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Malaria in the WHO Southeast Asia Region

A.V. KONDRASHIN*

REGIONAL BACKGROUND

Geography

WHO southeast Asia region consists of a total of 11 member states, of which only Mongolia and Democratic Peoples' Republic of Korea are not malaria endemic areas.

Malaria endemic countries of this region are Bangladesh, Bhutan, India, Indonesia, Maldives, Myanmar, Nepal, Sri Lanka and Thailand (Fig. 1).

The land area of the 11 WHO Southeast Asian countries is approximately 8,466,600 sq km, which is 6 per cent of the total world land area.

There is a great variation in landscape in the countries of the region, which can be grouped into 3 large zonal series:

(i) Tropical and sub-equatorial arid and semi-arid landscapes (central part of Indian subcontinent and some areas of eastern part; interiors of Indo-China).

(ii) Tropical and sub-equatorial forested landscapes (Western Ghats in India; deltaic regions in India, Bangladesh, Myanmar, and Thailand; hills in Assam and Myanmar).

(iii) Equatorial forested landscapes (Sumatra, Kalimantan, Western Java, Western Sulawesi, and southwestern part of Sri Lanka).

The diversity of landscape in the WHO southeast Asia region along with variation in the climate and economic activities of the population determines the zonal and intra-zonal distribution of malaria (Table 1).

Coasts are greatly influenced by the large biomass of their core areas, which determine the natural drainage systems, the latter in turn facilitate the mosquitogenic potential. Coastal areas with different vectors of malaria, such as *An. sundaicus* in Bangladesh deltaic regions, *An. annularis* in coastal areas of Orissa state and *An. culicifacies* along the rest of the eastern coast of India, support malaria systems of different stability.

Plains attract the largest concentration of human population, both in the rural and urban areas. These regions, being open terrain systems and exposed to extremes of climatic variation, produce unstable malaria systems and are prone to epidemics at periodic intervals.

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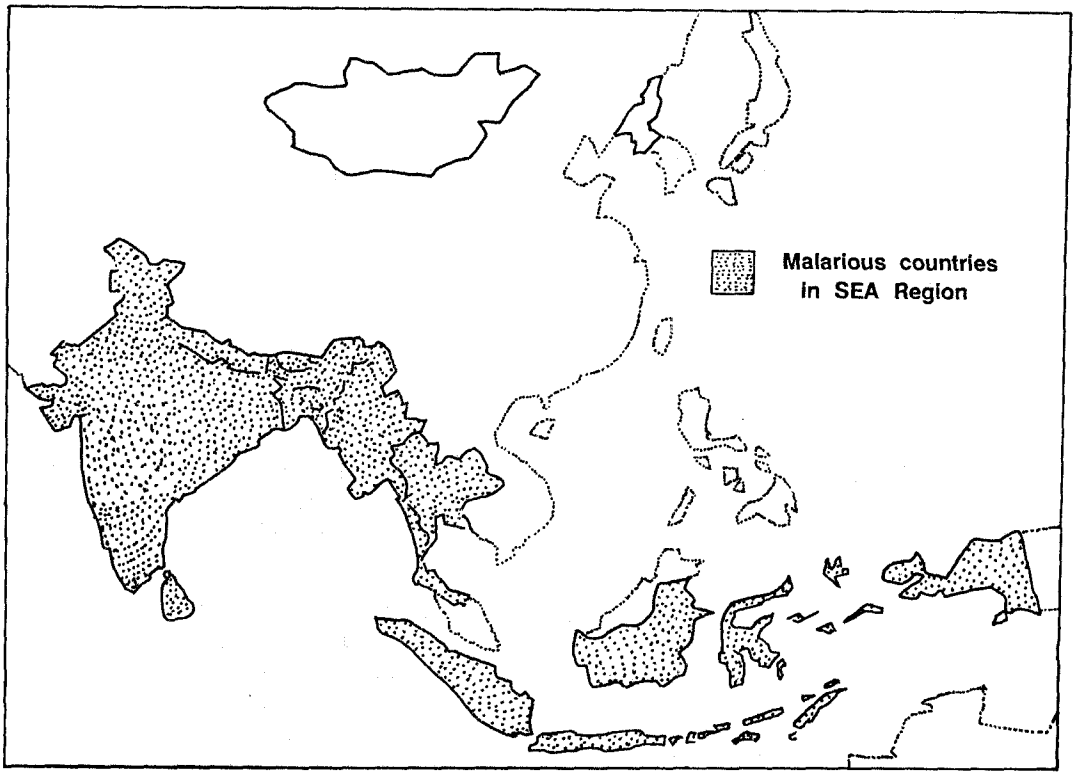

Fig. 1: WHO southeast Asia region.
The boundaries shown on this map do not imply official endorsement or acceptance by WHO.

Table 1. Zonal and intra-zonal distribution of malaria in the WHO southeast Asia region

Category	Zonal distribution			Intra-zonal distribution		
	Plains	Foothills/ Hills	Highlands	Urban areas	Peri-urban	Industrial areas
Plain	Irrigated –Canals	Forested –Deep forest	Valleys	Megapolis	Towns	Plants Development Projects
Forested	–Wells –Reservoirs	–Forest fringe				
Mangrove	*Non-irrigated* –Forested –Deserts *Marshy lands*	*Deforested* –Cereals cultivation –Others *Afforested* –Orchards –Plantations				

Foot-hill regions are next in order of concentration of population and provide good ground for rice cultivation and agro-based industries. These regions, being full of ravines, forests and rapids, have milder climatic conditions and thus generally support stable malaria systems with *P. falciparum* predominance.

Hilly regions are submountainous generally 3000 feet above mean sea level with rich fauna and flora covered by forests and criss-crossed by perennial hill streams. These regions in the majority of the countries are inhabited by national minorities and tribals and are sparsely populated. Ecologically, these regions give rise to highly stable malaria systems supporting long-lived vector populations giving rise to hyperendemic types of malaria.

Highlands, where normally, local malaria transmission in the countries of the region occurs, are mountain areas not higher than 4000-5000 feet. However, in some valleys in the highlands of Western Nepal and Kashmir, malaria occurs from time to time in the form of outbreaks.

The geographical distribution of malaria inside the ecological zones, however, is of a very mosaic character. The focal distribution of the disease is determined by various factors, including, most importantly, the degree of economic development of the area, the socio-economic status of population, and other factors. Urban malaria is overwhelmingly confined to India, while malaria in peri-urban areas is becoming a widespread phenomenon in some other countries of the region.

