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Short Note

Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors. This issue has been delayed for reasons beyond our control.

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Culture of *Gambusia affinis* with Food Fishes

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

Culture of *Gambusia affinis* along with carps in fish culture practice in village ponds in Shahjahanpur distt., U.P., has revealed very little difference in fish productivity (1539 kg/ha in ponds with *Gambusia*, 1572 kg/ha in ponds without *Gambusia*). The growth and survival of *Gambusia* were found to be good in most of the composite fish culture ponds. Culture of *Gambusia* along with edible fish in village ponds is, therefore, recommended to get the dual benefit of fish production and control of mosquito proliferation in village ponds.

INTRODUCTION

During 1970s a widespread resurgence of malaria was reported in India[1]. It was also noted that insecticides have a limited scope as the mosquitoes develop resistance against various insecticides and effective control of vectors cannot be achieved even at enormous costs. Moreover, environmental scientists also demanded restricted use of insecticides. In this situation there was an acute need to develop alternative methods of vector control. Recent studies have shown that larvivorous fishes can play an increasingly greater role in the control of mosquito breeding and consequent curtailment of transmission of the vector-borne diseases[1]. *Gambusia affinis* has been most widely used against mosquito larvae followed by *Poecilia reticulata* and the killifishes[2]. Under bioenvironmental control strategy implemented in Shahjahanpur distt. in 1986, a large-scale introduction of *Gambusia*, a first-priority ranking biological control agent, was carried out in rural areas to control the deteriorating malaria situation.

However, introduction of *Gambusia* in aquatic habitats of economic importance has been suggested as a possible hazard to natural fauna and flora as this may lead to imbalance in the ecosystems[3,4]. Fish farmers commonly carry out edible fish culture in village ponds for the economic benefit in this area. So it was important to know whether *Gambusia* could be cultured along with edible fishes without causing an adverse effect on the edible fish production. Therefore, a study was taken up to mass-culture *Gambusia* along with different edible fish species in the natural habitats. The results of this study are reported in this paper.

MATERIAL AND METHODS

Fish farmers of Shahjahanpur distt. were contacted to culture *Gambusia* along with edible

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fishes in village ponds. In this area six edible fish species are being cultured by the fish farmers:

(i) *Catla catla*, (ii) *Labeo rohita*, and (iii) *Cirrhinus mrigala* (all Indian major carps), (iv) *Hypophthalmichthys molitrix* (silver carp), (v) *Ctenopharyngodon idella* (grass carp), and (vi) *Cyprinus carpio* (common carp), the last three being exotic fishes.

The fry and fingerlings of edible fish species ranging from 2 to 6 cm were procured by fish farmers from Calcutta.

All the ponds are situated within a radius of 50 km from Shahjahanpur city. These ponds are natural and of *kutcha* type. The main source of water in these ponds is rain water. But in summer season, lifting water is supplied. Table 1 gives the total area of ponds, and the number of fry and fingerlings of different edible fish species introduced by the fish farmers.

Supplementary diet in the form of mustard oil cake and rice bran (1 : 3) was provided fortnightly to the fishes. However, the quantity of the supplementary diet varied from one pond to another as it was related to the convenience and economic status of fish farmers.

Gambusia affinis was initially procured from Haldwani, Nainital distt. (U.P.), in the months of March–April 1988 and introduced in different village hatcheries. The fish multiplied in these hatcheries and further distribution of *Gambusia* was carried out to other PHCs of Shahjahanpur. *Gambusia* was introduced in 18 ponds in which fish farmers were carrying out edible fish culture. These ponds were distributed in 5 PHCs, viz. Nigohi, Sindhauli, Powayan, Bhawal Khera and Khudaganj.

Three to six thousand *Gambusia* fish ranging from 1.5 to 5 cm in length were introduced in each pond during the months of February to March 1989. The population density of *Gambusia* was monitored during September–October 1989 in all the ponds using a seine net of 20 x 6 m. Netting was carried out 5 times at fixed corners of all the ponds between 0900 and 1000 hrs. The population density of *Gambusia* per metre water area was calculated by taking the average of five nettings.

Edible fishes were harvested during November 1989 to January 1990. The edible fish production per hectare was calculated on the total weight of the fish harvested from each pond. The fish production of experimental ponds with *Gambusia* and control pond without *Gambusia* have been compared considering the food and feeding habits of all the edible fish species and possible competition with *Gambusia*.

RESULTS AND DISCUSSION

The edible fish production of the ponds with *Gambusia* and without *Gambusia* are given in Table 1. The average fish production of ponds of both the categories have very little difference, i.e. 1539 kg/ha in *Gambusia* ponds and 1572 kg/ha in the ponds without *Gambusia* during the same period. Production of edible fish varied from one pond to another. This variation seems to be related to the productivity of different ponds. However, higher fish production was related to the composition of fish species comprising surface (Catla), column (Rohu) and bottom feeders (Mrigal) in most of the ponds except where the fish mortality was reported by the fish farmers.

Introduction of *Gambusia* led to good survival in most of the composite fish culture ponds. After about six months of introduction of *Gambusia*, the population density per metre water area ranged between 10.5 and 88.2. The average population density in all the ponds was 47.06, which shows excellent population growth of *Gambusia* in these ponds (Table 1). The high population density of *Gambusia* in these ponds seems to be related to the supplementary diet provided to the fishes. Moreover, fish farmers eliminate carnivorous and weed fishes from ponds before the

Table 1. Edible fish production with *Gambusia*

S. No.	PHC/Villages	Pond area (ha)	Fish species introduced (Month, Year : Number)	Edible fish production kg/ha (Nov 89_ Jan 90)	Introduction and population density of *Gambusia*	
					No. introduced (Feb_Mar 89)	Population denisty (per sq m water area) (Sep_Oct 89)
NIGOHI						
1.	Pachdeura	0.3	Rohu, Catla, Mrigal (Aug 87:5000; Jul 88:10000)	3333	6000	62.5
2.	Hasaua	0.4	Rohu, Catla, Silver Carp (Jul 88:10000; Aug 89:5000)	2500	6000	58.2
3.	Nigohi	0.6	Rohu, Catla, Silver Carp (Jul 88:42000)	1833	6000	24.5
4.	Dheerath	0.4	Rohu, Catla, Mrigal, Grass Carp (Aug 87:2000; Aug 89:4000)	2000	3000	38.0
5.	Bhatura	0.3	Rohu, Catla, Silver Carp, Common Carp (Jul 87:4000; Aug 87:5000)	1666	3700	69.0
6.	Jathora	0.6	Rohu, Catla, Mrigal (Jul 88:5000)	250	3000	18.0
7.	Dhullia	0.4	Rohu, Catla, Mrigal, Silver Carp (Jul 88:5000; Aug 89:12000)	1250	4000	43.8
8.	Shadevpur	1.0	Common Carp (Mar 88:3000)	400	5000	13.0
SINDHAULI						
9.	Charari	0.4	Rohu, Catla, Mrigal (Mar 88:12000)	1250	3000	51.0
10.	Gandharpur-I	0.6	Rohu, Catla, Mrigal, Grass Carp (Aug 88:35000)	1333	5000	87.0
11.	Gandharpur-II	0.6	Rohu, Catla, Mrigal, Grass Carp (Aug 88:50000)	1000	8000	71.0
POWAYAN						
12.	Chodera	0.8	Rohu, Catla, Mrigal (Jul 88:25000)	1250	4000	22.3
13.	Imalia	0.4	Catla, Mrigal, Grass Carp, Common Carp. (Jul 88:16000)	1500	5000	88.2

contd. ...

Table 1. Edible fish production with *Gambusia* (contd.)

S. No.	PHC/Villages	Pond area (ha)	Fish species introduced (Month, Year : Number)	Edible fish production kg/ha (Nov 89–Jan 90)	Introduction and population density of *Gambusia*	
					No. introduced (Feb–Mar 89)	Population denisty (per sq m water area) (Sep–Oct 89)
BHAWAL KHERA						
14.	Bandigawn	0.9	Rohu, Mrigal, Silver Carp, Common Carp (Jul 88:50000)	2222	3000	75.1
KHUDAGANJ						
15.	Laxmipur-I	1.2	Rohu, Catla, Mrigal, Grass Carp, Silver Carp, Common Carp (Jul 88:60000)	1166	3000	43.0
16.	Laxmipur-II	0.2	Rohu, Catla, Mrigal, Grass Carp, Common Carp (Jul 88:25000)	2500	4000	10.5
17.	Laxmipur-III	0.2	Rohu, Catla, Mrigal, Grass Carp, Silver Carp, Common Carp (Jul 88:25000)	2000	3000	19.0
18.	Jalal Nagar	0.4	Rohu, Catla, Mrigal, Grass Carp, Silver Carp, Common Carp (Jul 88:15000)	250	4000	53.0
			Average	1539		17.06
			Edible fish production without *Gambusia*			
NIGOHI						
1.	Arjunapur	0.75	Rohu, Catla, Mrigal (Jul 88:11000)	2500		
2.	Piparia (Udai Bhanpur)	1.5	Rohu, Catla, Mrigal, Grass Carp (Jul 88:10000)	2333		
3.	Patapur	1.25	Rohu, Catla, Mrigal, Silver Carp, Grass Carp (Aug 88:20000)	600		
SINDHAULI						
4.	Muria Pawar	2.0	Rohu, Catla, Mrigal, Grass Carp (Sep 88:35000)	857		
			Average	1572.50		

introduction of the edible fishes for their better survival and growth. This also contributed to population growth of *Gambusia* in these ponds.

The edible fish production of experimental ponds with *Gambusia* and control ponds without *Gambusia* was found to be more or less the same, although the former category of ponds was harbouring a considerable population density of *Gambusia* fishes which were sharing the diet and territory from the same aquatic ecosystem. This is indeed a significant finding. This may be due to the segregation in the food and feeding habits of all the edible fish species being cultured with *Gambusia*. It is well known that *Gambusia* is a surface feeder and insect larvae and zooplankton constitute the main diet of the fish. Except *C. catla* and *H. molitrix,* which are surface feeders, other fish species, viz., *L. rohita*, *C. mrigala* and *C. carpio*, are either column feeders or bottom feeders. Therefore, complete segregation in the feeding habits of *Gambusia* and the above mentioned fish species exists, thus reducing the chances of competition for food and territory.

However, the possible competition of *Gambusia* with surface feeders like *C. catla* and *H. molitrix* can be expected because these three fish species are surface feeders and feed on similar food items and exhibit clear interaction in their food and feeding habits. But similarity of diet is not the only indication of competition. One of the criteria for competition for food is that food must be in short supply[5,6]. The village ponds are generally rich in nutrient contents, being nearby agriculture fields, with organic manures being available in the form of human/cattle fecal matters. Zooplankton density increases with the increase in the nutrient content of the water and this increase in zooplankton is directly associated with an increase in the phytoplankton density, a function of increased nutrients in the water[7,8]. Therefore, it may be assumed that sufficient food was available to all the surface feeder fishes, so that edible fish production was not adversely affected with the introduction of *Gambusia*.

C. idella (grass carp) is a phytophagous fish which consumes filamentous algae in the initial stages but as the fish grows in size it takes other leafy aquatic plants[9]. Therefore, this fish is gaining world-wide popularity as a biological weed control agent. Owing to its ability in controlling a variety of aquatic weeds it indirectly helps the reduction of mosquito breeding through the destruction of shelter places of mosquito larvae and provides easy access for larvivorous fishes to feed on mosquito larvae. Thus, introduction of grass carp in village ponds is recommended.

A conclusion of the study is that composite fish culture with *Gambusia* can yield double benefits of fish production as well as the control of mosquito propagation in village ponds. Gupta *et al.*[10] have linked bioenvironmental control of malaria with edible fish production in Gujarat. They have mass-cultured *P. reticulata* with a fairly good edible fish yield from the village ponds. However, in the presence of *Gambusia* it is suggested that fingerlings of edible fishes should be preferred, as *Gambusia* is not harmful to them. Culture of *Gambusia* at village level is vey vital for the successful implementation of bioenvironmental control strategies for vector-borne diseases. Through active community participation the culture of *Gambusia* and its distribution in various mosquito-breeding sites may be carried out to control malaria and other vector-borne diseases under the technical guidance of malaria control agencies.

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Infection Rate and Vectorial Capacity of Malaria Vectors in Jeypore Hill Tract

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The role of different anophelines in malaria transmission in two geoclimatic zones, viz., Jeypore and Malkangiri plateau of Koraput distt., which are endemic for malaria, was studied. Out of 10,110 specimens belonging to 9 species collected and dissected over a period of 12 months by different methods in nine study villages, natural infection (sporozoites and oocysts) was detected in *An. fluviatilis* in both the zones. The sporozoite and oocyst rates were 0.28% and 1.41% in Jeypore zone and 5.09% and 0.85% in Malkangiri zone, respectively. Sporozoites were also found in a single specimen of *An. culicifacies* in Malkangiri area. *An. annularis* was incriminated in an earlier study. The vectorial capacities of *An. fluviatilis, An. culicifacies* and *An. annularis* were estimated for the three plasmodial species, viz., *P. falciparum*, *P. vivax* and *P. malariae* in different seasons. The definitive role played by each species in transmission is discussed.

INTRODUCTION

Vector incrimination is a prerequisite for understanding malaria situation and for planning and evaluating malaria control, particularly when several anopheline vector species co-exist. Three closely related species, *An. fluviatilis, An. minimus* and *An. varuna,* were incriminated as malaria vectors in hyperendemic Jeypore hills during pre-DDT period. *An. culicifacies* was considered to play no role in transmission, though oocyst infections were found[1,2]. After a decade, sporozoites were found only in *An. fluviatilis* and not in the other two[3]. A recent survey in 1986-88 indicated changes in the composition of anopheline species and natural infection was found in four species viz., *An. fluviatilis, An. annularis, An. culicifacies* and *An. aconitus*[4]. However, the actual role of different species in transmission and their vectorial status in different seasons have not been studied.

Therefore, a longitudinal study was undertaken in two of the four geoclimatic zones in Koraput distt., viz., Jeypore and Malkangiri, covering all the seasons of the year on vector bionomics, infection rates and vectorial capacity. Information on the resting and biting habits and breeding habitats has been published[5-7]. The results on infection rate and vectorial capacity for the period from January to December 1989 are given here.

MATERIAL AND METHODS

Anophelines were obtained from day-time indoor and outdoor resting shelters, from night-

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resting and all-night man-landing collections (hand catch) and with light traps and magoon traps, and these were identified. The nine recognized vector species, viz., *An. fluviatilis*, *An. culicifacies*, *An. annularis*, *An. minimus*, *An. jeyporiensis*, *An. aconitus*, *An. varuna*, *An. maculatus* and *An. philippinensis*, were dissected and examined for natural infection in gut/gland. The collections were done in five villages, namely Champapodar (top hill), B. Singhpur and Deulaguda (foothill), Seamalaguda (riverine) and Benasur (plain) in Jeypore zone (600 m altitude), and four villages, namely Kandhaguda (top hill), Bhandaguda (foothill), MV-7 (riverine) and Siadimal (plain) in Malkangiri zone (150 m altitude).

Vectorial capacity was calculated for three species, viz., *An. fluviatilis*, *An. culicifacies* and *An. annularis*, by using MacDonald's formula as modified by Garrett-Jones[8]:

$$C = \frac{ma^2 p^n}{-\log_e p}$$

The man biting rate (No. biting/man/night) (ma) was obtained from all-night man-landing collections[7], averaging indoor and outdoor results. Biting habit on man (a) was estimated by dividing the human blood index (HBI) (proportion of blood meals taken on man) by the interval (in days) between consecutive blood meals (i.e., gonotrophic cycle, assuming gonotrophic concordance). The HBI was obtained from the analysis of blood meals of mosquitoes collected from different day-time resting places using agar-gel diffusion method[9]. The gonotrophic cycle of *An. fluviatilis* was assumed to be 2 days for hot (March-June) and 3 days for rainy (July-October) and cold (November-February) seasons based on the studies in North Kanara[10]. The same duration was considered for the other two species also. Estimation based on the distribution of resting mosquitoes by gonotrophic stages plus the state of ovariolar sacs in biting population[11] is an indirect method. Direct observations of captive mosquitoes and recapture of the marked ones could not be done owing to low vector density and difficulty in colonization as well as for ethical reasons. The probability of daily survival (p) was computed from the proportion of parous females of biting population[12-13], and parity was determined by Polovodova's dilatation method. Other methods of estimation based on delayed and immediate sporozoite rates or analysis of decrease in total daily recaptures of marked mosquitoes[14] are time-consuming and require high density and so could not be used.

