; An. splendidus* with *An. theobaldi, An. annularis* and *An. stephensi.*
Negative : An. culicifacies with *An. annularis, An. nigerrimus* and *An. jamesii; An. subpictus* with *An. varuna* and *An. jamesii* (Table 2, e).

An. culicifacies and *An. subpictus* were present in almost equal proportions, an observation which is in agreement with that of Russell and Rao[9]. *An. pallidus, An. splendidus* and *An. tessellatus* showed preference for swamps over other habitats. Maximum larval associations were detected, which is suggestive of stable ecosystem, favourable microclimate and less predation. In comparison to drain, diversity index and homogeneity were less but higher than in other habitats. This ecosystem was a preferred breeding place for *An. stephensi* after wells, which is unique for this area (Table 1b).

Borrow-pits

Species : 11
Homogeneity : 0.06
Larval associations :
Positive : An. annularis with *An. culicifacies, An. stephensi, An. nigerrimus* and *An. barbirostris; An. nigerrimus* with *An. barbirostris* (Table 2, f).

An. subpictus was the most dominant species. Similar results have been reported from Tamil Nadu[7] and Orissa[8]. Very high larval density was observed owing to the small size of the habitat. Diversity index and homogeneity were minimum among all the habitats surveyed owing to the predominance of a single species. Occassional occurrence of weed might have attracted several minor species resulting in significant associtions, but none with *An. subpictus* (Table 1b).

Hoof/Tyre prints

Species : 7
Homogeneity : 0.21
Larval associations : Nil

Maximum larval density with poor diversity and homogeneity were observed owing to predominance of *An. subpictus*. *An. culicifacies* was the second dominant species, a finding in agreement with observations made in south-eastern Madras[9]. *An. aconitus, An. barbirostris* and *An. varuna* were not reported from this breeding place in a previous study[3]; however, their occurrence was rare (Table 1b).

CONCLUSIONS

Swamps and seepage drains support many

anopheline species and the diversity index and homogeneity values show that they are the most suitable habitats for anopheline breeding. Maximum larval associations recorded in these habitats may suggest high survival rate, ovipositional preferences and favourable physico-chemical characteristics of the habitats.

On the basis of associations observed between anophelines in different breeding habitats, it is possible to group them as a distinct community. Thus, maximum community groups occur in drains, whereas in ponds and borrow-pits, commoner species could form only one group, and *An. subpictus* breeds in isolation (Table 3).

Table 3. Anophelines grouped as community in different breeding habitats

Habitat	I	II	III	IV	V
Ponds	*annularis* *barbirostris* *culicifacies*				
Wells	*annularis* *barbirostris* *culicifacies* *nigerrimus*	*stephensi* *subpictus*			
Rice fields	*annularis* *barbirostris* *nigerrimus*	*culicifacies* *splendidus* *stephensi*	*culicifacies* *subpictus*		
Drains	*subpictus* and occasionaly *annularis*	*barbirostris* *jamesii* *nigerrimus* and occasionaly *annularis*	*aconitus* *fluviatilis*	*culicifacies* *stephensi* *theobaldi*	*fluviatilis* *splendidus* *theobaldi* *varuna*
Swamps	*culicifacieś* *stephensi* *subpictus*	*annularis* *barbirostris* *jamesii* *nigerrimus* *splendidus* *theobaldi*	*aconitus* *fluviatilis* *varuna*		
Borrow-pits	*annularis* *barbirostris* *culicifacies* *nigerrimus* *stephensi*				

Our study has clearly shown the contribution of larval habitats in supporting the breeding of different species and malaria vectors in particular. The diverse nature of habitats preferred by the vectors may be helpful in planning appropriate antilarval operations.

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SHORT NOTES

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Preliminary Observations on Mosquito Collections by Light Traps in Tribal Villages of Madhya Pradesh

NEERU SINGH*, A.K. MISHRA* and O.P. SINGH*

Key words: Light trap, Malaria, *Plasmodium falciparum*, Tribal villages, Vectors

The basic principle governing the use of light traps for sampling mosquitoes has been highlighted and reviewed by Service[1], and Ahmed and Panda[2]. The traps have been commonly employed to record changes in abundance of mosquitoes before and after control campaigns, and to compare seasonal and annual fluctuations in population size. Efficacy of these traps has been found to depend on the ecological zones and the dispersal pattern of the vector population within the zone[3]. This is a preliminary study undertaken to determine the period of peak activity in a forested region situated in deciduous wet forest belt to assess the feasibility of using impregnated bednets for controlling malaria.