Climate

Climatic conditions in the subtropical, tropical and equatorial belts of south and southeast Asia vary very widely and are influenced to a great extent by the summer and winter monsoons, particularly by the former, which bring in equatorial air masses from the sea.

The climate of the mainland as a whole may be broadly described as tropical monsoon type, with various seasons. Seasons do not correspond strictly to the characteristic temperature progression of a particular season. The distinction between wet and dry seasons is often more important than between hot and cool. The precise transition depends on the arrival of the monsoon although microclimates are affected quite strongly by the altitude as well. This type of climate usually determines the seasonal character of malaria transmission, which is of varied duration with the peak of malaria incidence occurring in the post-monsoon period (Fig. 2).

Climatic conditions in the archipelago and islands of the region are affected by the proximity of the areas to the equator, the elevation above sea level, and by an equatorial double rainy season. Variation is caused by two circulations – equatorial and meridional. Although high temperatures between 31°C and 35°C have been recorded in general, temperature alone cannot be taken as a limiting factor affecting malaria transmission, either for mosquito viability or for the extrinsic cycle of the malaria parasite. Humidity also does not operate as a limiting factor for transmission of malaria, since there is high relative humidity throughout the islands and the night humidity seldom falls below 80%. Though temperature and humidity are very favourable for perennial transmission of malaria in many areas, there are, however, generally two peaks of malaria incidence, in May-June and the major peak in November-February (Fig. 3).

Moisture conditions in the area are of a very distinct seasonal character and are determined by the rainfall pattern. Rainfall exceeding 2500-3500 mm occurs in many places of the sub-equatorial and equatorial belt, while in some arid/semiarid belts it hardly exceeds 80 mm a year. Rainfall prevails in the summer months; however, its pattern varies from one year to another and prolonged droughts may be alternated by devastating floods. Extreme variations in rainfall frequently

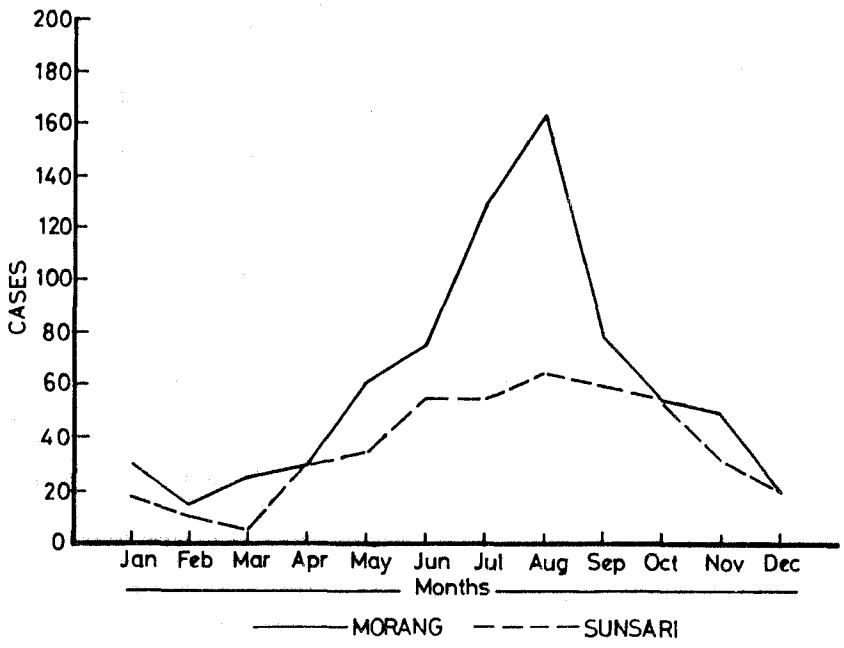

Fig. 2: Seasonal pattern of malaria, east region, Nepal. (Source: NMEO, 1986).

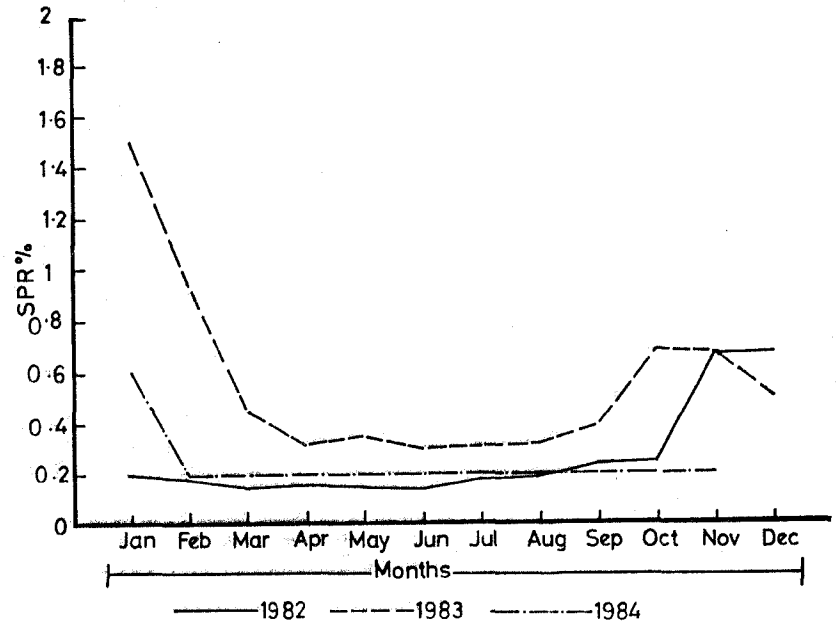

Fig. 3: Seasonal pattern of malaria in west Jaya, Indonesia (1982-1984). (Source: Ministry of Health, 1986).

result in the so-called "Regional epidemics" which occur either due to prolonged rainfall, as in north-western and western India, or due to drought conditions, as in the intermediate zone of Sri Lanka.

Socio-economic characteristics

Per capita gross national product (GNA): Table 2 presents the 1989 per capita GNP for 9 malarious countries of the region. Using the poverty range (US $275-370 per capita) defined by the World Bank based on the 1985 US dollar, and based on the 1989 per capita GNPs, four countries (Bangladesh, Bhutan, Myanmar, and Nepal), with 167 million or 13% of the region's population, were below the lower poverty level, while India, with 812 million people (65% of the 1989 regional population) remained within the poverty range. Only 4 countries of the region (Indonesia, Maldives, Sri Lanka and Thailand), with 22% of the total regional population, were above the upper level[1].