The duration of sporogony (n) was estimated as a function of temperature by using the formula

$$n = T/t - t_{min.}$$ (Ref. 11)

where n is duration of sporogony; T is total 'degree days' required for the sporogony; t is actual average temperature in °C; and $t_{min.}$ is threshold temperature required for the parasite development.

Data on minimum and maximum temperatures were obtained from the Rice Research Station, Jeypore, and Block Development Office, Malkangiri.

Since monthly samples were too small to calculate the survival rate, numbers collected in different months of a season were pooled together and the vectorial capacity was estimated season-wise. The vectorial capacity of *An. fluviatilis* was calculated from the data obtained from Champapodar (Jeypore zone) and Kandhaguda (Malkangiri zone), where sufficient samples on man-biting rate were available. Similarly, data collected from Kandhaguda (Malkangiri) and Deulaguda (Jeypore) were used in the case of *An. culicifacies* and *An. annularis*, respectively.

RESULTS AND DISCUSSION

Of the nine species dissected, infection (sporozoite/oocyst) was found in *An. fluviatilis* in both Jeypore and Malkangiri zones and *An. cu-*

licifacies only in Malkangiri zone (in a riverine village). None of the other anophelines was found infected (Table 1). The sporozoite rates of *An. fluviatilis* for these two areas were 0.28% and 5.09% and the oocyst rates, 1.41% and 0.85%, respectively.

In nine villages surveyed, infection in *An. fluviatilis* was found in five. Infection rate was higher in hill top and foothill villages. Infection was detected in all seasons of the year in Malkangiri zone, indicating perennial transmission. Of the total sporozoite positives, 62.5% was found in cold season, the period of peak malaria incidence[15]. In Jeypore zone, though sporozoite positives were obtained only in rainy and summer seasons, 60% of the total oocyst positives were found during cooler months (Table 2).

In riverine and plain villages, prevalence of *An. fluviatilis* was low owing to the absence of streams[6], and infections were found in some months only, indicating a short period of transmission. This correlated with the variation observed in the level of malaria incidence between the villages[15].

Table 3 summarizes the average man-biting rate, probability of survival through one day and estimated vectorial capacity of *An. fluviatilis*, along with the temperature and number of malaria cases (based on fortnightly fever survey) for three seasons.

There is a wide variation in the vectorial capacity of *An. fluviatilis* between the villages in the two zones. A higher value was obtained for Malkangiri zone owing to higher man-biting rate and human blood index, though cattle-to-man ratio is higher in this zone than in Jeypore. The possible explanation for such higher values of these two parameters in Malkangiri zone has been discussed[7]. The daily survival rate, however, did not vary between the two zones. The physiological ability of vector population in the two zones in acquiring as well as transmitting the acquired infection (vector competence) has been assumed

Table 1. Number of different Anopheline species dissected for gut and gland infections in two zones during January to December 1989

S. No.	Species	Jeypore zone			Malkangiri zone		
		No. dissected	Gland (%)	Gut (%)	No. dissected	Gland (%)	Gut (%)
1.	*An. fluviatilis*	1062	3 (0.28)	15 (1.41)	471	24 (5.09)	4 (0.85)
2.	*An. culicifacies*	3123	0	0	1131	1 (0.09)	0
3.	*An. annularis*	2596	0	0	171	0	0
4.	*An. aconitus*	187	0	0	3	0	0
5.	*An. jeyporiensis*	1142	0	0	16	0	0
6.	*An. varuna*	64	0	0	66	0	0
7.	*An. minimus*	1	0	0	0	0	0
8.	*An. maculatus*	70	0	0	3	0	0
9.	*An. philippinensis*	3	0	0	1	0	0
	Total	8248	3	15	1862	25	4

Table 2. Monthwise distribution of oocyst and sporozoite infection of *An. fluviatilis* in Jeypore and Malkangiri in 1989

Month	Jeypore			Malkangiri		
	No. dissected	Sporozoite (%)	Oocyst (%)	No. dissected	Sporozoite (%)	Oocyst (%)
Jan	130	0	3 (2.3)	107	8 (7.5)	1 (0.9)
Feb	78	0	0	9	1 (11.1)	0
Mar	73	1 (1.4)	1 (1.4)	28	0	0
Apr	74	0	1 (1.4)	12	2 (16.7)	0
May	37	0	0	14	0	0
Jun	76	0	1 (1.3)	13	2 (15.4)	0
Jul	35	1 (2.9)	0	44	3 (6.8)	2 (4.5)
Aug	112	0	3 (2.7)	26	2 (7.7)	0
Sep	104	0	0	14	0	0
Oct	123	1 (0.8)	0	23	0	0
Nov	120	0	5 (4.2)	103	4 (3.9)	1 (1.0)
Dec	100	0	1 (1.0)	78	2 (2.6)	0
Total	1062	3 (0.28)	15 (1.41)	471	24 (5.09)	4 (0.85)

to be the same. This parameter, however, is a dynamic one, varying both temporally and spatially[16], and this may also vary between the population in the two zones, but needs further investigation.

Though *An. fluviatilis* has been incriminated as a major malaria vector in several parts of India, vectorial capacity has not been calculated. Only critical densities expressed in number per man-hour have been estimated on the basis of indoor resting populations[17].

Season-wise analysis shows that in both Malkangiri and Jeypore zones, vectorial capacity was higher in rainy season. In Malkangiri, though man-biting rate was higher in cooler months than in rainy season there was no corresponding increase in vectorial capacity. This may be due to the prolonged sporogonic period estimated in cooler temperatures.

In both Jeypore and Malkangiri zones, a significant positive correlation ($r = 0.8574$, $p = 0.029$) between the estimated vectorial capacities and

Table 3. The average man-biting rate, probability of survival through one day, and estimated values of vectorial capacity of *An. fluviatilis* along with temperature and number of malaria cases in different seasons

Seasons	Zones	Temperature	ma	p	Vectorial capacity			No. of malaria cases		
					Pf	*Pv*	*Pm*	*Pf*	*Pv*	*Pm*
Hot (Mar_Jun)	Jeypore	Min: 20.5 - 26.9 Max: 28.5 - 38.7	3.71	0.76	0.147	0.256	0.086	37	7	4
	Malkangiri	Min: 23.6 - 29.8 Max: 29.7 - 35.5	5.25	0.79	1.421	1.795	0.889	29	7	0
Rainy (Jul_Oct)	Jeypore	Min: 20.8 - 24.4 Max: 27.7 - 31.5	7.38	0.82	0.361	0.537	0.198	60	8	0
	Malkangiri	Min: 22.5 - 25.6 Max: 26.5 - 27.6	12.50	0.85	3.025	4.197	1.854	131	24	0
Cold (Nov_Feb)	Jeypore	Min: 10.0 - 17.0 Max: 26.7 - 31.9	3.35	0.82	0.049	0.110	0.019	35	5	2
	Malkangiri	Min: 17.4 - 20.2 Max: 30.0 - 29.3	19.63	0.86	2.379	4.362	1.118	108	15	1

HBI 0.26 for Jeypore
0.84 for Malkangiri

Gonotrophic cycle: Hot season = 2 days
Rainy & Cold seasons = 3 days

Pf _ *Plasmodium falciparum*; *Pv* _ *P. vivax*; *Pm* _ *P. malariae*.

number of malaria cases, particularly *P. falciparum*, was observed (Table 3). The peak incidence was in July-August in Jeypore and in October-November in Malkangiri, when monthly figures are considered[15].

The vectorial capacity of *An. annularis* was calculated only for cold and rainy seasons. The calculation could not be done for hot seasons owing to very low man-biting rate. The vectorial capacity for *P. falciparum* was relatively higher in cold season (0.005) than in rainy season owing to a higher survival rate.

An. culicifacies biting occurred only during rainy and hot seasons and respective vectorial capacities for *P. falciparum* were 0.117 and 0.017. Though the values are slightly higher than those obtained in Pakistan, representing unstable malaria situation in a non-epidemic year[18], it is definitely a low estimate when compared to the other observations made in India[19, 20] and it is mainly so because of poor man-biting habit.

The difference in the estimated vectorial capacity of a particular species for different plasmodial species is a reflection of difference in the estimated values of sporogonic period. As a result, higher values have been obtained for *P. vivax*, which has a shorter sporogonic period than *P. falciparum*. However, *P. falciparum* has been the predominant species in the two zones, forming 70-80% of the total malaria cases[21]. A comparatively longer duration of gametocytaemia in untreated persons and lesser stimulation of immune response have been reported to favour the preponderance of *P. falciparum* in areas where there is prolonged transmission[22]. The vectors may also have different susceptibilities to the different plasmodial species[17], which will be contrary to the assumption made in the estimation of vectorial capacity.

The study indicates that *An. fluviatilis* is the main malaria vector in both the zones. *An. culicifacies*, though incriminated as vector in other parts of Orissa[23], has a low vectorial capacity owing to its poor man-biting habit. In cold season its density reduces to a very low level[5] and at higher altitudes (Bonda hills, 900-1200 m MSL) the prevalence is very low in most of the months (VCRC, unpublished data). Therefore, it is concluded that this species plays a secondary role in villages at low altitudes and its contribution to transmission, particularly in Jeypore zone, seems to be less.

An. annularis has been known as a vector in coastal plains and in inlands of Orissa[24, 25]. But in this hilly tract, it is found in higher numbers only in a few villages of Jeypore zone, where vegetated ponds — the preferential breeding habitat of this species[26] — are present. The man-biting rate is generally very low and only one sporozoite positive was found in a foothill village earlier[4]. Therefore, it is concluded that *An. annularis* is only a localized vector playing a subsidiary role in malaria transmission particularly when the density is very high.

The estimated values of vectorial capacity of the vector species could be useful in stratifying this area in relation to transmission potential and the type of control strategy to be adopted, as suggested by the Malariogenic Stratification Committee, 1986[27].

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Resistant falciparum Malaria in an Endemic Area of Allahabad (U.P.)

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Over the last three decades the problems of resistance in *P. falciparum* malaria to chloroquine have emerged in many parts of the world including India. The present study was carried out at PHC, Jasra, an endemic area for malaria in Allahabad distt. to ascertain the level of resistance to chloroquine in *P. falciparum* in selected villages according to WHO extended 28-day field test. One hundred one (58.4%) cases were positive for malaria. Amongst the positive cases, 92 (91.1%) showed *P. falciparum* and 9 (8.9%) *P. vivax* infection. Of the 92 cases, 31 (33.7%) showed resistance to chloroquine and 61 (66.3%) were sensitive to the standard dose of chloroquine. Out of the 31 resistant cases, 19 (61.3%) showed resistance at RI level and 12 (38.7%) at RII level.

INTRODUCTION

Chloroquine has been for many years the drug of choice for the treatment of acute attack of malaria and also for chemoprophylaxis. However, in spite of the extensive use of this drug, emergence of resistance to chloroquine in human malaria had not been reported till 1960, when it was first reported in *Plasmodium falciparum* malaria in Columbia[1]. Since then chloroquine resistance has spread to other regions, with increase in the number of resistant cases and in the degree of resistance. At present almost all the countries in southeast Asian region have reported resistance of *P. falciparum* to chloroquine. In India the first confirmed report of chloroquine resistance was from Diphu area of Karbi-Anglog distt. of Assam in 1973. Thereafter, a large number of tests were carried out under the NMEP and by the Malaria Research Centre of ICMR to determine the status of *P. falciparum*. The results show more than 74 foci of varying resistance, as determined by the *in vivo* and *in vitro* tests, in 19 states and union territories[2]. In Uttar Pradesh the foci of chloroquine resistance have been found in Mirzapur and Mathura districts[3]. It is feared that in the absence of effective measures directed against the transmission of the disease and the large-scale movement of the population in and out of the drug-resistant areas, the malaria situation will further deteriorate[2].

This study was carried out at the PHC, Jasra, an endemic area for malaria, where recrudescence after chloroquine treatment in *P. falciparum* malaria was observed to be a common phenomenon.

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The objective of the study was to determine the sensitivity status of *P. falciparum* malaria parasite to standard doses of chloroquine as suggested by WHO[4].

MATERIAL AND METHODS

The study was conducted at PHC, Jasra, which is situated about 22 km from Allahabad city in trans-Yamuna area. For the study conducted during November and December, 1990, the non-transmission months for malaria, twelve subcentre villages were selected where male multipurpose health workers (MPWs) were residing. Active fever survey was carried out in the selected villages and blood smears were prepared for all fever cases detected. The thick smear was stained with Giemsa stain and 100 fields were examined under oil immersion lens for malaria parasite. Cases showing asexual stages of *P. falciparum* were given chloroquine orally for three consecutive days, 600 mg (or 10 mg/kg body weight) choloroquine each on Day 0 and Day 1 and 300 mg (5 mg/kg body weight) on Day 2, respectively. Blood smears were collected from these cases on days 3, 7, 14, and 18 of the treatment. The intake of the drug and collection of blood smears on specific days were ensured by the MPWs of the subcentre under the overall supervision of the investigators. A detailed history from each case was recorded and the patient was also examined clinically for anaemia and splenomegaly. Sensitivity(s) or Resistance(r) of asexual parasite of *P. falciparum* was graded on the basis of the criteria of the WHO *in vivo* test for determining chloroquine resistance.

RESULTS

Out of 173 blood smears collected from fever cases in the 12 selected villages, 101(58.4%) were found malaria positive. Of the total of 101 positive malaria cases, 92(91.1%) were *P. falciparum* and 9(8.9%) *P. vivax* infections (Table 1).

Out of the 92 *P. falciparum* cases tested for chloroquine sensitivity, 31(33.7%) showed different levels of resistance and the rest were sensitive to the drug. Amongst those who showed resistance to chloroquine, 19(61.3%) had RI level of resistance. In the remaining 15(78.9%) there was delayed recrudescence. In 12(13%) cases, resistance was of RII level where there was only a marked reduction in parasitaemia.

Table 1. Distribution of positve cases by type of parasite

Parasite	Positive cases	
	No.	%
P. falciparum	92	91.1
P. vivax	9	8.9
Total	101	100.0

DISCUSSION

The study was carried out in an area endemic for malaria with a continuous increase in falciparum cases as evident from Table 2 and another report in which the AfI had increased for Uttar Pradesh from 0.02 in 1961 to 0.56 in 1989 according to MRC[5]. The proportion of falciparum cases which used to be nearly 10% in 1986 has now increased to 52% and crossed the proportion of vivax cases (Table 3). The situation was the same in the 1930s when there was no control programme in the country, as reported by Bazaz-Malik and Tatke[6]. With the introduction of the control and eradication programmes after independence the

Table 2. Distribution of *Pf* cases by level of sensitivity to chloroquine

Sensitivity and level of resistance	Falciparum cases	
	No.	%
S	61	66.3
RI	19	20.7
RII	12	13.0
Total	92	100.0

Table 3. Malaria cases in PHC, Jasra (1986-1990)

Year	Total blood slides examined	Total positive	*P. vivax*		*P. falciparum*	
			No.	%	No.	%
1986	7765	287	269	93.7	18	6.3
1987	7204	206	191	92.7	15	7.3
1988	8699	1723	1386	80.4	337	19.6
1989	10083	2351	1435	61.0	916	39.0
1990	12431	2772	1335	48.2	1435	51.8

proportion of falciparum cases markedly decreased, but the spurt in the cases in Allahabad distt. in recent years may be attributed to chloroquine resistance. Out of the 101 fever cases detected in our study, 91% were of falciparum malaria and amongst them, 33% were resistant to chloroquine (Table 2). A study by MRC[5] in Shankargarh just adjacent to our study area also reported 33% of *Pf* resistant cases. Mirzapur in 1981 reported a figure of 90% while for Sidhi in Madhya Pradesh 100% resistance was reported in 1980[2]. Observations from places around U.P. as from Bihar, Jabalpur[7] and Shahjahanpur varied between 40 and 55%[5]. The level of resistance as observed in our study was RI in 21% and RII in 13%. The number of RIII cases was noted. Nearly all the studies[7,8] stated so far have reported RI resistance between 30 and 40% but 90% RI resistance had been prevalent in Mirzapur in 1981[2]. RII and RIII are comparatively less except from Bihar and Mirzapur where RII level was observed in 29% and from Shahjahanpur (U.P.) where RIII level of resistance was noted in 28%, as reported by MRC[5]. Mutation, malnourishment and inadequate dosages of chloroquine against a compromised immune status of the host, where the parasite has a longer survival period, may have contributed to drug resistance as stated by Bazaz-Malik and Tatke[6].