The study was carried out in 4 tribal villages (85% Gond tribe) of Mandla district. Study villages comprise a group of hamlets (3-5) in which 5-25 families live. The hamlets are scattered and located on hill slopes or at the foot of hills with a stream nearby. The means of communication are scanty because of natural barriers like thick forest patches, rocky streams and their tributaries. Teak (*Tectona grandis*) forests are common with plenty of *Bambusa nutanus* (bamboo) and *Madhuca indica* (mahua).

Generally houses have attached cattlesheds and are made of bamboo with mud plaster on both the sides. Outdoor sleeping is very com-

*Malaria Research Centre (ICMR), Field Station, Medical College Building, Jabalpur - 482 003, India.

Table 1. Records of anopheline species* collected by light trap (September 1991 to August 1992)

Time interval hrs	*An. culici-facies*	*An. sub-pictus*	*An. annu-laris*	*An. flu-viatilis*	*An. theo-baldi*	*An. splen-didus*	*An. jam-esii*	*An. var-una*	*An. jeop-oriensis*	*An. tur-khudi*	*An. nige-rrimus*	Total
1800-1900	104	19	7	8	3	3	0	0	0	1	0	145
1900-2000	170	60	15	1	1	5	3	0	0	1	2	258
2000-2100	183	56	15	12	4	1	1	0	0	1	0	273
2100-2200	128	49	11	10	5	1	1	1	1	2	1	210
2200-2300	101	31	13	6	7	2	1	0	0	1	0	162
2300-2400	80	29	5	11	4	1	2	0	1	0	0	133
2400-0100	71	25	7	3	3	0	0	3	0	0	0	112
0100-0200	65	8	4	1	8	1	0	0	0	0	0	87
0200-0300	58	27	6	1	8	0	1	1	3	0	0	105
0300-0400	42	16	2	0	4	0	0	2	1	0	1	68
0400-0500	44	16	5	1	1	0	0	0	0	0	0	67
0500-0600	55	25	1	0	3	0	0	0	1	0	0	85
Total	1101	361	91	54	51	14	9	7	7	6	4	1705

**An. vagus, An. barbirostris* and *An. moghulensis* were captured in very small numbers.

mon. All the houses have only one electric point connection.

The light trap experiments were carried out from September 1991 to August 1992. A CDC light trap with black light was used in the study. The collections were made in each of the villages from 1800 to 0600 hrs once a week except in December, January and June when only 3, 3 and 2 collections respectively were made. The trap was always sited in a fixed room (indoor) in human dwellings at a constant height of $5^1/_2$ ft. The room was without windows and electricity, but people slept inside the room. The domestic light (20 W bulb) outside the room was switched on from 1900 to 2000 hrs. The trap was manually emptied at hourly intervals until morning. Mosquitoes collected every hour were kept in labelled test tubes. Temperature and humidity were also recorded hourly at the site of trap collection. Mosquitoes were identified with the help of Christophers' key[4].

A total of 1711 anophelines representing 14 species was collected in 44 trap nights (Table 1). In addition, 446 *Culex* and 24 *Aedes* mosquitoes were caught. *An. culicifacies* was the predominant species forming 64.34% of the total anopheline catches followed by *An. subpictus* (21%), *An. annularis* (5.3%) and *An. fluviatilis* (3.1%). The average catches of *An. culicifacies* and *An. fluviatilis* per trap per night were 25 and 1.2 respectively. Fig. 1 shows monthwise collections of total anophelines (man-hour, trap-night) and *An.*