Association between poverty and malaria is well known. Analysis of the malaria situation, state-wise, in India revealed that there is a correlation between the magnitude of malaria in a state and the proportion of people below the poverty line. Other studies demonstrated a higher malaria experience with a declining socio-economic status value (Kondrashin and Kalra, in press).

Imbalance of economic growth: The most striking feature in the malarious countries of the region is an increasing imbalance of economic growth between urban and rural areas and between various economic zones within the countries. Epidemiologically, this results in large-scale uncontrolled population movement, rapid urbanization, deterioration of environment, etc. It also results in alteration of the malaria pattern, including urban malaria, occupation-related malaria and drug-resistant malaria.

Food production and supply: Though there was an improvement in food production and in per capita daily calorie supply for a few countries of the region in 1989, due to green revolution, there was also deterioration in daily calorie supply in a few other countries (Table 2). Since the figures in Table 2 represent the average status of the population, it does not take into account the disparity in calorie intake between various population groups. Studies in India, for example, revealed that intake of protein energy and iron among women was low not only during pregnancy, but was even lower

Table 2. Basic socio-economic indicators in SEAR countries (1980-1989)

Country	Per capita GNP, US $	Average annual rate of inflation (%)	Population below absolute poverty level urban/rural (%)	Average index of food prod. per capita	Daily calorie intake per capita % of requirment
Bangladesh	180	10.6	86/86	98	83
Bhutan	180	8.9	–	127	–
India	340	7.7	28/40	118	100
Indonesia	500	8.3	26/44	124	116
Maldives	420	6.4	–	–	–
Myanmar	220	3.0	40/40	120	119
Nepal	180	9.1	55/61	106	93
Sri Lanka	430	10.9	–	87	110
Thailand	1220	3.2	15/34	108	105

Sources : The world bank, 1990/1991; UNICEF, 1991.

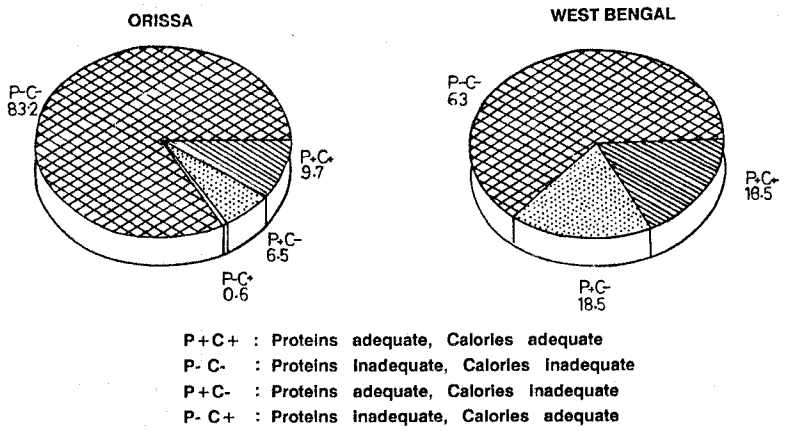

Fig. 4: Protein-calorie adequacy per cent in tribal children (1-3 yrs). (Source: NNMB tribal surveys, 1985-87).

during lactation because of stricter food taboos observed during this period. Nutritional deficiency among the children of various ages continues to be another common phenomenon in many countries of the region, with dire epidemiological consequences (Fig. 4).

Literacy and education: Literacy rates have increased in the last 10 years for several countries (Table 3), but the absolute number of illiterate adults has also increased. Although all countries reported male literacy rates of above 35% by 1990, women in most SEAR countries have much lower rates. In some countries, female literacy rates are more than half of those of men (Bangladesh, Bhutan and Nepal). The low level of literacy among women is one of the factors contributing to their low social status and is instrumental in the non-active participation of women in antimalaria activities.

Table 3. Adult literacy rates (per cent) by gender in countries of southeast Asia region.

Country	Male		Female	
	1980	1990	1980	1990
Bangladesh	39.7	47.1	18.0	22.0
Bhutan	36.0	36.8	10.0	10.0
India	54.8	58.0	25.7	33.7
Indonesia	77.5	84.1	57.7	68.0
Maldives	—	91.2	91.5	—
Myanmar	85.8	89.1	71.7	72.3
Nepal	31.7	37.6	9.2	13.2
Sri Lanka	91.3	93.4	82.0	83.5
Thailand	92.3	96.1	84.0	89.9

Source: UNESCO, 1990, 1991.

Unemployment and underemployment: Unemployment is one of the major factors contributing, in the countries of the region, to the phenomenon of population movement, and thus is closely related to the malaria problem as well. The problem of unemployment affects primarily young men and women, the foremost mobile groups of population, with the 20-29 year age-group being the most affected. In India and Indonesia, for example, the number of unemployed constitute about 10 per cent of the total labour force.

Population characteristics

Size and Density: At mid-year 1990, the total population of the world was estimated to be 5.3 billion persons, of which 1.3 billion (24.5%) lived in the 11 countries of the southeast Asian region.

The average population density for the region in 1990 was 155 persons per sq km in comparison to the world density of 39 (variation being 33 in Bhutan to 825 in Bangladesh).

Recent studies in some of the countries of the region revealed a negative correlation between the population density and the prevalence of malaria. However, within the areas with low population densities, a direct relationship was observed between the prevalence of malaria and the density of particular ethnic, or occupational groups of population. The prevalence of *P. falciparum* in India, for example, closely correlates with the distribution and density of tribals.

Population growth: One of the most serious problems which the majority of the SEAR countries have been experiencing for the last two decades was uncontrolled population growth. The average annual rate of increase was more than 2% in all countries during the 1960s and 1970s, and only during the 1980s did some of the malarious countries of the region show a decline (Table 4).

The enormous increase in population has resulted in population pressure on the available agricultural land, thus increasing the number of landless farmers and promoting migration to urban areas,

Table 4. Average annual rate of population growth in malarious SEAR countries, the world, and more developed and less developed regions (1960, 1970, 1980-85, 1985-90)

Country	Percentage			
	1960	1970	1980-85	1985-90
Bangladesh	2.5	2.7	2.7	2.7
Bhutan	1.8	1.9	1.8	2.2
India	2.2	2.5	2.2	2.1
Indonesia	2.1	2.3	2.1	1.9
Maldives	—	3.1	3.2	3.5
Myanmar	2.1	2.3	2.1	2.1
Nepal	1.9	2.1	2.6	2.5
Sri Lanka	2.4	2.3	1.7	1.3
Thailand	3.0	3.0	2.0	1.5
World	—	—	1.7	1.7
More developed	—	—	0.65	0.54
Less developed	—	—	2.1	2.1

Source : WHO/SEARO, New Delhi, 1991.