Although the phenomenon of resistance is not very serious as yet in the study area, effective monitoring and surveillance are called for to prevent further spread of the resistance phenomenon to other areas. With the *Pf* cases on the increase throughout the country and pockets of resistant cases reported from a large number of states, timely intervention in the form of alternative drugs and stringent surveillance measures for detection and treatment of cases can impede the progress of the disease.

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Malaria Related to Constructions in Panaji, Goa

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A marked difference in malaria incidence amongst labour imported for construction and local residents was observed in a study following the outbreak of malaria in Panaji (Goa) in 1986. Water stagnation in and around the construction was the main breeding site for *An. stephensi* and the intensity of malaria transmission was dependent on the area under construction.

INTRODUCTION

In India, in the post-resurgence phase, malaria has been recognised as one of the major health problems in urban areas and its origin has been often linked with the migration of population, especially labourers from rural areas[1, 2].

Panaji, the capital city of Goa, experienced a severe outbreak of malaria in 1986. Earlier, in a population of 50,000 less than 10 cases of malaria had been recorded per year. The focus of malaria, which was initially confined to a labour camp near a major construction site in the Campal area, gradually spread to the entire city in 1987[3]. At that time, blood smear test for malaria and treatment facilities were available only at the Urban Health Centre, and larviciding for mosquito control was done by Panaji urban unit of the National Filaria Control Programme. In the years that followed, malaria cases increased in epidemic form from 352 in 1986 to 5677 in 1988.

Construction activity in Panaji has increased enormously in the last decade. Labour come from many malaria endemic areas. A large number of malaria cases were found in the immigrant labour engaged in the construction of multistorey complexes and two new bridges over river Mandovi. A study was therefore taken up on malaria incidence in workforce engaged in construction in Panaji and its epidemiological implications. Results of the study are reported in this paper.

MATERIAL AND METHODS

Panaji, with an area of 7.5 sq km and a population of 54,122, is located at 15° 31′N latitude and 73° 52′E longitude on the western coast of India. River Mandovi runs all along in the north and, major portion of the land is water-logged with backwaters of this river in the south. Main area of

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the city surrounds a hillock known as Altinho and the city extends about 4 km on either side to Ribandar in the east and Donapaula area in the west. The weather is mild in most part of the year as maximum and minimum temperatures fluctuate from 28 to 34°C and 18 to 25°C, respectively. The normal rainfall is 2500 mm spread usually from April–May to October–November. In 1990, Panaji received 2623 mm rainfall in 141 days from May to November. A maximum of 759.6 mm rainfall was reported in June followed by 601.1 mm in August. It rained almost daily from June to August. Average relative humidity fluctuated between 75% and 95%.

The wardwise updated figures of population were taken from the Health Intelligence Bureau of the Directorate of Health Services, Goa. An independent census was conducted to ascertain the number of constructions and labourers in each ward, their origin and home-visiting frequency. Blood smears from fever cases in the labour hutments and residential colonies were collected during fortnightly active surveillance and daily passive collection done in the Malaria Clinics at the Malaria Research Centre and the Urban Health Centre, Panaji. The slides were stained with JSB stains for microscopical exami-nation. *Plasmodium vivax* cases were treated with chlo-

Table 1. Mosquito breeding in construction sites (1990)

Month	Nos. checked	Nos. breeding (%)	Nos. *An. stephensi* breeding (%)
Jan	244*	15 (6.1)	5 (2.0)
	86†	7 (8.1)	2 (2.3)
Feb	171*	11 (6.4)	4 (2.3)
	52†	9 (17.3)	2 (3.8)
Mar	420*	28 (6.6)	6 (1.4)
	76†	8 (10.5)	2 (2.6)
Apr	314*	24 (7.6)	10 (3.2)
	194†	14 (7.2)	4 (2.1)
May	475*	45 (9.5)	10 (2.1)
	242†	27 (11.2)	20 (8.3)
Jun	688*	111 (16.1)	42 (6.1)
	356†	57 (16.0)	32 (9.0)
Jul	386*	21 (5.4)	7 (1.8)
	591†	38 (6.4)	22 (3.7)
Aug	356*	17 (4.8)	3 (0.8)
	643†	19 (2.2)	4 (0.6)
Sep	292*	34 (11.6)	7 (2.4)
	675†	24 (3.5)	6 (0.9)
Oct	338*	24 (7.1)	5 (1.5)
	596†	37 (6.2)	8 (1.3)
Nov	197*	9 (4.6)	4 (2.0)
	232†	12 (5.2)	3 (1.3)
Dec	809*	30 (3.7)	9 (1.11)
	684†	39 (5.7)	11 (1.6)
Total	4609*	369 (8.0)	112 (2.43)
	4427†	291 (6.57)	116 (2.62)

* Masonry tank; † Curing and rain water stagnations.

roquine @ 10 mg/kg body weight on the day of slide collection and primaquine @ 0.25 mg/kg body weight daily on subsequent 5 days. *Plasmodium falciparum* cases on the other hand, were given chloroquine in a single dose of 10 mg/kg body weight on Days 1 and 2 and 5 mg/kg body weight on the third day. They were also administered a single dose of primaquine @ 0.75 mg/kg body weight on Day 2. Primaquine was not given to infants and pregnant women[4]. Throughout 1990, various construction sites were visited weekly for the detection of mosquito breeding. Bowls (300 ml) were used to draw water samples from masonry tanks and curing water stagnations. From the shallow water, mosquito immatures were picked up with the help of pasteur pipettes. The immatures were brought to the laboratory in plastic containers with screw caps and reared at room temperature by providing dog biscuit powder and yeast extract in a ratio of 3 : 1. The adults emerging from the field-collected samples were identified under a stereomicroscope using the key of Christophers[5].

RESULTS AND DISCUSSION

The survey in labour camps in Panaji revealed that 2829 persons including actual construction workers and their family members had migrated from 13 states and resided in hutments close to construction sites. Labour from Karnataka constituted 72.5%, most of whom belong to Bijapur, Hubli, Dharwad and Belgaum districts. Labourers from other places comprised 0.04 to 5.4% of the total labour force, and only 10 skilled workers belonged to Goa who returned to their native places daily. Investigations revealed that 566 (20%) labour visited their home state once in every six months, 55.9% between 6 months and one year, 10.8% once in two years, 11.1% after two years and 60 labourers were uncertain about their return.

In all, 4609 masonry tanks and 4427 curing and rain water stagnations were checked for mosquito breeding in different construction sites. Table 1 gives the results of mosquito breeding surveys at construction sites. Results revealed that 369 (8%) masonry tanks were supporting mosquito breeding but *An. stephensi* breeding was found in 112 (2.43%) tanks. Vector breeding varied considerably throughout the year as 0.8 to 6.1% masonry tanks and 0.6 to 9.0% curing water and rainwater stagnations respectively were harbouring immatures of *An. stephensi* in different months. *An. stephensi* habitat positivity increased from 2.1% in April to 8.3% in May in the curing and rain water collections whereas in the masonry tanks the increase in breeding was noticed in June only, i.e., from 2.1% in May to 6.1% in June. The peak in *An. stephensi* breeding started building up from March with maximum breeding in June and decline in July and August (Fig. 1).

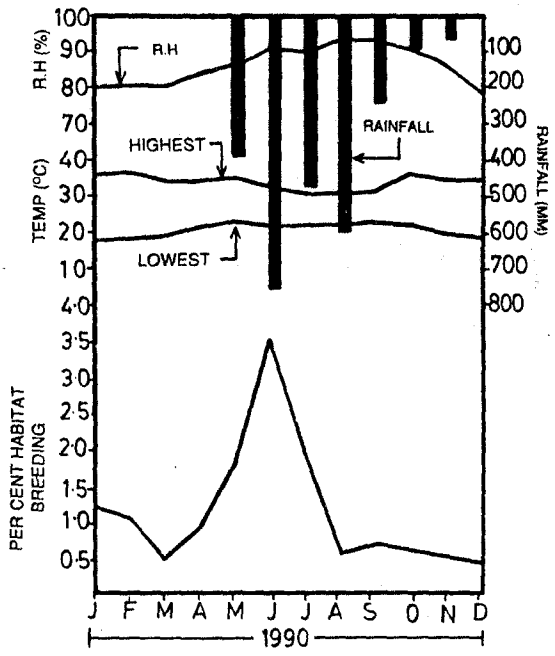

Fig. 1: Habitats found positive for *An. stephensi* breeding in Panaji.

Table 2 gives monthwise comparison of parasitological data of malaria in immigrant labourers

Table 2. Results of active and passive surveillance in labour camps and local residents during 1990

Month	BSE		BER	Pos.	Pv	Pf	Pv + Pf	SPR	SfR	Pf%	PI
Jan	A	469	16.5	56	42	14	0	11.9	3.0	25.0	19.7
	B	609	1.1	80	68	12	0	13.1	2.0	15.0	1.5
Feb	A	259	9.1	33	27	6	0	12.7	2.3	18.2	11.6
	B	405	0.7	58	45	13	0	14.3	3.2	22.4	1.0
Mar	A	257	9.0	43	37	6	0	16.7	2.3	13.9	15.2
	B	338	0.6	52	48	4	0	15.4	1.2	7.7	0.9
Apr	A	250	8.8	42	35	7	0	16.8	2.8	16.6	14.8
	B	348	0.6	60	51	9	0	17.2	2.6	15.0	1.1
May	A	410	14.5	207	178	28	1	50.5	7.0	14.0	73.2
	B	549	1.0	141	128	11	2	25.7	2.4	9.2	2.6
Jun	A	954	33.7	238	207	31	0	24.9	3.2	13.0	84.1
	B	1657	3.0	347	321	25	1	20.9	1.5	7.5	6.4
Jul	A	887	31.3	274	223	50	1	30.9	5.7	18.6	96.8
	B	2430	4.5	472	409	60	3	19.4	2.6	13.3	8.7
Aug	A	676	23.9	211	147	63	1	31.2	9.5	30.3	74.6
	B	1489	2.7	308	214	85	9	20.7	6.3	30.5	5.7
Sep	A	602	21.3	153	103	50	0	25.4	8.3	32.7	54.0
	B	846	1.5	174	108	65	1	20.5	7.8	37.9	3.2
Oct	A	450	15.9	116	73	41	2	25.8	9.5	37.0	41.0
	B	606	1.1	138	96	41	1	22.8	6.9	30.4	2.5
Nov	A	362	12.8	70	54	16	0	19.3	4.4	22.8	24.7
	B	588	1.1	133	117	15	1	22.6	2.7	12.0	2.5
Dec	A	235	8.3	51	42	9	0	21.7	3.8	17.6	18.0
	B	494	0.9	92	75	17	0	18.6	3.4	18.5	1.7
Total	A	5811	205.4	1494	1168	321	5	25.7	5.6	21.8	528.1
	B	10359	19.1	2055	1680	357	18	19.8	3.6	13.4	37.97

A - Labour population (2829); B - Local population (54122).

and Goans. The results show that fever rate in labour camps was higher, ranging from 8.3% in December to 33.7% in June as compared to 0.6% noticed in March and April to 4.5% observed in Goans during July. Slide positivity rates in two demographic groups were comparable up to April 1990 but in labour camps there was a sharp increase from 16.8 to 50.5% in May, whereas SPR in the local population increased from 17.2% in April to 25.7% in May, i.e., an increase of 8.5% as against 33.7% witnessed in labour camps. The increase in SPR coincided with the abrupt increase in *An. stephensi* per cent positivity in construction sites (Fig. 1). In subsequent months, SPR remained high in labour camps as compared to local population except in November. Similarly, slide falciparum rate (SfR) was higher in labour camps ranging from 2.3 to 9.5% as compared to 1.2 to 7.8% in local population. Annual parasite incidence (API) for 1990 was 528.1 in labour camps and 37.97 in the local residents, i.e. malaria incidence was high in the immigrant labour by a factor of 14 (Fig. 2).

To study the relationship between the number of constructions and malaria cases amongst these two social groups, the city of Panaji was divided into two zones A and B (Fig. 3). Breeding sites

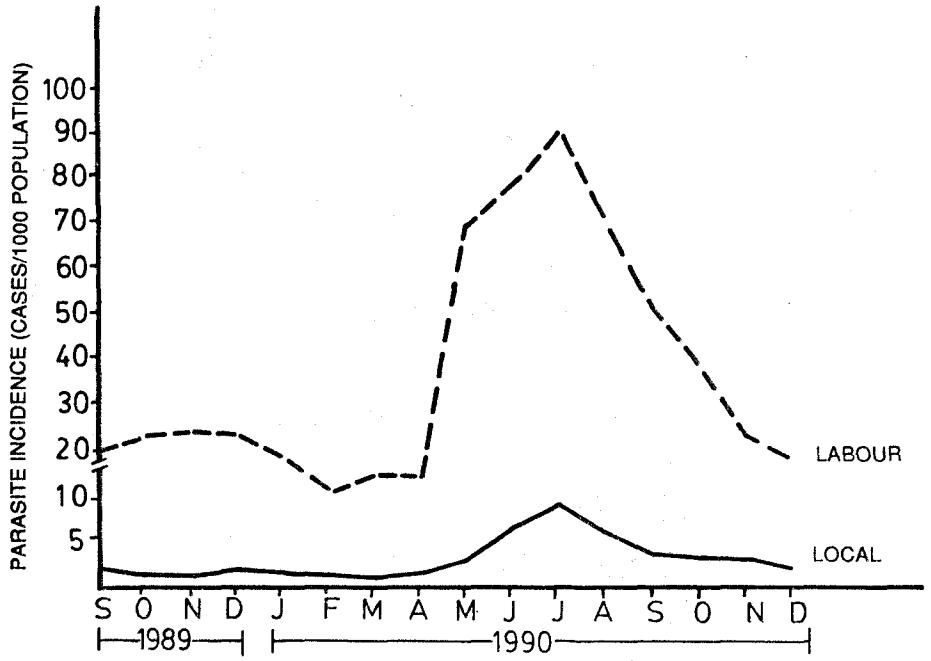

Fig. 2: Monthly parasite incidence of malaria in the labour camps and local residents.

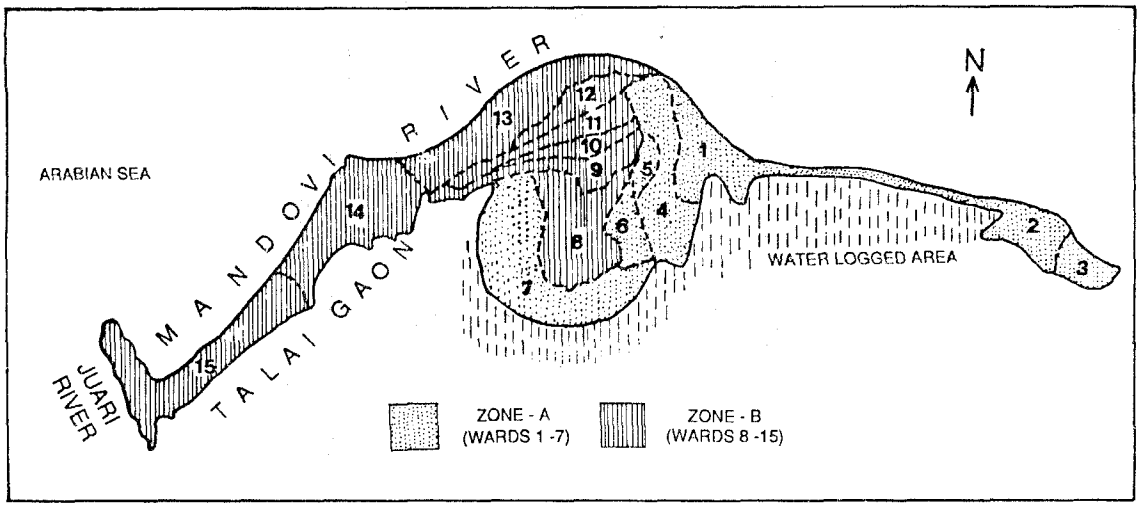

Fig. 3: Map of Panaji showing Municipal Wards 1-15.

preferred by *An. stephensi*, number of constructions and malaria cases amongst local population and labourers were separated for these zones. Zone A comprised wards 1 to 7 with 40% of the city area. Zone B, with wards 8 to 15, covered 60% of the city area and was under heavy construction activity. In zone A, there were 625 permanent breeding sites of *An. stephensi* includ-

Table 3. Malaria incidence in Panaji in labour camps and local residents during 1990

Municipal wards	Permanent *An. stephensi* breeding sites	Floor area under construction sq m	Epidemiological data									
			Labour camps					Local residents				
			Population	BS checked	Malaria cases	SPR	API	Population	BS checked	Malaria cases	SPR	API
Conservation Zone 1-7	625	22693	491	750	167	22.2	340.1	21231	2321	377	16.2	17.7
Accelerated construction Zone 8-15	3979	114025	2338	5061	1327	26.2	567.6	32891	8032	1678	20.9	51.0
Total	4604	136718	2829	5811	1494	25.7	528.1	54122	10353	2055	19.8	37.96

ing 288 overhead tanks, 46 masonry tanks, 53 groundwater tanks and 237 wells and one natural fountain spring.