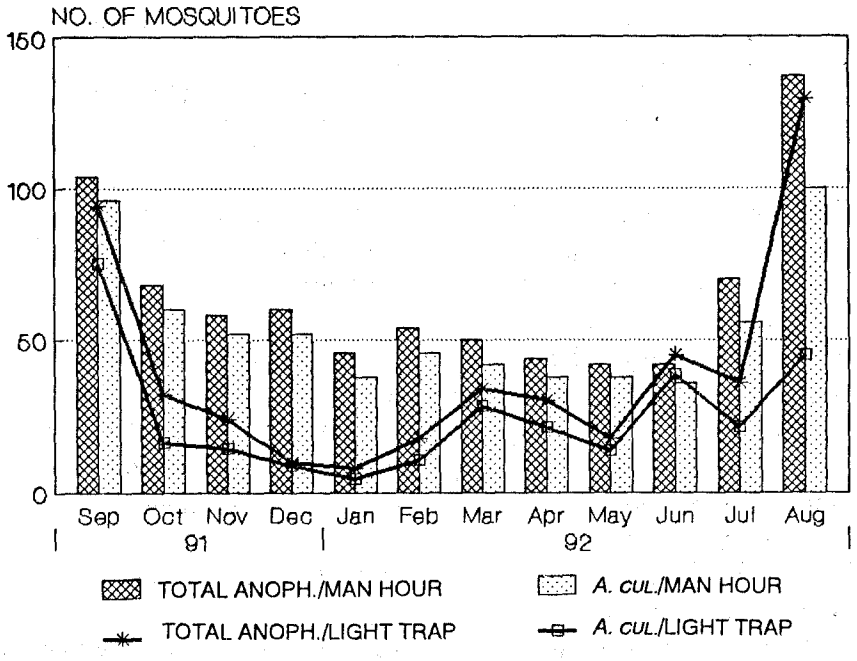

Fig. 1: Monthwise collection of anophelines by two methods; indoor resting collection by suction tube/light trap.

Table 2. Sexwise records of anophelines in light trap collections (September 1991—August 1992)

Time interval hrs	*An. culicifacies*		Other anophelines		Total anophelines (%)	
	Female	Male	Female	Male	Female	Male
1800-1900	76	28	33	8	109 (75.17)	36 (24.82)
1900-2000	129	41	81	8	210 (81.10)	49 (18.90)
2000-2100	168	15	83	8	251 (91.60)	23 (08.40)
2100-2200	117	11	80	4	197 (92.90)	15 (07.07)
2200-2300	86	15	61	2	147 (89.60)	17 (10.36)
2300-2400	78	2	51	2	129 (96.90)	4 (03.00)
2400-0100	63	8	41	0	104 (92.80)	8 (07.14)
0100-0200	57	8	22	0	79 (90.80)	8 (09.20)
0200-0300	49	9	44	3	93 (88.57)	12 (11.42)
0300-0400	36	6	24	2	60 (88.20)	8 (11.80)
0400-0500	33	11	20	3	53 (79.10)	14 (20.90)
0500-0600	44	11	27	3	71 (83.50)	14 (16.50)
Total	936	165	567	43	1503 (87.84)	208 (12.16)

culicifacies (Man-hour, trap-night) from September 1991 to August 1992. There was a substantial number of males in the light trap catches (12%). Out of 1101 *An. culicifacies,* 165 (15%) were male (Table 2). This indicates that breeding of these species was proximal to houses sampled.

Analysis of data collected shows that peak activity for *An. culicifacies* appears to be between 1900 and 2100 hrs and for *An. fluviatilis* between 2000 and 2200 hrs and very few mosquitoes were caught after 0200 hrs. It appears that both the species were most active at dusk. During the study poor catches of *An. culicifacies* were obtained when the temperature was 20°C or lower. Similar results were obtained in Bastar[2].

Our study shows that feeding of *An. culicifacies* starts soon after dusk, i.e. at 1800 hrs with a peak between 1900 and 2100 hrs. Therefore, use of impregnated bednets for control of malaria is not feasible.

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Resting Sites of *Anopheles stephensi* Liston in Calcutta

K.K. CHATTERJEE*, D. BISWAS*, D.K. CHOUDHURI+, H. MUKHERJEE* and A.K. HATI*

Key words: *An. stephensi*, Vector ecology

Determination of indoor resting sites of any arthropod vector is of paramount importance for establishing population indices before and after control operation for an appropriate evaluation; for adopting proper chemical control measures; for collecting specimens for bloodmeal analysis; as well as for calculation of infection rates, age grading and other population analysis[1]. *Anopheles stephensi* is the long-recognized vector of malaria in the city of Calcutta[2,3]. Information about the resting sites of the vector species in this city is not available due to the practical non-availability of its adults in their conventional resting places, i.e. rooms of brick-built houses[4-8]. An attempt was made to study this aspect by collecting adults of the vector species from three different types of shelters, i.e. temporary huts, brick-built rooms and cattlesheds, throughout the year in an area of Calcutta. This paper reports data collected during the investigation.