Table 5. Median age of the population in SEAR malarious countries (1980 & 1990)

Country	Median age (yrs)	
	1980	1990
Bangladesh	16.8	17.7
Bhutan	19.7	20.0
India	20.6	21.8
Indonesia	19.3	21.6
Maldives	16.0	NA
Myanmar	20.0	20.9
Nepal	19.5	18.8
Sri Lanka	21.9	24.2
Thailand	19.4	22.9
World	22.6	24.2

NA – Not available.
Source : WHO/SEARO, New Delhi, 1991.

to areas where new crops or industries have created a demand for cash labour, or to those areas with previously uncultivated lands.

Age and sex structure: In general, southeast Asians are "younger" than the world average person, as can be seen from Table 5, thus suggesting high sensitivity to malaria infection due to low community immunity.

The sex ratio for the SEA region as a whole was estimated at 105 males per 100 females in 1990, higher than that of the world.

Urbanization: The urban population of the countries of southeast Asia at present constitutes about 23% of the total population, the highest being in India and Indonesia (25% and 26.2% respectively) and the lowest being in Bhutan (6.4%). However, it has been estimated that the urban population of the region will reach 52% of the total population by 2025. The problem of urban malaria is confined mainly to the Indian subcontinent. *An. stephensi* is the principal vector in urban areas of India[2]. Urban malaria constitutes about 50% of total malaria cases in Tamil Nadu State, while 66% of total malaria cases of West Bengal State are contributed by Calcutta city itself. During malaria resurgence in India in the mid 1970s, urban malaria constituted not less than 10% of total malaria incidence in the country (Table 6).

Large-scale population movement: Although the importance of population migration and circulation in malaria epidemiology was recognized a long time ago, the present-day magnitude and diversity of population movement is not analogous with anything in the past (Table 7)[3].

The exact extent of population movement is not known; however, it is estimated that the present degree of internal migration within India is much greater than previously believed and amounts to not less than 15 to 20% of the total population every year. In Thailand, the number of temporary migrants and mobile local villagers living in and near forested areas and in the foothills near the borders is estimated at about 10.5 million (more than 15% of total country population)[4]. In Sri

Table 6. Urban malaria in India (1988-90)

Year	Total malaria cases	Percentage to total cases of India	Total *P. falciparum* cases	Percentage to total *P. falciparum* cases of India
1988	146,000	7.88	15,000	2.19
1989	203,000	10.06	29,000	3.89
1990*	191,000	11.33	19,000	3.39

*Provisional.
Source : NMEP, India, 1991.

Table 7. Typology of population movement and associated activities in relation to malaria in southeast Asia (Modification of Prothero's scheme, 1989)

Space/Time	Population movement						
	Internal					External	
	Circulation		Migration			Migration	
	Daily/Periodic	Seasonal	Long-term	Irregular	Regular	Neighbouring countries	Remote countries
A. Rural → Rural Endemic ← Non-Endemic	Cultivating Collecting (firewood etc.) Marketing Grazing	Pastoralism Hunting Trading (Fairs etc.) Labouring	Labouring	Gathering	Planned settle-ment	Labouring Pastoralism Nomadism Pilgrimage Tourism	Labouring Pilgrimage Tourism
B. Rural → Rural Endemic ← Endemic					Marriage	Trading Refugees	
C. Rural → Urban Endemic ← Non-Endemic	Commuting Cultivating Pilgrimage Tourism	Trading Labouring Pilgrimage Tourism	Labouring Trading	Natural disaster victims	Labouring Retire-ment	Labouring Pilgrimage Tourism Trading Refugees	Planned settlement Labouring Pilgrimage Tourism
D. Rural → Urban Non-Endemic ← Endemic				Refugees	Marriage		
E. Urban → Urban Endemic ← Non-Endemic	Intra-urban Commuting Trading Pilgrimage Tourism	Trading Labouring	Official Commercial	Refugees Trading	Change of residence Marriage	Official Commercial Pilgrimage Tourism	