In zone B there were 3979 breeding sites of *An. stephensi* including 2716 overhead tanks, 145 masonry tanks, 736 ground water tanks, 377 wells and 5 swimming pools. The central portion of these two zones was separated by Altinho hillock which represented ward 8. Zone A, in which 20 (15.9%) constructions were in progress, had 491 (17.3%) migrated labourers and registered 167 (11.2%) malaria cases amongst them (Table 3). On the other hand, zone B, in which 106 (84.1%) complexes were under construction, had a labour force of 2338 (82.6%) of which 1327 (88.8%) were found malaria positive. Also there existed a good degree of similarity in the proportion of malaria cases amongst migrant labourers and local residents (11.2% and 18.35%, respectively) in zone A as well as in zone B (88.8% and 81.65%, respectively). The annual parasite incidence of malaria in labourers was greater by 19.2 and 11.1 folds as compared to that in local residents in wards 1 to 7 and 8 to 15, respectively. It was also observed that API was 2.88 times greater in the local population residing in wards 8 to 15 as compared to that in wards 1 to 7. This could be due to the presence of more permanent breeding sites and lesser number of people (310.2) exposed to risk per construction in wards 8 to 15 as compared to lesser number of breeding sites and more population (1061.5) exposed per construction in wards 1 to 7.

The study showed that the tropical aggregation of labour was a major risk group, and 126 buildings under construction were the main problem areas. To prevent the spread of malaria, it would be essential to quarantine the immigrant labourers in 3-4 camps with proper sanitation facilities. This will also facilitate screening and treatment of malaria. Weekly foolproof vector control measures should be instituted in all construction sites to control *An. stephensi* breeding and reduce the risk of malaria transmission. If these measures are not implemented, the immigrant labour will continue to spread malaria which might adversely affect tourism in Goa.

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Bioenvironmental Control of Malaria at the Indian Drugs and Pharmaceuticals Ltd., Rishikesh (U.P.)

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Bioenvironmental control of malaria was achieved at the Indian Drugs and Pharmaceuticals Ltd. (IDPL) complex, Rishikesh, Dehra Dun distt., Uttar Pradesh, India. The IDPL complex is in 15 sq km area with about 25,000 population. One major mosquito-breeding site of about one sq km was eliminated by diverting the factory effluents into a drain. Filling borrow pits, ditches and low-lying areas with burnt coke ash, cleaning blocked drains, mosquito-proofing the overhead water tanks, application of larvivorous fishes and improved case detection and treatment were the additional malaria-control measures. Impact assessment of the interventions revealed that there was 25% reduction in malaria incidence as well as 90% reduction in the use of antimalarials, and a considerable reduction in nuisance mosquito species and malaria vector densities.

INTRODUCTION

Malaria control using residual insecticides has resulted in the development of insecticide resistance in vector populations, thereby making malaria control problematical and unattainable. An alternative technique is to control malaria by the application of bioenvironmental methods[1]. Bioenvironmental malaria control was launched in July 1986 at an industrial site, Bharat Heavy Electricals Ltd. (BHEL), Ranipur, Hardwar. Biological control with larvivorous fish, *Gambusia affinis* and *Poecilia reticulata,* and environmental modification and manipulation methods combined with active weekly surveillance and prompt radical treatment successfully controlled indigenous malaria transmission at the BHEL campus in two years. This was the first successful demonstration of industrial malaria control in India without the use of any chemicals[2]. Based on the success achieved at the BHEL, it was decided to extend this strategy to the Indian Drugs and Pharmaceuticals Ltd. (IDPL), Virbhadra, Rishikesh, Dehra Dun district. This is an important public sector pharmaceutical industry established in early 1950s. The incidence of malaria in this industrial complex was high with very high populations of nuisance mosquitoes. The results of this study are reported in this paper.

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MATERIAL AND METHODS

Study area

The IDPL complex, situated about 25 km north of Hardwar (U.P.), covers an area of 15 sq km. Average annual rainfall is 1400 mm and the temperature ranges from 2 to 42°C. Topography of the area constitutes mainly foothills of the Shivalik mountain range along the Ganga. The township has 2,500 residential quarters, three unauthorized labour colonies and a population of about 25,000. A 25-bed hospital provides medical facilities to the IDPL staff, but there was no blood smear examination of malaria parasites and all suspected malaria cases were treated on clinical basis. Both anopheline and culicine populations were high owing to innumerable natural and man-made larval habitats, such as slow-running streams, subsoil water seepages, poor factory effluent drainage, uncovered overhead and underground water tanks, artificial water containers from factory scrap disposal, tin cans, pots, tyre dumps, desert coolers, frequently blocked surface drains, borrow pits, and stabilization tanks of biological treatment plant (BTP). There was no well-organized antimalaria programme, except for occasional malathion fogging and spraying of hutments with DDT or HCH.

The bioenvironmental malaria control project was initiated in July 1987. Potential larval habitats were mapped and an action programme was developed. For comparison, a control area of 6,000 population was selected, 6 km away from the IDPL complex. Larval habitats at the control site were similar to those at IDPL, such as streams, subsoil water seepages, borrow pits, irrigation channels and cement tanks. Malaria intervention measures were not applied in the control area.

Source reduction and biological control

The specific components of intervention strategy adopted at IDPL complex are given in Table 1. Burnt hard coke ash, an industrial waste from a nearby industry, was used for filling a large number of the borrow pits, stagnant water pools and other low-lying areas. Three hundred twenty-three tractor trollies (capacity 7 m^3) of industrial waste or soil were used for this purpose. A front dozer-mounted tractor was used for about 60 hrs to level the filled-up areas. Work relating to the improvement in drainage, mosquito-proof-

Table 1. Intervention strategy adopted at the IDPL complex

S. No.	Problems	Methodology
1.	Large ditches/borrow pits/low-lying areas	Filling with industrial waste (Burnt hard coke ash)
2.	Water stagnation due to community taps	Construction of cemented stand posts and proper disposal of wastewater
3.	Domestic wastewater	Construction of soakage pits
4.	Water stagnation in uncemented drains	Construction of cemented drains with proper gradient
5.	Uncovered Overhead Tanks (OHT)	Mosquito-proofing of OHTs with proper covers
6.	Small underground tanks/sluice valve chambers and choked man-holes	Application of EPS beads
7.	Open blocked small drains	Desilting and canalization
8.	Storm-water drains and bigger underground tanks	Introduction of larvivorous fishes: guppy and *Gambusia*
9.	Intradomestic breeding containers	Emptying at weekly intervals
10.	Fever cases in the campus	Early case detection and administration of radical treatment within 24 hrs.

ing of overhead tanks and covering of open manholes was done with the assistance of the IDPL civil maintenance department. Larvivorous fish, *Gambusia affinis*, were released in underground tanks and storm-water drains for the control of mosquito breeding.

Factory wastewater used to stagnate in a sq km area. This area was a major source of mosquito production. The factory effluents were evaluated for toxic materials and found suitable for irrigation. Hence, a major source reduction was accomplished by diverting factory recycled wastewater for irrigation to nearby farms. To clear the water logging, a drain was constructed and factory wastewater was diverted through the drain. Thus the pond dried up and the whole area was protected from water-logging and consequently from mosquito breeding on a permanent basis.

Health camps and group meetings were organized to educate people about mosquitoes, malaria and personal protection methods. Their cooperation and active involvement was solicited in source reduction work. Smokeless chulhas (wood stoves) were provided in labour colonies to create a healthy and pollution-free environment.

Entomological and parasitological surveys

Entomological and parasitological monitoring was done for the entire duration of the study. Adult mosquito densities were monitored by the suction tube method in three experimental areas and one control area at fortnightly intervals. For this purpose, indoor resting collections were made in the morning between 0600 and 0800 hrs from three human dwellings and three cattlesheds in each locality. Mosquitoes were identified, and man-hour densities of total mosquitoes, total anophelines and vector species determined. Weekly peridomestic and intradomestic larval surveys were conducted and positive larval habitats subjected to intervention methods. Weekly active surveillance was carried out by the project staff in labour colonies to collect blood smears from fever cases. Passive surveillance at the IDPL hospital was maintained by the hospital staff. All slides were examined at the project malaria clinic on the same day and radical treatment was provided to all malaria cases within 24 hrs. Adult vivax malaria patients were treated with 900 mg chloroquine in divided dosages (D1-600 mg; D2-300 mg) and 15 mg primaquine daily for 5 days. Falciparum malaria patients were treated with 1500 mg chloroquine base (D1-600 mg; D2-600 mg; D3-300 mg) and a single dose of 45 mg primaquine. Children were given proportionately lower dosages of chloroquine and primaquine. Primaquine was not given to pregnant women and infants. All *P. falciparum* cases were subjected to *in vivo* WHO 28 days' extended test. A record of all control activities was maintained and data were tabulated on a monthly basis.

RESULTS AND DISCUSSION

A total of 10,797 mosquitoes comprising eleven species of the genera *Anopheles* and *Culex* were collected from the experimental and control areas from January 1988 to December 1989. The percentage prevalence of each species in experimental and control areas is given in Table 2. Of the total adult mosquitoes collected in the experimental area, 72.60% were anophelines and 27.40% *Culex quinquefasciatus* Say. Among the anopheline species, *An. subpictus* Grassi was the most abundant (46.30%) followed by three malaria vectors in the order of prevalence, i.e., *An. fluviatilis* James (11.40%), *An. culicifacies* Giles (9.27%) and *An. stephensi* Liston (1.92%), while in the control area, 81% were anophelines and 19% *Culex quinquefasciatus*. The most prevalent anopheline species was *An. culicifacies* (27.40%) followed by *An. subpictus* (21.90%), *An. fluviatilis* (21.65%) and *An. stephensi* (3.32%). *An. culicifacies* immatures were most frequently collected in subsoil seepage water pools, hoof prints and irrigation channels, while *An. fluviatilis* immatures were collected exclusively in slow running streams.

Table 2. Results of adult mosquito collections made at 15-day intervals for man-hour density estimates at IDPL complex during 1988 and 1989

S. No.	Species	Experimental		Control	
		No. collected	%	No. collected	%
1.	*An. culicifacies*	717	9.27	840	27.40
2.	*An. fluviatilis*	881	11.40	664	21.65
3.	*An. stephensi*	149	1.92	102	3.32
4.	*An. subpictus*	3580	46.30	672	21.90
5.	*An. splendidus*	160	2.10	91	2.96
6.	*An. maculatus*	58	0.75	84	2.73
7.	*An. annularis*	53	0.68	32	1.04
8.	*An. vagus*	10	0.13	0	0.0
9.	*An. pulcherrimus*	3	0.04	0	0.0
10.	*An. aconitus*	2	0.02	0	0.0
	Total anophelines	5613	72.60	2485	81.00
11.	*Culex quinquefasciatus*	2117	27.40	582	19.00
	Total mosquitoes	7730	100.00	3067	100.00

Note: Time spent in the collection of mosquitoes from experimental and control areas was 144 and 48 hrs, respectively.

Overhead tanks and underground tanks were the primary sources of *An. stephensi* immatures.

Source reduction and developmental work done at the IDPL complex since July 1987 to July 1989 is given in Table 3. Intervention activities were stopped in August 1989 due to low incidence of malaria. From July 1987 to July 1989, a large number of ditches were filled and low-lying areas levelled. Blocked drains were cleaned and canalized weekly. Soakage pits were constructed in labour colonies for disposal of the domestic wastewater. Hoof prints alongside slow running streams were filled with soil. Intradomestic larval habitat surveys of 85,838 artificial containers (pooled data of weekly surveys) revealed 4.6% positivity.

Immatures in the positive artificial containers were eliminated manually. Forty-three overhead tanks (0.33%) out of 12,671 surveyed were positive for anopheline larvae. All the overhead tanks were made mosquito-proof with proper covers. Ten health camps and 24 group meetings were organized. Smokeless chulhas (wood stoves) were

Table 3. Source reduction and development work

S. No.	Type of work	No.
1.	Positive breeding places eliminated	16461
2.	Earth work	
	(a) No. of pits filled	1060
	(b) No. of trucks/tractors of burnt hard coke ash/soil dumped	323
	(c) No. of hours for which dozer/tractor was used in leveling	59
3.	No. of drains cleaned	1240
4.	Canalization (no. of sites)	1942
5.	No. of soakage pits constructed	16
6.	No. of stand posts constructed	4
7.	Health camps	10
8.	Group meetings	24
9.	Improved chulhas installed	205

encouraged and during this period 205 chulhas were installed in labour hutments with the help of other agencies as part of the environmental improvement programme.

Mosquito densities observed during 1988 and 1989 are given in Table 4. Mosquito densities, particularly anophelines and vectors, were lower in the experimental areas than in the control area for most part of the year except for some monsoon months when water-logging increased and mosquito-breeding was difficult to control in natural streams. The impact of intervention was more pronounced on the vectors, viz., *An. culicifacies*, *An. fluviatilis* and *An. stephensi*, and their densities were significantly reduced as compared to those of the control area. Anopheline populations, primarily *An. subpictus*, increased during monsoon period due to continuous rains and difficulty in reducing larval habitats. The high density of *Culex quinquefasciatus* in the IDPL township was mainly due to profuse breeding in 24 stabilization tanks at the biological treatment plant where no engineering or biological method worked effectively. Use of expanded polystyrene (EPS) beads[3] was not applicable in these stabilization tanks because of their large size and these were not wind-protected. The increase in mosquito density after July 1989 in the experimental areas was attributed to withdrawal of intervention activities.

Within the experimental area, comparison of vector density in the township and labour colonies (Fig. 1) shows that malaria vector densities in labour colonies were higher than in the township because labour colonies were situated near perennial water sources, such as slow running streams, subsoil water seepages, etc., which supported anopheline breeding throughout the year. The township area was virtually free from permanent sources of anopheline larval habitats. Low vector densities at the township at times increased owing to infiltration. The peak populations of *An. culicifacies* was recorded during monsoon whereas peak populations of *An. fluviatilis* occurred during post-monsoon months. Although *An. fluviatilis* is regarded as an efficient malaria vector even at low densities, studies on the blood meal analysis of this mosquito revealed a human blood index (HBI) of 0.005, thereby suggesting that it is primarily a zoophilic species in this area.

Table 5 shows the malaria incidence at the IDPL complex recorded at the malaria clinic and through active surveillance since the beginning of the project, i.e., July, 1987. The slide positivity rates (SPR) at IDPL in 1987 (July to December), 1988 and 1989 were 4.9, 4.1 and 3.5, respectively (Table 5). There was 25.5% reduction in malaria incidence in 1989 as compared to 1988. Out of the total malaria cases at the IDPL complex, about 60% cases were from labour colonies and the rest from township areas. Follow-up of all malaria cases revea'ed that 10% cases were from outside the study area. *In vivo* study of all the ten *P. falciparum* cases showed full sensitivity to chloroquine.