Temporary hutments (*jhupries*) exist at many places in the city, even in posh residential areas. These structures have thatched roofs, made of bamboo, tile, polythene sheet and asbestos or corrugated sheets. Often there is no window, and doors are so small that they do not allow sufficient light to enter. Very dark corners are found with used-up clothes, folded umbrellas, folded mosquitoe nets, etc. hang-

*Department of Medical Entomology, Calcutta School of Tropical Medicine, Calcutta-700 073, India.
+Academic Staff College and Department of Zoology, University of Burdwan, Burdwan-713 101, India.

Table 1. Monthwise collection of *Anopheles stephensi* from fixed 42 temporary hutments, 8 cattlesheds and 36 brick-built rooms in an area of Calcutta

Year/Month	Temporary hutments		Cattlesheds		Brick-built rooms	
	No.	PMD	No.	PMD	No.	PMD
1986						
Oct	19	0.34	8	0.75	—	—
Nov	23	0.41	5	0.47	1	0.02
Dec	1	0.02	2	0.19	—	—
1987						
Jan	—	—	1	—	—	—
Feb	2	0.04	—	—	—	—
Mar	5	0.09	1	0.09	—	—
Apr	3	0.05	5	0.47	—	—
May	11	0.19	3	0.28	—	—
Jun	29	0.52	9	0.84	1	0.02
Jul	98	1.75	28	2.63	3	0.06
Aug	62	1.10	11	1.03	—	—
Sep	32	0.57	14	1.31	2	0.04
Total	285	0.42	87	0.68	7	0.01

PMD - Per man-hour density.

ing disorderly, and cobwebs are also found in corners and under the roof. Brick-built pucca houses are occupied mostly by rich people. Brick-built rooms are well ventilated and brighter. Owing to protective steps taken by the government of West Bengal against health hazards, cattlesheds are becoming rare in Calcutta. Wherever found, they often exit in proximity to temporary shelters or brick-built rooms. Structures that exist are similar to temporary shelters. Inside it is very dark, often foul smell emanating. Cattlesheds may also be regarded as mixed dwelling, as human beings sleep there at night.

Employing 10 min per shelter per day, mosquitoes were collected from fixed 42 temporary shelters, 36 brick-built rooms and 8 cattlesheds in an area of about 0.04 sq km in Central Calcutta. Collection was made with the help of test tubes and aspirators between 0600 and 0800 hrs twice a week from October 1986 to September 1987. Mosquito collections were brought to laboratory for identification. Per man-hour collection of *Anopheles stephensi* was noted every month shelterwise as a measure of its adult density. Surfaces on which adults of the species were collected were also recorded.

The overall man-hour collections of *Anopheles stephensi* were as: 0.42 (0 - 1.7) in temporary hutments, 0.68 (0 - 2.6) in cattlesheds, and 0.01 (0 - 0.06) in brick-built rooms (Table 1). Prevalence of adults of the vector species was higher in both temporary hutments and cattlesheds than in brick-built rooms. In Calcutta, *Anopheles stephensi* breeds predominantly in man-made containers in and around brick-built houses[9], but our study shows that brick-built houses are not conducive to the resting of the vector species. Temporary hutments and cattlesheds seem to be the preferred resting places of *Anopheles stephensi*. Paucity of mosquitoes in brick-built rooms may possibly be due to excito-repellent action of insecticides, used in bygone days, as pointed out by Hati *et al*[10]. The species was found in greater numbers during the rainy season (July to October), i.e. 211 out of 285 (74.0%) in temporary hutments, 61 out of 87 (70.1%) in cattlesheds and 6 out of 7 in brick-built rooms, with a common peak in July, which suggests that in Calcutta, rainy season is more conducive to the building up of adult population of *Anopheles stephensi* than winter and summer seasons.