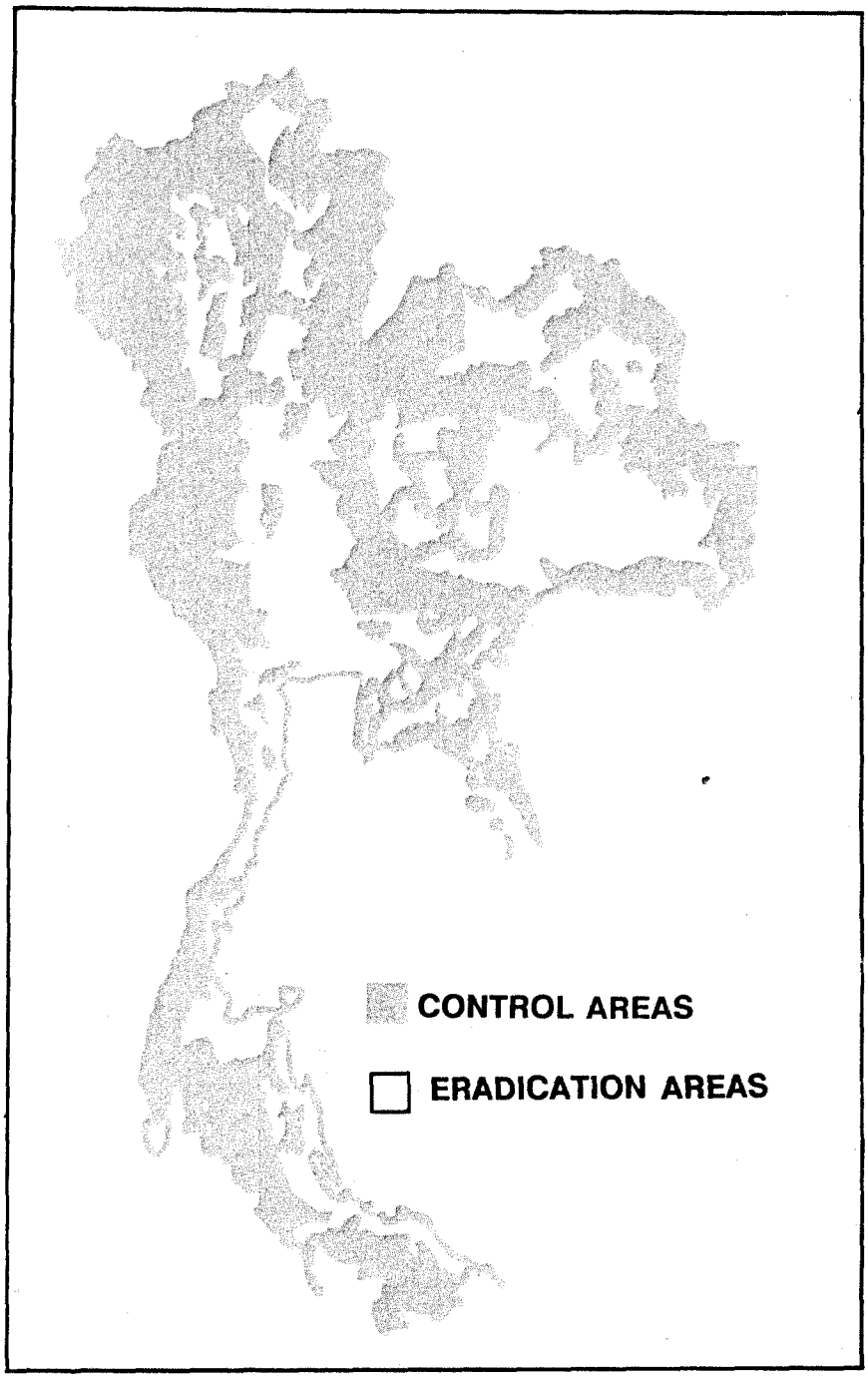

Fig. 5: Map of Thailand showing control and eradication areas.
Source : Malaria Division, 1991.

Lanka, some 13.9% of the total population are interdistrict migrants. In Nepal, it is estimated that as much as 70% of all able-bodied males in the hills migrate. Migration of populations in all its various forms in SEAR is viewed as a serious public health problem because it usually increases the spread of disease. Operational constraints caused by uncontrolled population movements result in obstructing malaria control measures, development and spread of resistant malaria, establishment of urban malaria and changing epidemiological pattern of the disease.

Ethnic diversity: Ethnic diversity in populations is a common phenomenon in many countries of the region, particularly in India, Bangladesh, Myanmar and Thailand.

An example of ethnic diversity among the indigenous inhabitants of forest areas is that of the tribes in India.

Tribals constitute nearly 8 per cent of the population of India, or 54 million as per the 1981 Census. The Scheduled Tribes number over 250, and speak over 100 languages and dialects. They usually occupy inhospitable, highly malarious terrain with difficult accessibility, which has not only a direct bearing on the mosaic picture of malaria but also poses considerable operational problems.

EPIDEMIOLOGICAL DATA

Malaria priority areas

Analysis of various factors, such as the level of malaria endemicity, seasonality of transmission, vector behaviour and its density, vector bionomics, transmission dynamics and a few others has revealed that forests, forested hills, forest fringe areas, developmental project sites and border areas should be accorded highest priority in all malaria endemic countries of the region (Figs. 5 and 6). Rubber plantations, tea gardens, irrigated plains and urban areas in some countries were also named as priority areas.

Population at risk

Identification of population groups: Infants, young children and pregnant women have been identified as malaria high risk groups in all countries of the region, followed by mobile population groups, particularly those engaged in forest-related economy, gem mining, fishing, industrial and construction work and the like.

Ethnic minorities, refugees, displaced persons, tourists and pilgrims constitute malaria high risk groups in a few countries of the region.

The size of the population at risk: This can be seen from Table 8. It appears that, apart from the appreciable number of people under high and moderate malaria risk, there is also a considerable number of people residing under low malaria risk. Thus there is still a great malaria potential in all countries of the region.

Malaria among different age- and sex-groups: Alterations in the community's immunity which has been brought about by various intervention measures have influenced the present pattern of age- and sex-related malaria.

In India, there is wide variation observed from state to state. As for the whole country, only 0.5 per cent of total malaria cases are accounted for by infants, who constitute 2.5 per cent of the country's total population. In the age-groups of 1-4 and 5-15 years, there is a marginal difference between the percentages of population in those age-groups and percentages of malaria cases detected in them. Considerable difference was observed in the age-group of 15 years and above, thus showing that malaria at present is confined to older age-groups, probably because of the higher mobility of adults[5]. In Nepal, after a few years of successful implementation of the malaria eradication programme, malaria became mainly a disease of adults. Statistical analysis of current malaria data in Sri Lanka, Thailand, Myanmar indicates that malaria incidence is greater among higher age-groups than in lower age-groups.