A malaria prevalence study was conducted in 1988 and 1989 to assess malaria prevalence in the surrounding areas where malaria control was based on insecticide spraying. The slide positivity rates in the surrounding area in 1988 and 1989 were 15.3 and 15.2, respectively, which indicated a clear difference in malaria transmission in the two sites. Although the reduction in malaria incidence was only 25.5%, there was a drastic reduction in the distribution of antimalarial drugs. The total numbers of antimalarials distributed by the IDPL hospital before and after the launching of the bioenvironmental control of malaria project are given in Table 6. The consumption of antimalarials during 1989-90 was reduced from 90 to 100% as compared to 1985-86. Besides, antimalarials were administered on the basis of clinical examination and although in most cases there was no need of antimalarials, the attending physicians felt that patients responded to such treatment although parasitologically they may be negative for malaria parasite. Continuing medical education to medical personnel is necessary since

Table 4. Results of monitoring of mosquito densities (per man-hour) at the IDPL, Rishikesh

Year/species		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1988													
An. culicifacies	E	1.00	1.66	0.83	1.30	0.50	3.83	8.66	14.50	7.66	4.83	0.83	1.16
	C	6.00	2.00	2.00	2.50	2.00	16.50	44.00	34.50	32.00	8.50	8.50	1.00
An. fluviatilis	E	0.83	1.50	4.33	2.66	0.16	2.00	0.0	1.00	8.16	10.00	5.00	5.66
	C	44.00	23.50	83.00	12.50	2.00	9.50	0.0	1.00	5.50	4.00	10.00	14.50
An. stephensi	E	0.50	0.83	0.16	0.12	1.00	1.16	1.33	0.33	0.33	3.00	1.50	2.00
	C	0.0	0.0	0.50	0.0	0.0	1.50	1.00	1.50	1.50	6.00	10.50	3.50
Total vectors*	E	2.23	4.00	5.32	4.08	1.66	7.00	10.00	15.83	16.16	17.83	7.33	8.83
	C	50.00	25.50	85.50	15.00	4.00	27.50	45.00	37.00	39.00	18.50	29.00	19.00
Total anophelines	E	3.50	8.83	7.83	5.00	3.66	17.33	80.83	125.66	65.83	38.66	42.16	34.66
	C	50.00	36.00	90.00	17.00	12.00	136.00	163.50	146.00	42.50	20.50	37.00	26.00
Total mosquitoes	E	6.66	16.66	34.50	63.90	40.16	44.16	101.66	129.83	73.83	42.16	42.33	37.50
	C	50.00	37.50	101.50	106.00	43.50	153.50	169.59	148.00	47.00	22.00	38.00	32.50
1989													
An. culicifacies	E	0.00	0.00	0.33	0.16	0.50	5.33	10.66	10.66†	20.66	18.16	5.16	1.66
	C	0.00	0.00	0.50	1.00	2.50	41.50	113.50	21.75	27.00	31.00	20.00	4.50
An. fluviatilis	E	2.33	3.83	9.00	2.33	0.16	0.0	0.33	2.33	13.50	28.66	26.16	19.66
	C	9.50	12.50	26.50	13.50	4.50	3.00	0.0	0.50	19.50	11.50	20.50	23.00
An. stephensi	E	0.33	0.33	1.33	1.66	0.83	2.00	1.83	0.16	0.83	2.33	1.00	0.50
	C	1.50	0.50	6.50	4.00	1.00	2.50	7.50	0.0	0.0	0.50	1.50	0.50
Total vectors*	E	2.66	4.16	10.66	4.16	1.50	7.33	12.83	13.16	35.00	49.15	32.33	21.83
	C	11.00	13.00	33.50	18.50	8.00	47.00	121.00	22.25	46.50	43.00	42.00	28.00
Total anophelines	E	3.33	6.16	15.50	7.33	2.33	9.83	46.16	111.00	91.50	101.33	80.50	31.33
	C	15.50	29.50	38.00	22.00	11.00	54.50	139.50	58.25	58.00	51.50	55.50	49.00
Total mosquitoes	E	7.16	9.50	34.66	41.00	41.00	42.33	60.66	113.66	94.16	102.16	83.66	33.16
	C	19.50	34.00	55.50	40.00	41.00	84.50	141.00	58.25	60.00	52.00	56.00	49.00

E—Experimental area; C—Control area; **An. culicifacies + An. fluviatilis + An. stephensi*; †Intervention work stopped in experimental area.

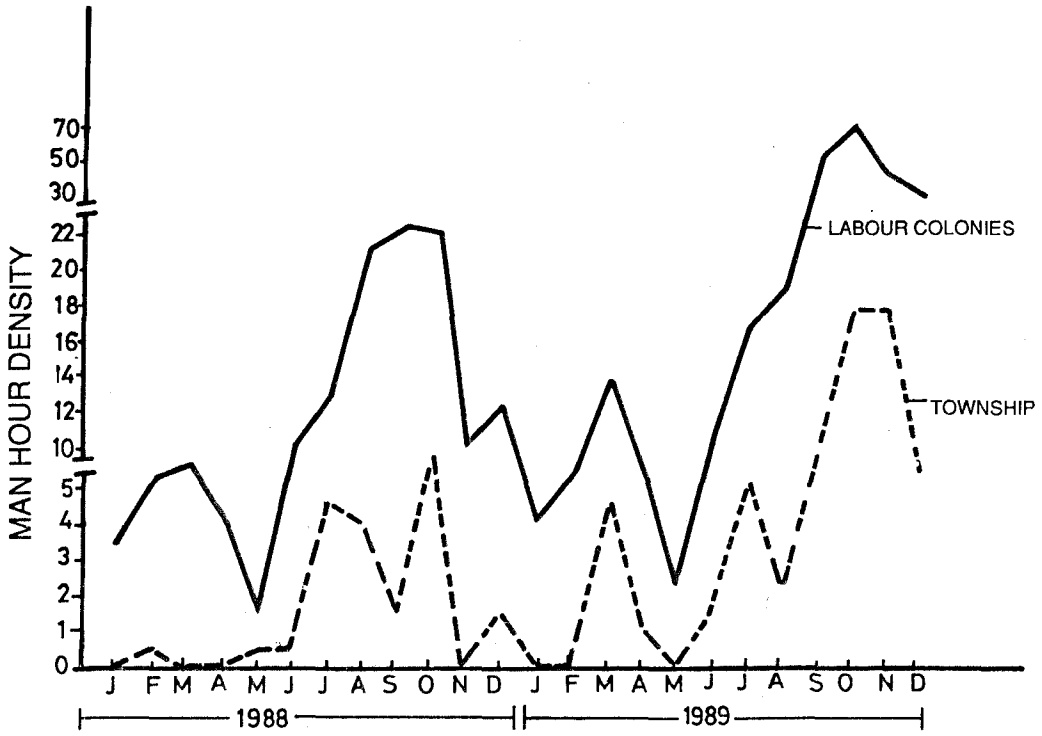

Fig. 1: Vector density in township and labour colonies at IDPL complex.

malaria is often missed or misdiagnosed and invariably treatment is inadequate or incomplete. The reduction in consumption of antimalarials was a clear index of the improvement in malaria situation, particularly in the diagnosis and correct treatment of fever cases at the IDPL complex.

The annual budget of IDPL hospital is Rs. 5.5 million, of which about 0.8 million (15%) was spent on malaria. The expenditure on bioenvironmental control was only Rs. 0.15 million, suggesting a net saving of 80% on malaria budget. Bioenvironmental control strategy has advantages over the insecticidal application as it is simple, cost-effective, socially acceptable, reduces pollution and improves the environment. The strategy has world-wide application and dates back to the early part of the century when the Panama Canal was constructed. Landfilling, drainage operations, house screening combined with chemotherapy, prophylaxis and judicious use of insecticides practically eliminated malaria from the canal zone[4]. The water control projects of Tennessee Valley Authority in USA is another successful example of a systematic application of environmental management measures for prevention and control of malaria[5]. Simple environmental management methods had been successfully applied in the control of malaria in Malaysia[6] and in recent years in India in Kheda[7,8], Mandla[9] and an industrial area in Hardwar[2].

The study demonstrated successful malaria control by bioenvironmental methods which were unattainable by insecticidal methods as was being practised at the IDPL. The study also revealed that malaria control should be based on a study of local problems and the application of appropriate and specific methods to tackle each situation.

Table 5. Parasitological data (1987-1989)

Year		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
	BSC	—	—	—	—	—	—	546	572	650	373	363	210	2714
1987	Total (+)ve	—	—	—	—	—	—	39	48	21	14	4	7	133 (4.9)
	Pf	—	—	—	—	—	—	0	0	0	2	2	0	4
	BSC	207	155	116	179	186	210	339	709	450	369	162	140	3222
1988	Total (+)ve	3	1	0	1	10	12	22	19	40	18	3	4	133 (4.1)
	Pf	0	0	0	0	0	0	0	0	1	2	1	0	4
	BSC	173	145	179	275	187	262	282	562	366	205	121	65	2822
1989	Total (+)ve	4	0	1	6	7	10	12	16	22	12	6	3	99 (3.5)
	Pf	0	0	0	0	0	0	0	0	0	2	0	0	2

Note: There was no parasitological examination of fever cases till the time of launching of the bioenvironmental control strategy in July 1987.
Figures in parentheses indicate slide positivity rate; BSC — Blood smear collected; Pf — *Plasmodium falciparum* cases.

Table 6. IDPL Hospital record of antimalarial drugs used for the treatment of clinically diagnosed malaria cases

Antimalarial drugs	1985-86	1986-87	1987-88	1988-89	1989-90	% reduction in 1989-90 against 1985-86
Chloroquine (150 mg tablet)	42300	44000	7100	1100	1300	97
Primaquine (7.5 mg tablet)	11700	6300	3000	1000	984	92
Metakelfin (500 mg sulphalene + 25 mg pyremethamine tablet)	6040	8000	4500	2100	307	95
Chloroquine (injection 30 ml)	100	88	40	0	0	100

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Dietary Modulation of Malaria Infection in Rats

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Feeding of wistar albino rats on low protein and energy diet (4% protein) caused suppression of parasitaemia when infected with *Plasmodium berghei* besides causing a depressed immune response. The refeeding of protein energy deficient rats on normal protein and energy diet (18% protein) for four weeks resulted in the normal course of parasitaemia after *P. berghei* infection. The present study was carried out to find the cause of suppression of malaria in protein energy deficient rats. The experiments revealed re-elevation of malaria parasitaemia when rats were fed on low protein diets supplemented with *p*-amino-benzoic acid (PABA). Moreover, the parasite persisted at subpatent levels in tissues in protein energy deficient rats and resulted in the development of antimalarial antibodies. Low protein energy diet could cause deficiency of certain essential nutrients required for the parasite, PABA being one of them, and hence suppresses the parasitaemia to subpatent levels. As a result, sufficient antigenic stimulus is provided to the host so that the host develops an immune response which might in turn help in further suppression of parasitaemia to subpatent levels.

INTRODUCTION

Infection and malnutrition coexist so perniciously that it is difficult to treat either of them in isolation. Malnutrition invariably lowers the resistance to infection[1, 2] and infectious diseases in turn exaggerate the sequelae of malnutrition[3]. Though most of the infections and malnutrition have synergistic effects, some conflicting observations have been reported. Some of the epidemiological records reveal increased prevalence rates and increased child death rates in malnourished individuals[4, 5], whereas others have shown decreased prevalence and lower mortality rates[6] due to malaria. Starvation[7], low iron diet[8, 9], low vitamin diet[1, 10], low milk diet[11, 12], and low protein diet[13-16] have been reported to suppress the malarial parasitaemia. Though the mechanism underlying the suppression of malaria infection with starvation[17], low iron diet and milk diet[18, 19] has been elucidated, it is not clear how low protein diet alters the course of parasitaemia. PABA has been shown to be an essential nutrient for the growth and metabolism of the parasite[20, 21]. Supplementation of PABA in diet could correct the irregular course of malaria parasite[22]. Targett[23] observed that low protein diet fed animals resisted the superinfection with *P. berghei*. Our own studies revealed a depressed cellular as well as humoral immune response in low protein and

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energy diet fed animals[14]. These studies made us suppose that suppression of parasitaemia in low protein diet fed animals could be due to both dietary and immunological factors. Hence, the present study was designed as follows.

Experiment No. 1 (PABA supplementation): This was designed to find whether low protein diet causes low availability or nonavailability of an essential nutrient (PABA) for the parasite.

Experiment No. 2 (Superinfection and immunity): This was carried out to find whether the parasite exists at subpatent levels in low protein diet fed rats and helps to build up the specific immunity.

MATERIAL AND METHODS

Animals – Wistar albino rats, 3-4 weeks old, were housed in separate cages and fed on special diet.

Diet – Diet containing 4 or 18 per cent protein was prepared and made isocaloric by supplementing the protein deficiency with starch and sugar as given by Bhatia and Vinayak[13].

Parasite – *P. berghei* (NICD) maintained by weekly passage in swiss albino mice was employed. Before infecting the experimental rats the parasite was once passaged through the wistar albino rats. The infection was given by intraperitoneal inoculation of 1×10^7 parasitized red blood cells (PRBC).

Groups of animals

Experiment No.1 (PABA supplementation): Animals for this experiment were divided into following four groups–

Group I P (n = 16): This group of rats was fed on 4 per cent protein diet throughout the experiment. The animals were infected with *P. berghei* on Day 28 of diet.

Group II P (n = 16): This group of rats was pair fed with group I P throughout the experiment. The animals were infected with *P. berghei* on Day 28 of diet.

Group III P (n = 16): The animals of this group were fed on 4 per cent protein diet throughout the experiment. Two days prior to inoculation of the parasite the diet was supplemented with additional PABA (5 mg/rat), which was continued till the end of the experiment. The animals were infected on Day 28 of feeding on specific diet.

Group IV P (n = 16): The rats in this group were pair fed with those on 4 per cent protein diet in group III P and were given 18 per cent protein diet throughout the experiment. The PABA supplementation and infection were given as in group III P.

Follow-up of animals of Groups I,II,III and IV P: The course of parasitaemia was observed in all the groups of rats for 20 days post-inoculation (P.I.) of the parasite. The PABA levels were checked in the blood by the method of Bratton and Marshall[24] at three stages, i.e., pre-inoculation (Day 0), during patent infection (Day 7 post-inoculation) and post-patent (Day 14 post-inoculation).

Experiment No. 2 (Superinfection and immunity): The rats were divided into following three groups–

Group I S (n = 55): The animals in this group were fed on 4 per cent protein diet and inoculated with the parasite on Day 28 of diet. Out of 55 animals,11 died during the course of experiment and were not included in the study. Thirteen animals which did not develop patent parasitaemia for 20 days were rechallenged on Day 21 P.I. Those animals which did not develop parasitaemia for 7 days after rechallenge were superinfected with the parasite on Day 28 P.I.

Group II S (n = 55): Rats in this group were pair fed with those in group I S and fed on 18 per cent protein diet. The pair fed animals with those which died in group I S were excluded from the study.

Group III S (n = 6): Rats of this group were fed on 18 per cent protein diet *ad libitum*. The animals were infected with *P. berghei* on Day 28 of diet.

Follow-up of animals of Experiment No. 2: The course of parasitaemia in blood was observed daily. The serum was checked at weekly intervals after the inoculation of the parasite for the appearance of precipitin lines against *P. berghei* antigen by applying countercurrent immunoelectrophoresis. The tissue smears were prepared on the day of sacrifice and stained with Giemsa. The tissue smears were prepared from brain, liver, kidney and spleen.

RESULTS

Experiment No. 1 (PABA supplementation): The PABA levels in blood were higher in animals fed on low protein energy or low energy diets supplemented with additional PABA as compared to those on non-PABA supplemented diet (Table 1). Moreover, the animals on PABA supplemented diet (4 or 18% protein) had a tendency to develop more parasitaemia than those on non-PABA supplemented diets but with the same protein content (Fig. 1). With the increase in parasitaemia, a fall in PABA levels was observed. The animals on PABA supplemented diet (Groups III P and IV P) had lowest PABA levels during patent infection as compared to pre-patent or post-patent levels (Table 1). On the other hand, the animals on non-PABA supplemented diet showed a continuous fall in PABA levels during the post-patent period also.

Experiment No. 2 (Superinfection and immunity): The parasitaemic levels of the 4 per cent protein fed animals were lowest and those of 18% protein *ad libitum* fed animals highest (Fig. 2). In the group of low protein diet fed animals, 24/44 (54%) had prolonged pre-patent period and 13/44 (30%) did not show any patent parasitaemia (Fig. 2). Five of the thirteen animals which did not develop patent parasitaemia showed positive precipitin lines against *P. berghei* antigen 7 days after rechallenge with the parasite.

The tissue impression smears revealed that 36, 34, 43 and 5% slides were positive for the parasite, respectively, in spleen, liver, kidney and brain. None of the animals from which these slides were prepared showed any parasite in blood.