Altogether 285 and 87 adults of *Anopheles stephensi* were obtained respectively from temporary hutments and cattlesheds whereas only 7 were obtained from brick-built rooms. Taken as a whole, 37% (139/379) of the adults were collected on hanging objects, i.e. umbrellas, nylon strings, cobwebs, mosquito nets, clothes, gunny bags, etc.; 19% (71) on furniture; 25% (96) from ceiling; 11% (41) on room materials, i.e. in and outside earthen pitchers, inside empty tin drums, iron pillars, etc.;6% (24) on walls; and 2% (8) on miscellaneous objects (Table 2). Hanging objects seemed to be the preferred resting sites of

Table 2. Resting sites of *Anopheles stephensi* in different types of shelters in an area of Calcutta (October 1986 to September 1987)

Type	Temporary shelters		Cattlesheds		Brick-built rooms		Total	
	No.*	%	No.	%	No.	%	No.	%
Hanging objects	122	42.8	13	14.9	4	57.1	139	36.7
Furniture	64	22.4	6	6.9	1	14.3	71	18.7
Ceiling	54	18.9	42	48.3	—	—	96	25.3
Room materials	31	10.9	9	10.3	1	14.3	41	10.8
Walls	14	4.9	9	10.3	1	14.3	24	6.3
Miscellaneous objects	—	—	8	9.2	—	—	8	2
Total	285	100.0	87	100.0	7	100.0	379	100.0

*Number of adults collected.

Anopheles stephensi in both temporary huts (43%) and brick-built rooms (4 out of 7) but in cattlesheds, mosquitoes preferred taking shelter on ceiling (48%). Information collected about the resting sites of *Anopheles stephensi* may be of considerable help in formulating an effective control strategy against the ageold problem of malaria in Calcutta.

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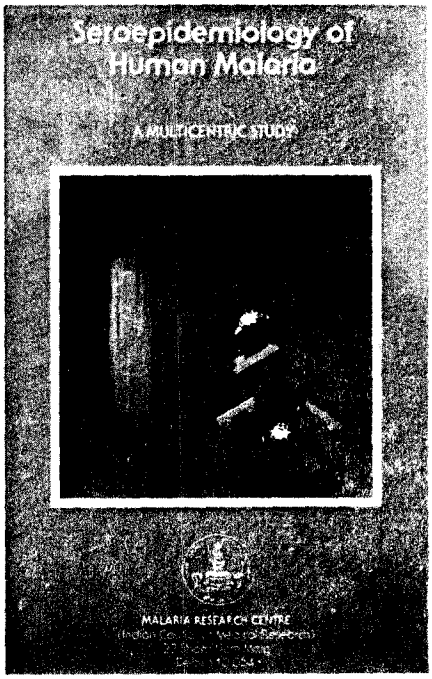

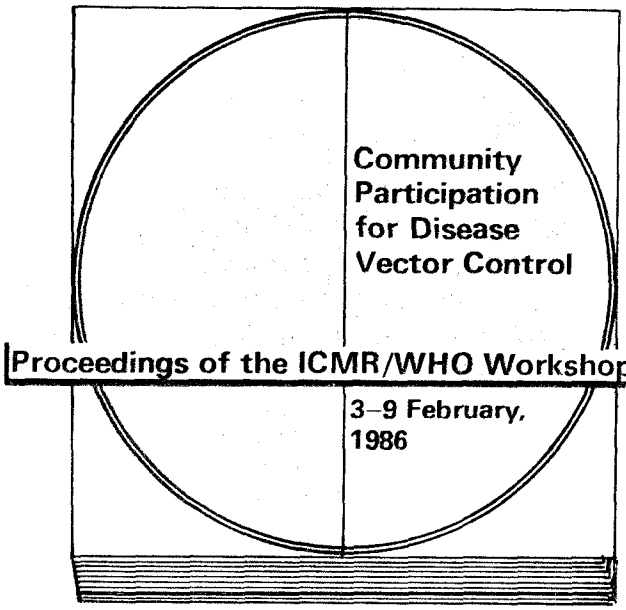

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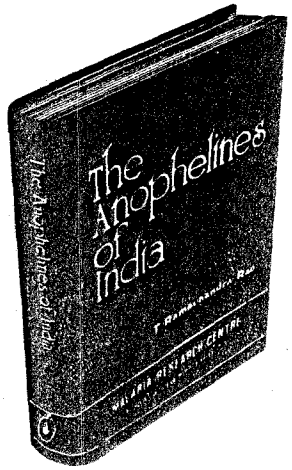

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