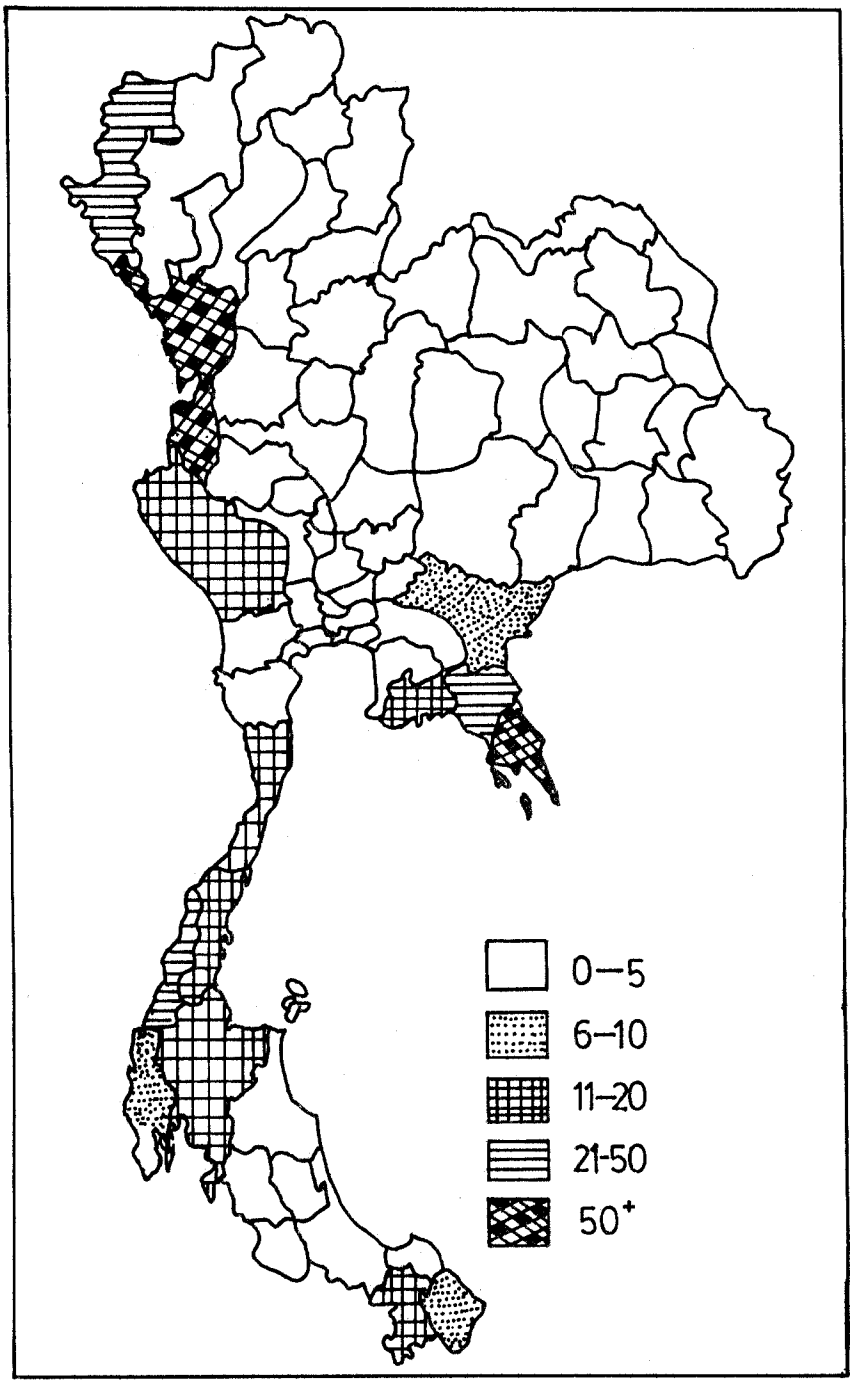

Fig. 6: Annual parasite incidence (API per 1000) for the fiscal year 1990.
Source : Malaria Division, 1991.

Table 8. Population at malaria risk in SEAR countries (1990 estimations)

Country	Total population (million)	Population at risk (million)		
		High	Moderate	Low
Bangladesh	118.9	10.0	11.5	71.0
Bhutan	1.3	0.35	NA	NA
India	843.7	75.0	240.0	500.0
Indonesia	179.3	3.1	1.2	NA
Maldives	0.22	+	+	+
Myanmar	40.0	14.8	10.0	NA
Nepal	18.4	3.6	5.0	2.2
Sri Lanka	17.2	4.0	NA	NA
Thailand	56.0	19.0	NA	NA

+ = No indigenous transmission.
Source: Country Reports, 1991.

There is a marked preponderance of malaria among males as compared to females. It is not clear whether this is due to better detection of cases among adult males or to a higher exposure of adult males to malaria. Sex differences in malaria incidence might probably be influenced by local socio-economic status, ethnic groups, the attitude of the parents especially mothers towards male and female children, treatment for malaria and ignorance sometimes about the availability of free services in the villages.

Malaria morbidity and mortality

The malaria situation in the region has remained somewhat stationary for the past 5-6 years, with the case incidence remaining between 2.5 and 2.8 million cases and the slide positivity rate (SPR) remaining at about 3 per cent. The proportion of *P. falciparum* continues to be about 40 per cent of the total malaria cases, the highest being in Myanmar (more than 85 per cent) and the lowest in Nepal (approximately 10 per cent). There has been no indigenous case of malaria in Maldives for the last few years; however, the number of imported cases from neighbouring countries shows an upward trend.

The overall malaria situation in India, Nepal, Sri Lanka and Thailand showed a slight improvement in 1990 compared with the previous years, while it remained unchanged in Indonesia and showed an upward trend in Bangladesh. The malaria profile in the countries of the region is shown in Figs. 7 and 8.

Though it is very difficult to assess accurately the actual extent of malaria mortality in each endemic country of the region, on the whole it appears that it is somewhat low compared to what could be expected, probably because of shortcomings in the reporting procedures for malaria deaths. The existing system of registration possibly underestimates the number of deaths as only parasitologically confirmed cases are taken into account, so that cases with no access to medical institutions are not reported. However, studies conducted in many endemic areas of the region have indicated that malaria deaths, although they occur in fact more frequently than officially reported, are not as major a demographic factor as they used to be before the initiation of malaria eradication programmes.

In India, malaria deaths varied between 268 in 1989 and 222 in 1990. In Bangladesh, it is esti-

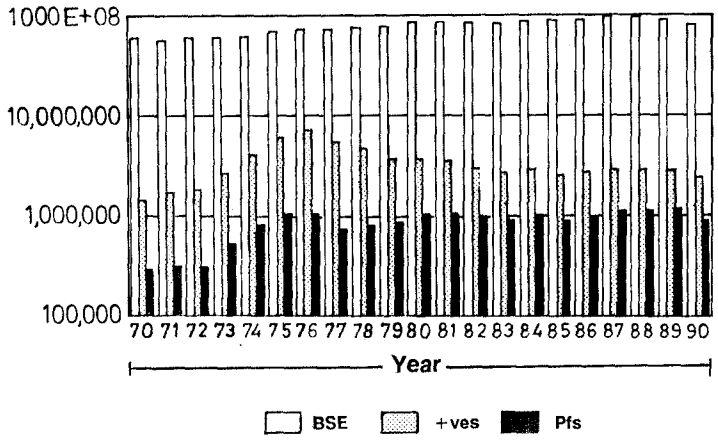

Fig. 7: Malaria profile of southeast Asia region.
Source : WHO/SEARO, New Delhi, 1991.

mated that between 200 and 800 cases of malaria death occur annually. In Nepal, according to official statistics, only sporadic deaths due to malaria have occurred. In Thailand, malaria is now the seventh ranking cause of death nationwise.

Drug resistance

P. falciparum resistance to various antimalarials is a most pronounced phenomenon of contemporary malaria in the countries of the WHO southeast Asia region.

Resistance to 4-aminoquinolines is highly prevalent in all the countries of the region except Maldives (Fig. 9). Resistance of *P. falciparum* to sulfapyrimethamine combination (SP) has also developed in vast areas of Thailand, and in some parts of Myanmar, Bangladesh, Bhutan and Indonesia (Fig. 10).