DISCUSSION

Protein restriction in diet results in the deficiency of other nutrients by causing loss of appetite and malabsorption. In the present experiments also the outcome of malaria infection in low protein energy or energy deficient diet fed animals could be due to the diet itself or to the secondary effects produced by the diet resulting in the deficiency of certain essential nutrients like PABA, vitamins and iron which have been reported to affect the course of parasitaemia. A number of workers have attributed the suppression of para-

Table 1. *p*-Aminobenzoic (PABA) acid levels in blood with or without PABA supplementation in diet

Group	Pre-patent period PABA (µg/ml)	Patent period PABA (µg/ml)	Post-patent period PABA (µg/ml)
4% protein	2.6 ± 1.2	2.26 ± 0.4	0.58 ± 0.8
4% protein + PABA	4.4 ± 1.6	3.6 ± 2.1	4.5 ± 1.3
18% protein	3.6 ± 0.4	2.12 ± 0.4	0.56 ± 0.52
18% protein + PABA	4.7 ± 1.2	2.64 ± 1.6	6.3 ± 2.84

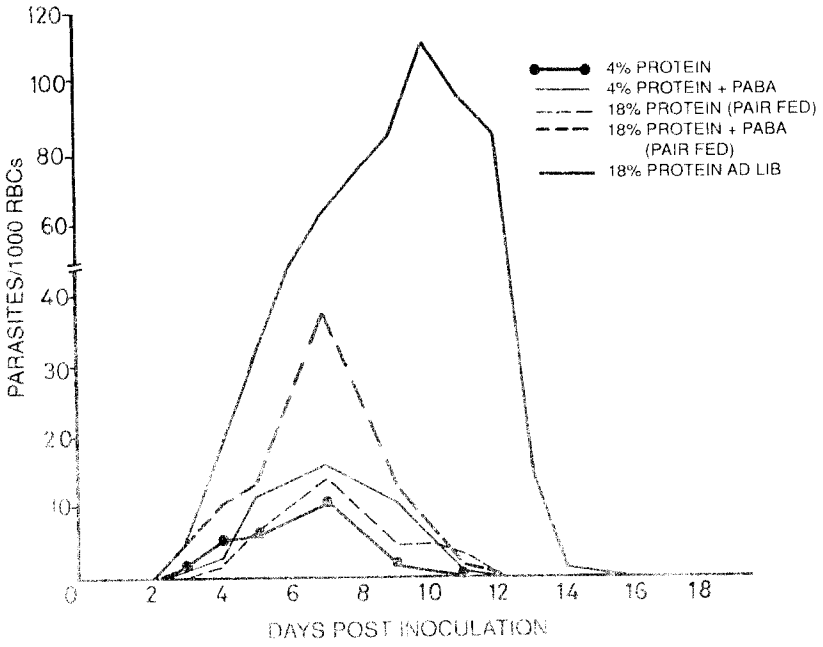

Fig. 1: Parasitaemia in rats fed on 4 or 18% protein, PABA supplemented or nonsupplemented diets.

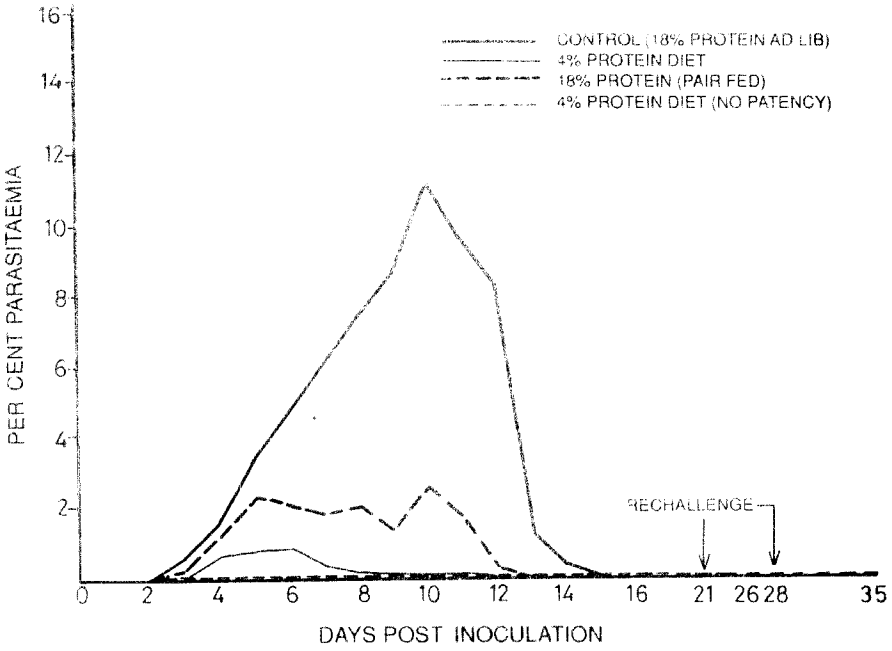

Fig. 2: Parasitaemia in rats fed on 4 or 18% protein before and after rechallenge.

sitaemia by milk or meat diet wholly or partly to PABA deficiency[11,18,19,25,26]. The role of PABA as an essential growth factor for *Plasmodium* was confirmed by Kretschmar[27] when the supplementation of milk diet with PABA increased the growth of parasite and also the mortality of the animals. Ferone[28] corroborated that PABA is required by the parasite and is metabolized by the parasite itself, which was later confirmed by Homewood[20]. Recently Gilks *et al.*[22] found that PABA supplementation in the diet corrected the irregular course of parasitaemia and normalized the mortality due to cryopreserved stabilates of malaria in rodents.

In our present study, PABA levels in blood were seen to be significantly low in protein energy and energy deficient rats after 4 weeks' feeding on the specific diets. The levels of the PABA were lowest in protein deficient animals. The supplementation of the low protein diet with PABA increased not only the PABA levels in blood but also modulated the parasitaemia by increasing the degree of parasitaemia. Our finding that PABA levels were lowest during the patent infection as compared to pre-patent or post-patent period indicated the utilization of the PABA by the parasite as has been shown by Ferone[28] and Homewood[20].

The patency of the parasite in low protein diet fed animals after the supplementation of the diet with PABA, the increase in parasitaemia and the correction of the irregular course of parasitaemia with PABA supplementation (as has been observed by Gilks *et al.*[22] also) indicate PABA as one of the many factors responsible for the suppression of parasitaemia in these animals.

The extreme deficiency of proteins and essential nutrients could be the cause of non-patency of the parasite in blood of 30 per cent of rats fed on protein calorie deficient diets. Although the animals which failed to develop patent parasitaemia even after rechallenge could not mount a detectable immune response before rechallenge, 39 per cent of them were able to develop detectable circulating antibodies after rechallenge. Moreover, the parasite could be located in impression smears of the tissues. It appears that the parasite had been persisting at subpatent levels and continuously providing antigenic stimulus resulting in the development of antibodies. Probably the built-up immunity had been arresting the growth of parasitaemia after the rechallenge of infection. Our findings corroborate those of Targett[23] who demonstrated resistance to superinfection of low protein diet fed animals.

PABA deficiency and persistence of the parasite at subpatent levels could be one of the factors for the suppression of parasitaemia in low protein diet fed animals.

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Histopathological Studies in Relation to Protection Induced by using MDP as an Adjuvant in Malaria

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Different immunomodulators were tried for their efficacy to protect against the lethal infection of *Plasmodium berghei* in mice. Since MDP was the most effective non-FCA adjuvant imparting a significant degree of protection, histopathological studies were undertaken in mice protected by using this adjuvant in comparison with mice suffering from acute malaria. The malarious mice revealed abnormalities of the liver, spleen and kidney, whereas these abnormalities were minimal in the MDP-immunized mice. The results are consistent with the degree of protection induced by MDP, and are significant with respect to the effectiveness of the non-FCA adjuvant in causing minimal histopathological abnormalities.

INTRODUCTION

Histopathological studies have shown extensive damage of liver and spleen tissue in malaria[1,2]. Kidney damage has also been reported in *P. falciparum* and *P. malariae* malaria[2,3] whereas lesions of the central nervous system in cerebral malaria due to *P. falciparum* are well known. However, histopathological studies in relation to protection have not been done.

In a study utilizing a batch of adjuvants tested for their efficacy to protect mice against the lethal malaria parasite *Plasmodium berghei*, MDP was found to be the most effective non-FCA adjuvant imparting a significant degree of protection[4,5]. The histopathological findings in mice protected by using this adjuvant compared with non-immunized mice dying of malaria are reported.

MATERIAL AND METHODS

Animals: Inbred NMRI mice weighing 20-25 g were used in the study. The animals were kept on pellet diet (Hindustan Lever) with water *ad libitum*.

Parasites: The rodent malarial parasite *Plasmodium berghei* (Vinckei & Lips strain, 1948) obtained from National Institute of Communicable Diseases, Delhi, was maintained in the laboratory by weekly blood passage using 1×10^6 infected RBCs per mouse intraperitoneally. Giemsa-stained smears were examined and the per cent parasitaemia was calculated.

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Antigen : Antigen was prepared by colloidal silica (Percoll) gradient centrifugation[6]. Heparinized blood of infected mice with a parasitaemia of 30% and above was mixed with an equal volume of 6% dextran (mol. wt 275,000 daltons) and allowed to sediment for 30 min. The leukocyte-rich plasma was removed, RBCs were washed with cold Hanks' balanced salt solution (HBSS), and diluted 10 times with HBSS. Aliquots (1 ml) were overlaid on 1 ml of 55% Percoll HBSS and centrifuged at 3000 g for 15 min at 4°C. Cells collected from the interphase were washed with HBSS, suspended in 10 times their volume of saponin solution (1:7500 in saline), incubated at 37°C for 15 min and centrifuged at 3000 g for 15 min. The supernatant was discarded and the liberated parasites were washed in large volumes of chilled PBS (pH 7.2). Free parasites were counted and stored at –70°C. Protein content was determined in the supernatant of the sonicated (20 kc/s, 30 sec) free parasites.

Immunization : A group of 25 normal mice were given MDP mixed with squalene (BDH, England) and antigen as described earlier[4]. Each dose consisted of MDP (100 µg per mouse), squalene (100 µg per mouse) and antigen (100 µg per mouse).

Three such doses were given subcutaneously at five-day intervals. Five days after the last dose, the treated as well as a group of 21 untreated mice were challenged with $1x10^4$ PRBC per mouse. Parasitaemia and mortality were observed sequentially. Of the 25 immunized mice, 15 could survive the lethal challenge with *P. berghei*[4] and were termed MDP-protected mice. The untreated mice developed a lethal infection with high parasitaemia. An antigen control group was also set up in which the antigen was injected alone in similar doses as the MDP-protected group. However, antigen alone could not afford any protection to the immunized mice.

Histopathology : Twelve acute malaria mice (Day 12 post-challenge) and nine MDP-protected mice were autopsied for histopathological studies. The organs assessed included liver, spleen, kidney and brain. The tissues were fixed in 10 per cent buffered formaline for 24 hrs. After embedding the

Table 1. Comparative histopathology of the acute malaria and the MDP-protected group

Organ	Acute malaria group*	MDP-protected group*
Liver		
—Pigment deposition in kupffer cells	Prominent	Mild
—Extramedullary haemopoiesis (EMH)	Prominent	No
Spleen		
—Pigment in red pulp	Extensive	Mild
—EMH	12.5	0
Kidney		
—Mesangial hypercellularity	50	12.5
—EMH	50	0
Brain		
—Small haemorrhagic focus + Pigment	10	0
—Few ependymal cells + Pigment	10	0
—Small focus of polymorphs + Pigment	10	0

* Numbers indicate percentage of animals.

tissue in paraffin, 5 micron thin sections were cut, stained with Ehrlichs haematoxylin and Eosin (H & E method) and examined under the light microscope.

RESULTS

Acute malaria group

The classical observation in all the animals dying of significant parasitaemia was intense pigmentation of liver and spleen (Table 1).

Liver : The liver showed prominent pigment-laden kupffer cells and the pigment was more conspicuous in the vicinity of central hepatic veins (Figs. 1 and 2). Nine out of 12 mice showed evidence of extramedullary haemopoiesis (EMH). Three animals, however, showed very prominent areas of extramedullary haemopoiesis with enlargement of portal tracts. One mouse showed focal scarring, moderate EMH in portal areas and extensive pigment.

Spleen : The spleens of a majority of the animals showed prominent germ centres with extensive pigment in the red pulp (Fig. 3) and parasitized erythrocytes lying in the red pulp. EMH was also observed in one out of eight animals.

Kidney : Kidneys of nearly 50 per cent of the animals showed mesangial hypercellularity and 50 per cent showed evidence of EMH. Kidneys of a few mice (five) contained tubular casts with lumen-containing pigment.

Brain : Most of the brain sections showed no defects at all (seven out of a total of ten). However, one animal showed a small haemorrhagic focus with pigment. A few ependymal cells containing pigment were seen in one mouse and one small focus of polymorphs and pigment was seen in the molecular layer of cerebellum in one mouse.

MDP-protected group

Liver : A very mild deposition of pigment was seen in eight out of nine animals. No pigment was seen in one mouse. EMH was not seen in any animal. Thus the changes were much less conspicuous than in animals with acute infection.

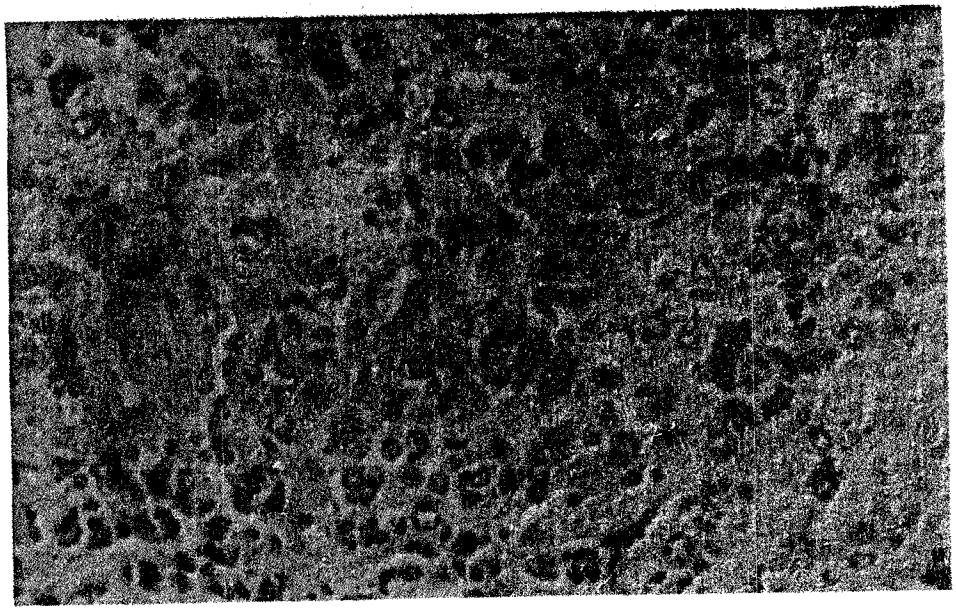

Fig. 1: Section of a liver of an animal with acute malaria (Day-12) showing prominent pigment-laden kupffer cells (H & E x 440).

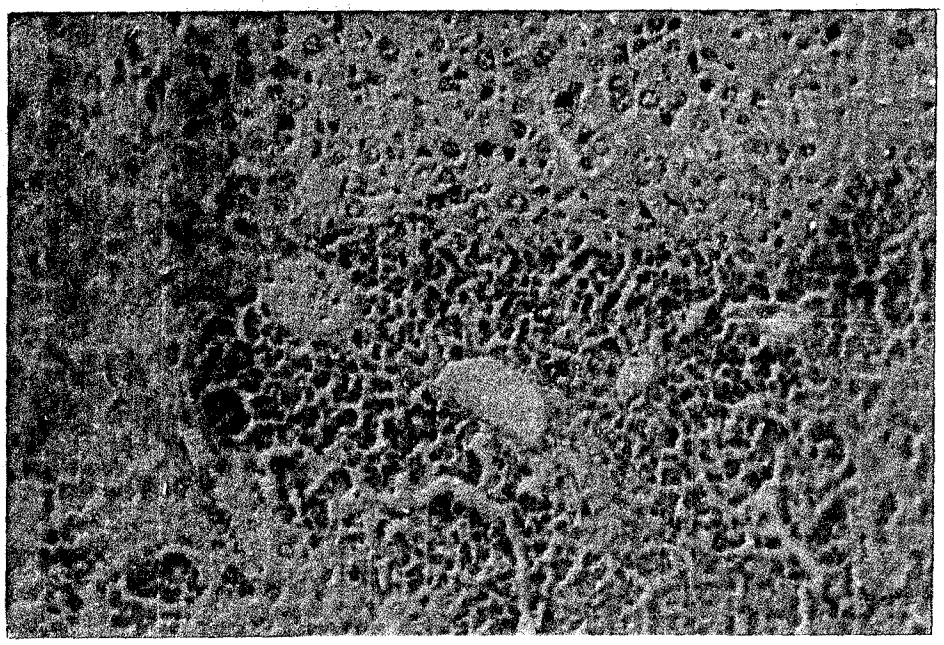

Fig. 3 : Section of the spleen of an animal with acute malaria (Day-12) showing extensive pigment in the red pulp (H & E x 160).

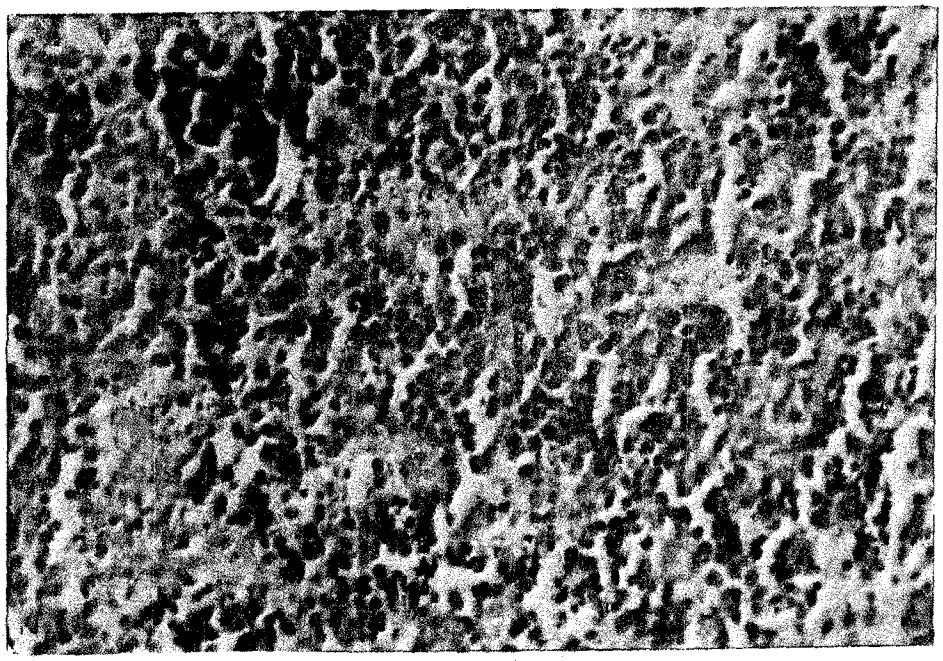

Fig. 2 : Section of a liver of another animal dying of acute malaria showing marked extramedullary haemopoiesis (H & E x 160).

Spleen : The spleens of most of the animals of this group did not reveal extensive pigmentation of the red pulp. Six out of a total of seven mice showed a mild pigment. One animal showed no pigment at all. No animal showed intense pigmentation. EMH was not seen in the spleen of any of the animals.

Kidney : Kidneys of most (seven out of eight) animals did not reveal any significant mesangial hypercellularity or evidence of EMH.

Brain : Brain sections of all the animals showed no defect.

DISCUSSION

Histological examination of the liver of the acute malaria group showed prominent pigment-laden kupffer cells. EMH was seen in a majority of the animals. A few animals showed enlargement of the portal tracts and focal scarring. Van Zon *et al.*[1] also showed extensive damage of liver tissue consisting of subcapsularly located areas of necrosis; the cellular accumulations found in the sublobular veins consisted of pigment-containing macrophages, plasma cells, lymphocytes, polymorphonuclear cells and parasitized erythrocytes, suggesting an ongoing immunological reaction. Degeneration and necrosis in the centrilobular regions have been commonly seen in the liver[2]. This is probably due to local anoxia as a result of stagnation of local circulation. In a recent study on patients of smear-positive *P. falciparum* malaria having jaundice, histologically the most consistent finding in liver biopsies was reticuloendothelial cell hyperplasia[7]. Pigmentation in kupffer cells, fatty change, sinusoidal and portal infiltration were the other features seen. In our study with the MDP-protected group, only a mild deposition of pigment was seen. No EMH was seen in any animal and the changes were much less conspicuous than in animals with acute infection.

The spleen of a majority of the infected animals showed prominent germ centres with extensive pigment in the red pulp and parasitized erythrocytes lying in the red pulp. Studies by others have also shown congestion of the organ, accumulation of pigment in the parasitized cells in capillaries and sinusoids, increase in the number of macrophages, diffuse cellular hyperplasia, dilated sinuses, foci of necrosis in the splenic pulp and splenic trapping, pitting and destruction of both infected and non-infected erythrocytes[2,3,8]. In this study with the MDP-protected group, a majority of the animals did not reveal extensive pigmentation of the red pulp. None of the animals showed intensive pigmentation or EMH.

In lethal *Plasmodium yoelii* murine malaria also, the splenic response has been shown to be vigorous, displaying marked phagocytosis, erythropoiesis, lymphopoiesis, plasmacytopoiesis and, from Day 3 of infection, increasing levels of parasitized erythrocytes[9].

Stevenson and Kraal[10] have shown that the level of resistance to infection in inbred mice with the murine malaria species *Plasmodium chabaudi AS* is genetically determined. The results demonstrated marked histological changes in the spleen and liver during the course of infection in both resistant and susceptible mice. These changes include depletion of cells from the marginal zone of the spleen which, in the case of the marginal metallophilic macrophages, appear to be more severe in susceptible A/J mice.

The kidneys of nearly 50 per cent of the acute malaria mice showed mesangial hypercellularity and evidence of EMH. Kidneys of a few mice contained tubular casts with lumen-containing pigment. In the case of *P. falciparum* infection, congestion and punctate haemorrhages in the cortex and medulla have been seen[2]. In the nephrotic syndrome associated with *P. malariae*, focal hyalinizing lesions of the tuft of the glomerules and segmental endothelial cell proliferation have been seen. Thickening of the capillary walls of the basal membrane is due to the deposition at this site of antigen-antibody complexes[3].

In our study with the MDP-protected group, kidneys of a majority of these animals did not reveal any significant mesangial hypercellularity or evidence of EMH.

The brain sections of most of the infected mice showed no defect. However, very few mice showed small foci of polymorphs or haemorrhagic foci with pigment. The MDP-protected mice revealed no defect at all. The central nervous system showed severe lesions in cerebral malaria due to *P. falciparum* infection. The changes seen are gross congestion of meninges and the brain itself, occlusion of capillaries and precapillaries and scattered areas of softening due to degeneration/demyelination of the perivascular nerve tissue[2,3].

ACKNOWLEDGEMENT

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Evaluation of Trebon (Ethofenprox) for Insecticidal Efficacy against Mosquito Larvae and on Non-target Organisms

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Ethofenprox (Trebon), a new compound, was evaluated against three species of mosquito larvae in the laboratory. Larval LC50 values revealed that *Culex quinquefasciatus* was more susceptible than *Ae. aegypti* and *An. stephensi*. The residual toxicity of Trebon was studied against culicine larvae in the rice agroecosystem at rates ranging from 0.05 to 0.2 kg ai/ha. Effective control was obtained for even beyond five weeks at the larvicidal rate of 0.2 kg ai/ha. Trebon is not safe to nontarget aquatic organisms.

INTRODUCTION

The inadequacy of insecticides for mosquito control due to development of resistance and cross-resistance on the vectors has been clearly demonstrated by various investigators[1-3]. Attempts are, therefore, being made to search new compounds which are more effective against target species and safer to non-target organisms. Amongst the newer insecticides, synthetic pyrethroids hold great promise because of their high toxicity to target organisms but low toxicity to other animals. Ethofenprox, closely related to DDT and similar to the pyrethroids in its site of action[4], has been found promising. At the larvicidal rates, it was harmless to freshwater fishes. It is reported that the pyrethroids caused a moderate reduction in the population of non-target insects, such as boat man, damselfly and dragonfly naiads; the recovery of some of the affected fauna was noted within a week after treatment[5]. The present study was carried out to find out the efficacy of ethofenprox against larvae of various species of mosquitoes and to determine its impact on some of the non-target organisms in rice fields in south India.

MATERIAL AND METHODS

Ethofenprox (Trebon 20% WDP) [2-(4-ethoxyphenyl)-2-methylpropyl-3-phenoxybenzyl ether] is a newly synthesized compound and its analogues are typical pyrethroids.

A sample of technical-grade ethofenprox (96.3%) was obtained from Coromandel Indag Products

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India Ltd. The pyrethroid fenvalerate (Sumicidin 20% EC) was used as standard.

Larvae of *An. stephensi* and *Ae. aegypti* were drawn from laboratory colonies; in the case of *Cx. quinquefasciatus*, field-collected strain was used. The procedure followed for determining the susceptibility status is the same as described by WHO[6]. Technical-grade materials were used in preparing 1% stock solutions (w/v) in acetone, and serial dilutions were prepared as per requirements. To obtain different target dosages, 1 ml of stock solution of appropriate concentration was added to 249 ml of water in beakers (500 ml) and the contents were vigorously stirred to ensure uniform mixing. The same quantity of acetone alone was used in control. Twenty-five third instar larvae were introduced into each beaker. Each concentration had three replicates with appropriate control. After 24 hrs exposure, larval mortality was recorded. LC50 values were obtained by probit analysis. Control mortality, if any, was corrected by using Abbott's formula. All tests were conducted at 28 ± 2°C and 60-70 per cent humidity.

Field evaluation

Ethofenprox was evaluated against culicine larvae in the rice agroecosystem in Melur, Madurai distt., Tamil Nadu, from November to December 1988 using WHO's modified method[7]. A newly transplanted rice field of 20 sq m in triplicate was taken as the mosquito-breeding site. Care was taken to avoid plant protection measures.The pre-application larval density was determined using laddle dips (9.5 cm dia.) 24 hrs prior to the chemical application from thickly populated areas. Five doses of ethofenprox, viz., 0.05, 0.075, 0.1, 0.15 and 0.2 kg ai/ha and fenvalerate at 0.2 kg ai/ha, diluted in the required amount of water, were applied by means of a pneumatic knapsack sprayer. Periodical observations were made on the mortality of different stages of larvae till the original larval population was attained. The percentage of reduction in the mosquito larval population was calculated on the basis of the differences between the post-treatment and pretreatment. In addition, the percentage reduction of the non-target organisms was also recorded[8].

RESULTS AND DISCUSSION

The larvicidal efficacy of Trebon under laboratory conditions against different species of mosquitoes are given in Table 1. From the larval LC50 values it was found that *Cx. quinquefasciatus* (field strain) was more susceptible than *Ae. aegypti* and *An. stephensi*, of which the latter is most tolerant to this insecticide. In larvicidal efficacy, ethofenprox was apparently 30 times more potent than fenvalerate against *Cx. quinquefasciatus*.

The relative susceptibilities of the above three species to ethofenprox showed that *Cx. quinquefasciatus* and *Ae. aegypti* were 70 and 11 times, respectively more susceptible than *An. stephensi*. A similar trend was reported by many workers[1-3,5,9] in the case of compounds like K-othrine (Decamethrin), zolone and fenvalerate. The residual effect of ethofenprox against culicine larvae observed under field conditions are given in Table 2. At the rates of 0.05 a.d 0.075 kg ai/ha, it

Table 1. Larvicidal efficacy of Ethofenprox against different species of mosquitoes

Species	Regression equation $y = a + bx$	LC50 ppm	95% Fiducial limits	RTI
An. stephensi	$y = 1.0687 + 5.5181x$	0.0126	0.0119 — 0.0133	1
Ae. aegypti	$y = 5.58 + 0.62x$	0.00114	0.00014 — 0.031035	11.05
Cx. quinquefasciatus	$y = 0.787 + 2.45x$	0.00018	0.00019 — 0.00020	70

RTI — Relative toxicity index.

Table 2. Evaluation of Ethofenprox (Trebon 20 WDP) against mosquito larvae (culicine) in rice agroecosystem

Products	Dosage kg ai/ha	Pre-application count of culicine larvae	Corrected pre cent morality at different intervals (days)														PTI	ORE
			1 & 5	7	9	12	15	18	21	24	27	30	35	40	Persis-tent days	Average persistent toxicity		
Trebon (20% WDP)	0.05	157	97	82	64	59	43	29	28	8	1	—	—	—	30	45.67	1370.1	6
	0.075	78	98	89	71	77	68	41	32	10	4	4	3	—	35	45.18	1581.3	4
	0.1	140	100	95	87	85	79	56	52	18	14	2	1	—	35	53.65	1874.3	3
	0.15	153	100	100	91	88	80	71	63	39	29	13	4	—	35	61.63	2157.05	2
	0.2	146	100	100	99	82	85	86	69	58	29	22	7	2	40	67.18	2687.2	1
Sumicidin (20% EC)	0.2	121	100	90	70	83	48	50	20	2	6	—	—	—	27	52.11	1406.97	5
	Control	137	177	112	121	111	128	87	69	117	112	69	73	22				

PTI—Presistent toxicity index; ORE—Order of the relative efficacy based on average persistent toxicity values.

Table 3. Effect of Ethofenprox and Fenvalerate on various species of non-target organisms in the rice agroecosystem

Test compounds and dosages kg ai/ha	Average no. of non-target organisms/5 dips pre- and post-treatment (days)											
	Anisops bouvieri			Dragonfly naiads			Mayfly naiads			Damselfly naiads		
	Pre	5	15	Pre	5	15	Pre	5	15	Pre	5	15
Trebon (20% WDP) 0.1 kg ai/ha	5	1	6	17	2	4	32	0	11	21	1	1
Trebon (20% WDP) 0.2 kg ai/ha	8	0	2	18	1	7	21	0	0	6	1	3
Sumicidin (20% EC) 0.2 kg ai/ha	3	0	1	9	0	3	15	0	0	4	0	1
Control	9	18	7	13	5	27	11	64	21	9	2	4

showed good to complete control of culicine larvae up to 4 days after application. Other treatments (0.1, 0.15 and 0.2 kg ai/ha) showed good to complete control for 7 days.

The residual toxicity of ethofenprox at 0.05 kg ai/ha was nil after 27 days of application, and in other treatments there was no effect after 35 days. But with a higher dosage (0.2 kg ai/ha) the effect was apparent up to 40 days. Fenvalerate 0.2 kg ai/ha gave total protection up to 5 days and the residual effect lasted 27 days after treatment. The study shows that ethofenprox is highly effective against mosquito larvae with longer residual action.

Effects on non-target organisms

During the course of the study on the efficacy of new mosquito larvicides in the field, several non-target organisms were sampled by the dipping method. Quantitative assessment of the number of these organisms sampled prior to and after treatment with the larvicides provides valuable preliminary information on the acute toxicity of the larvicidies to the organisms sampled. Among the aquatic invertebrates, mayfly naiads were dominant in our mosquito breeding sources. Mayfly naiads have been found to be highly susceptible to most mosquito larvicides such as (Deltamethrin, FMC - 45497 and fenvalerate)[5]. Ethofenprox (20% WDP) and fenvalerate (20% EC) proved toxic to mayfly naiads at the larvicidal rate of 0.2 kg ai/ha (Table 3). Both the test compounds caused reduction in the population of naiads of dragonfly, damselfly and adults of *Anisops bouvieri*.

The study shows that ethofenprox is highly effective against mosquito larvae with moderate reduction in the population of non-target organisms in comparison with fenvalerate. Literature reveals that pyrethroids are nontoxic to non-target organisms. However, the test compounds used in this study exhibited toxicity.

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Evaluation of Ayush-64 for Blood Schizontocidal Activity against Rodent and Simian Malaria Parasites

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Ayush-64, a new herbal antimalarial drug developed by the Central Council for Ayurveda and Siddha, was evaluated for direct parasiticidal action against *P. berghei* and *P. yoelii nigeriensis* in swiss mice and *P. cynomolgi* B and *P. knowlesi* in rhesus monkeys. No blood schizontocidal activity could be demonstrated against any of the four malaria parasites.

INTRODUCTION

Medicinal plants and their products have been extensively used in traditional systems of medicine in various parts of the world and plants of diverse genera are believed to be useful for treatment of malaria[1]. It is vital that their antimalarial activities are evaluated for direct parasiticidal action using well-established experimental malaria models. A new herbal drug Ayush-64, commercialized in recent years by the Central Council for Research in Ayurveda and Siddha, has been reported to control clinical attacks of *P. vivax*[2]. Each 500 mg tablet of Ayush-64 comprises extracts from four plants, namely *Swertia chirata* (100 mg), *Picrorhiza kurroa* (100 mg), *Alstonia scholaris* (100 mg) and *Caesalpinia cristata* (200 mg). In this study, the blood schizontocidal action of Ayush-64 was evaluated against two species of rodent malaria parasites and two simian malaria parasites.