An increasing trend of *P. falciparum* resistance to the triple combination of Mefloquine-Sulfadoxine-Pyrimethamine (MSP) has been reported recently from the Thai-Cambodian border, particularly from Borai district. Somewhat reduced sensitivity to quinine has also been seen in the same areas (Fig. 11).

Vector resistance to insecticides

Vector resistance to insecticides is considered to be one of the contributory factors impeding malaria control efforts in the countries of the region. There are about 19 Anopheline species known to be primary or secondary vectors of malaria in endemic areas of the region (Table 9). Of these, six species have major operational implications for disease control, three due to their resistance to one or more insecticides (*An. culicifacies, An. aconitus, An. annularis*), and three species according to their refractory behaviour (*An. dirus, An. sundaicus, An. minimus*).

Vector resistance to DDT has been one of the major factor in setbacks to malaria control programmes. The countries worst affected are India, Indonesia, Nepal and Sri Lanka. In Bangladesh, Myanmar and Thailand, anti-vector programmes with domiciliary spraying were also impeded on account of the refractory behaviour of *An. dirus* and *An. minimus*.

Epidemics

Malaria outbreaks and epidemics have become a very frequent event since the middle of the 1970s, when a wave of post-eradication epidemics oc-

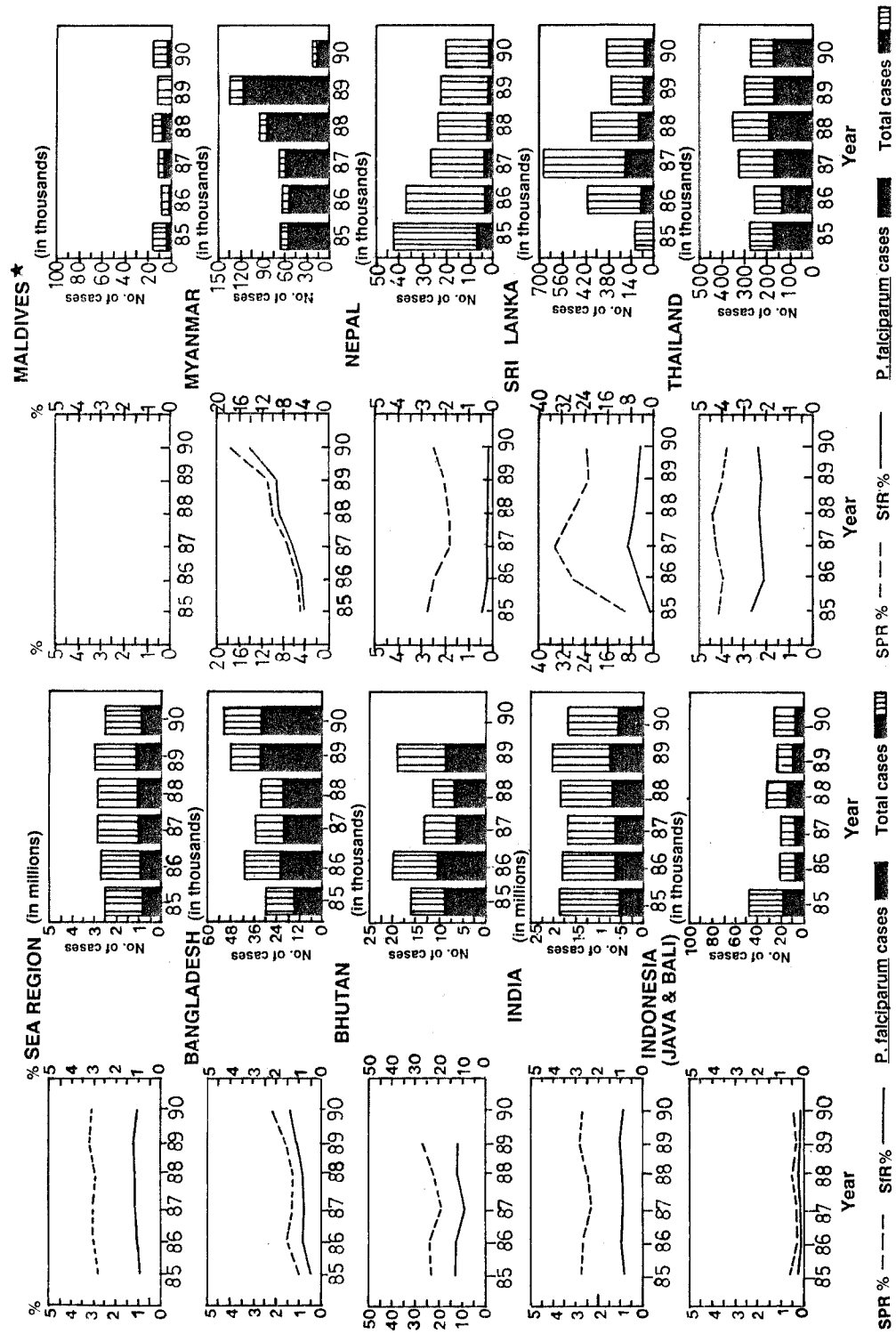

Fig. 8: Malaria profile in the countries of WHO southeast Asia region (1985-90).

Note: SPR – Slide positivity rate (per 100 slides); SFR – Slide falciparum rate (per 100 slides); 1990 Data: provisional.

*All cases detected since 1984 are imported.

Source : WHO/SEARO, New Delhi, 1991.