MATERIAL AND METHODS

Four experimental malaria parasites, viz., *P. berghei* and *P. yoelii nigeriensis* (resistant to chloroquine, mefloquine and quinine) in swiss mice and *P. cynomolgi* B and *P. knowlesi* in rhesus monkeys which are being maintained routinely at this Institute, were used for the study. The daily regimen of Ayush-64 to both mice and monkeys was administered orally in two divided doses at 0900 hrs and 1700 hrs and blood parasitaemia from infected animals was recorded from Giemsa-stained thin blood smears.

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RESULTS

Activity against rodent malaria (*P. berghei* and *P. yoelii nigeriensis*)

In the first series of experiments, the antimalarial activity of Ayush-64 was evaluated against two rodent parasites initially, using the standard 4-dose drug schedule. The results (Table 1) show that 12 mice inoculated with 2.5×10^7 *P. berghei* parasites developed 8.17±1.27% parasitaemia on Day 4 and all the animals died, showing a mean survival time of 9.00±1.87 days. There was no significant difference in the Day 4 parasitaemia level or mean survival time in animals treated with 1.0 or 3.0 gm/kg/day x 4 days. Similarly, no significant difference in parasitaemia level and the mean survival time was observed in mice treated with Ayush-64 (1.0 and 3.0 gm/kg/day x 4 days) and control groups of mice inoculated with *P. yoelii nigeriensis*.

In the second experiment, treatment with Ayush-64 at 1:0 and 3.0 gm/kg dose levels was initiated 5 days prior to infection with *P. berghei* or *P. yoelii nigeriensis*, and treatment was continued till Day+3. Day 4 parasitaemia levels in these animals also did not show any significant difference in the control and Ayush-64 treated groups. Moreover, the mean survival times in control and Ayush-64 treated groups were comparable (Table 1).

Activity against *P. cynomolgi* B

Four rhesus monkeys inoculated with $1x10^6$ *P. cynomolgi* parasites became patent on Day 3. Three monkeys were treated with Ayush-64 at 3 gm/monkey/day dose for 5 days (Day 4-8). The results (Table 2) show that treatment with Ayush-64 did not alter the course of infection as primary peak parasitaemia levels ranging between 233856 and 292192/mm^3 were attained on Day 9-10. The untreated monkeys also developed peak parasitaemia of 178130/mm^3 on Day 9. Since *P. cynomolgi* B infection in rhesus monkeys produces non-fatal infection, there was a gradual decline in parasitaemia levels in the control as well as Ayush-64 treated monkeys.

Activity against *P. knowlesi*

Four rhesus monkeys inoculated with 1×10^5 *P. knowlesi* parasites became patent on Day 4. Three monkeys were treated with Ayush-64 at 3 gm/monkey/day from Day 4 onwards.

Table 1. Blood schizontocidal activity of Ayush-64 against *P. berghei* and *P. yoelii nigeriensis* in swiss mice

Daily dose gm/kg	*P. berghei*			*P. yoelii*		
	No. of mice	Day-4 parasit-aemia (%)	Mean survival time (days)	No. of mice	Day-4 parasit-aemia (%)	Mean survival time (days)
(A) *Dose schedule from Day 0 to +3 (4 days)*						
3.0	12	6.42±1.51	11.61±4.22	11	6.43±1.92	6.82±1.25
1.0	12	5.61±0.91	11.37±3.86	12	8.05±2.65	6.00±0.95
Control	12	8.17±1.27	9.00±1.87	12	7.17±3.62	6.46±1.05
(B) *Dose schedule from Day -5 to +3 (9 days)*						
3.0	10	4.40±0.84	10.30±4.55	10	5.13±2.25	6.00±0.82
1.0	10	4.24±0.97	10.90±5.15	12	4.65±1.12	5.91±0.70
Control	12	5.97±1.47	10.58±4.14	10	4.58±0.87	6.00±0.67

Table 2. Blood schizontocidal activity of Ayush-64 against *P. cynomolgi* B in monkeys

Monkey No.	Treatment		Parasitaemia/mm^3									
	Dose/day	Days	3	4	5	6	7	8	9	10	11	12
5970	3gm	4-8	680	1368	3078	7182	35340	86072	208436	233856	51330	67270
5971	3gm	4-8	600	1150	5520	9085	41630	225990	254340	131625	45880	80250
5972	3gm	4-8	480	1308	6104	14824	58424	125849	292192	214380	50750	26250
5969	Control	—	520	1526	4360	6322	28776	90960	178130	27255	39790	29900

Table 3. Blood schizontocidal activity of Ayush-64 against *P. knowlesi* in monkeys

Monkey No.	Treatment		Parasites/10^4 RBC on day								
	Dose/day	Days	4	5	6	7	8	9	10	11	12
5974	3gm	4-11	1	3	60	170	530	1240	2160	4500	Died
5975	3gm	4-10	1	8	160	420	930	1850	3500	Died	—
5976	3gm	4-11	1	8	130	350	750	1160	1850	3500	Died
5973	Control	—	1	6	50	180	470	1060	2380	5000	Died

The treatment, however, did not produce any effect on the course of parasitaemia and all the three treated monkeys died on Days 11, 12 and 12. The untreated control monkey also succumbed to infection on Day 12 (Table 3).

DISCUSSION

Ayush-64 has been used by the Central Council for Research in Ayurveda and Siddha in several clinical studies at out-patient and in-patient levels and has recorded 72-90% efficacy against *P. vivax* cases at a dose of 3 gm/day (in divided doses) for 4-5 days[2]. Moreover, the drug has also been documented to produce clearance of parasitaemia in most of the cases by the time the full course of drug had been completed. However, in the present antimalarial screening of Ayush-64 the parasiticidal action of this drug could not be observed against any of the rodent/simian malaria parasites. Our results with *P. berghei* agree with those obtained by the Central Council for Ayurveda and Siddha who have also reported that the drug has no action against rodent malarial parasite. This drug may be boosting the non-specific defence mechanism of the host which is able to gradually control benign malarial infection produced by *P. vivax*.

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SHORT NOTE

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Insecticide Susceptibility of *Anopheles flavirostris* in the Philippines

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An. flavirostris Ludlow (Culicidae, Diptera), the main malaria vector in the Philippines, developed resistance to Dieldrin in 1959[1]. This paper deals with its susceptibility to DDT and Bendiocarb. In October 1989 a series of tests on the susceptibility status of *An. flavirostris* to DDT and Bendiocarb were conducted on mosquitoes collected from Mangatarem, Bugallon and Aguilar municipalities within the Pangasinan province. In the test, LC50 of DDT and Bendiocarb were found to be 0.33-0.36% and 0.006-0.0067%, respectively.

In October 1989, a series of tests on the susceptibility status of *An. flavirostris* to DDT and Bendiocarb were conducted on mosquitoes collected from Mangatarem, Bugallon and Aguilar municipalities within Pangasinan province. These places are about 200 km from Manila, the capital of the Philippines. Topographically these municipalities have perennial sources of water, and the lands are planted mainly with rice, and are characterized by rolling hills and fringe-forested areas. DDT spraying for malaria control had been withdrawn from Mangatarem and Bugallon three years ago. But Aguilar had not been protected by any insecticide for ten years, though the people use different kinds of insecticides like pyrethroids, phosphates, B.T., etc. for agriculture purposes.

To determine the susceptibility status of the test species to DDT, 25 specimens were exposed to DDT-impregnated papers supplied by WHO in five different concentrations. In the case of Bendiocarb, the five different concentrations of the insecticide were calculated according to Symes *et al.*[2] and Magalona[3]. The basic principle of Busvine and Barnes[4] to impregnate paper, which was modified by Busvine and Nash[5], Fay *et al.*[6] and Mathis *et al.*[7], also followed by Apperson and Georghiou[8] by using acetone w/v, was used in this study. For test control of Bendiocarb the test papers were impregnated with acetone alone and a uniform interval of 1 hr was given between treatment of the paper and exposure of the insects to it[9, 10]. For each replication, papers were freshly impregnated. The test was carried out by exposing 25 blood-fed, uninjured females in each tube. Mortalities were noted after 1 hr exposure and 24 hrs recovery period. During recovery period adults were supplied with glucose solution in cotton wool pad to prevent death by desiccation or starvation[11-18]. The experiment was replicated four times for both the insec-

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Table 1. Toxicity of DDT and Bendiocarb against *Anopheles flavirostris* Ludlow in the Philippines (1989)

Insecticides	Test places	LC50	95% confidence limits	Slope
DDT	Mangatarem	0.36	0.296 - 0.437	2.36
	Bugallon	0.34	0.286 - 0.404	2.13
	Aguilar	0.33	0.271 - 0.402	2.39
Bendiocarb [Ficam]	Mangatarem	0.0066	0.0056- 0.0077	2.39
	Bugallon	0.0067	0.0057- 0.0077	2.26
	Aguilar	0.006	0.0052- 0.0068	2.04

ticides. On the basis of concentrations used and mortality obtained logarithmic dose mortality probit line (ld-p line) was drawn. LC50 and 95% confidence limits were calculated[19].

Among the different concentrations, 4% DDT effected 100% mortality of *An. flavirostris* from all the places. Almost a similar result was obtained from the 2% concentration. On 1% DDT the mortality effect ranged from 89 to 95%. There was no significant difference on the mortality rate as effected by DDT in sprayed (Mangatarem and Bugallon) and unsprayed (Aguilar) areas. Similarly, 100% mortality of the adults from all the places was observed with 0.024% and 0.048% concentrations of Bendiocarb. Reducing the rate to 0.012% of Bendiocarb led to mortality ranging from 75 to 80% in the three different places. The data shown in Table 1 indicate that *An. flavirostris* adults collected from all the three areas were susceptible to both the insecticides. LC50 of DDT was 0.33-0.36%, while that of Bendiocarb was 0.006-0.0067%. This result with Bendiocarb is in agreement with the result of Rosario and Rosario[12] who obtained LC50 for *An. flavirostris* as 0.0064%. They also observed LC50 for *An. mangyanus* as 0.0086% and for *An. litoralis* as 0.0082%, in the Philippines. Hence there is no indication of any cross-resistance even after insecticidal pressure due to crop protection. Since DDT is cost-effective it is suggested that it should be used to control *An. flavirostris*, the malaria vector of the Philippines. If there arises a need to replace DDT, then Bendiocarb, although twice expensive, may be a right substitute.

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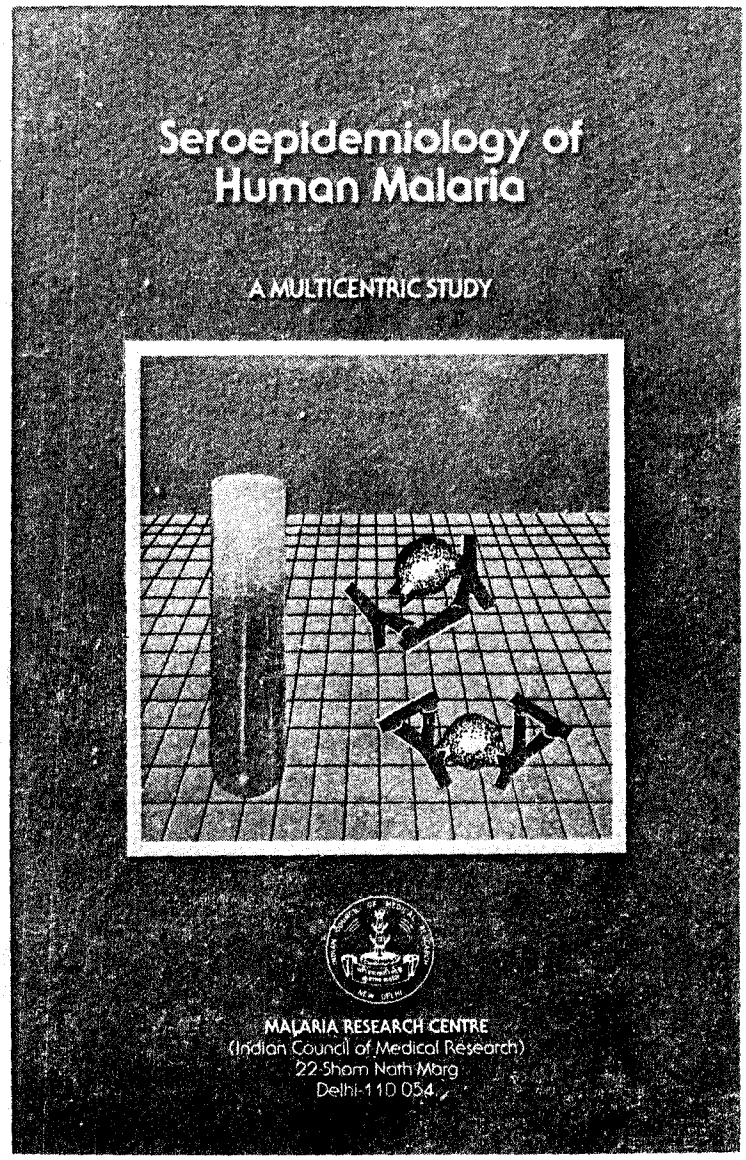

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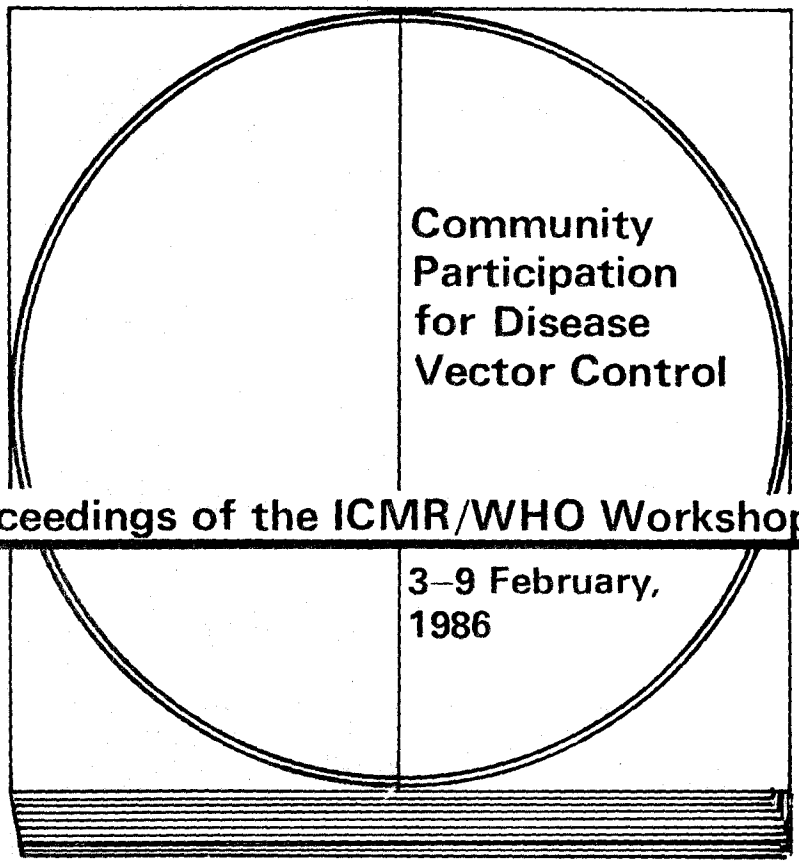

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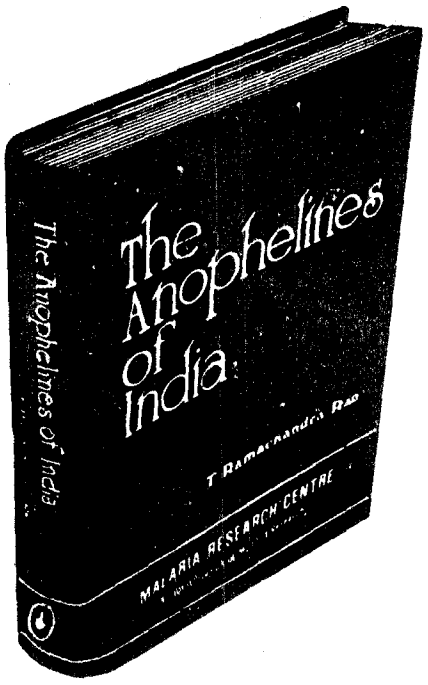

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