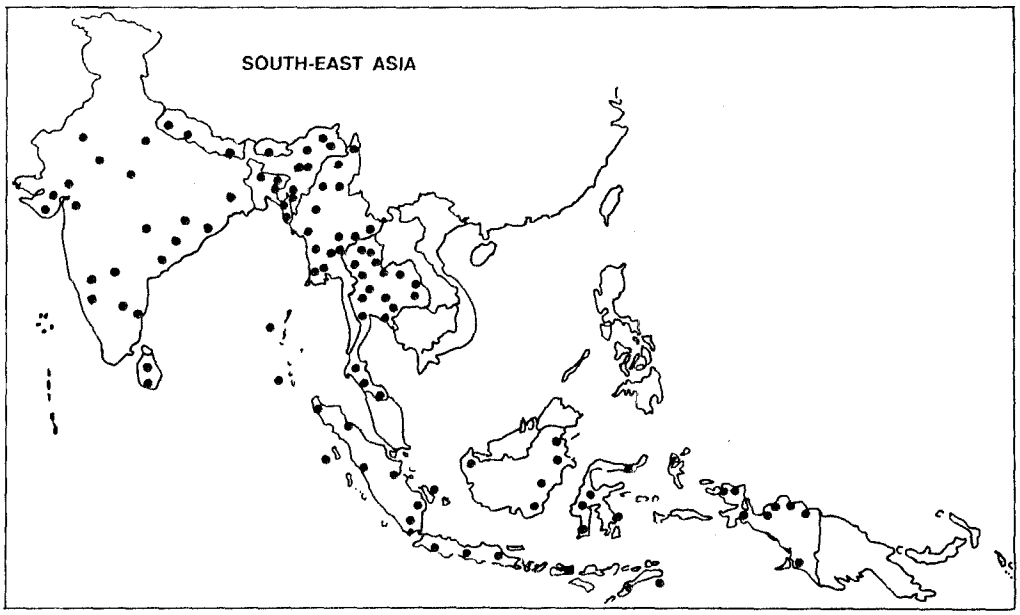

Fig. 9: Distribution of chloroquine-resistant *P. falciparum* foci 1990.
Source : WHO/SEARO, New Delhi, 1991.

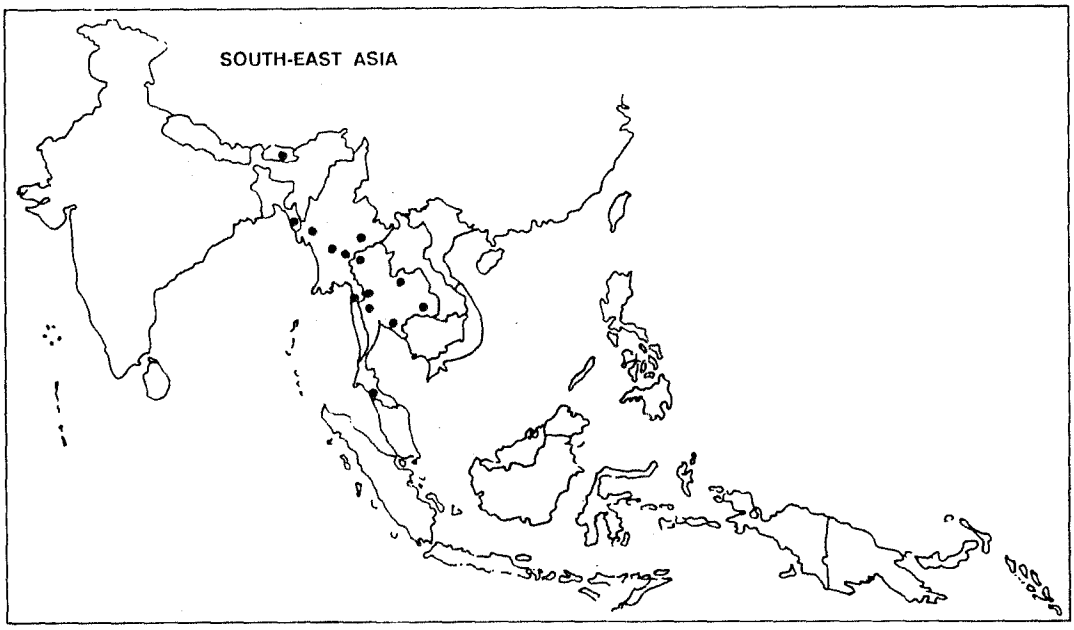

Fig. 10: Distribution of sulphadoxine-pyrimethamine-resistant *P. falciparum* foci 1990.
Source : WHO/SEARO, New Delhi, 1991.

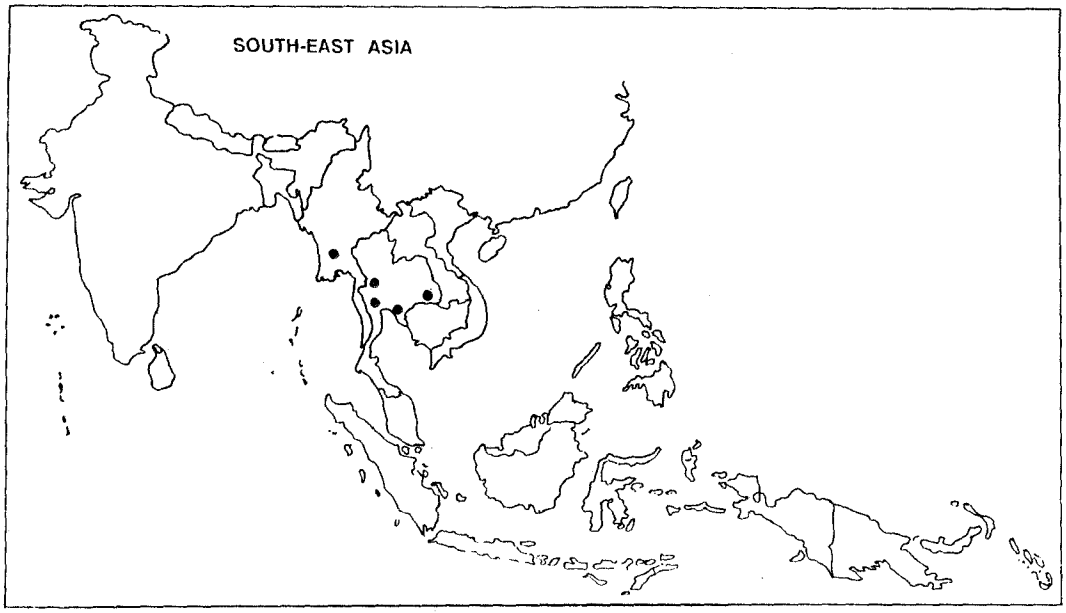

Fig. 11: Distribution of mefloquine-resistant *P. falciparum* foci 1990.
Source : WHO/SEARO, New Delhi, 1991.

curred practically in all the countries of the region. A number of epidemics of varying magnitude have occurred during the last decade in epidemic prone areas of Punjab, Haryana, Delhi and the northwestern sections of Uttar Pradesh states and union territories of India. Recently, a large-scale malaria epidemic occurred in Gujarat state (Table 10).

In Myanmar and Thailand, epidemics occur mainly among non-immunes moving into highly malarious areas. To detect these epidemics in time is very difficult, since a great deal of population movement is illegal.

In Nepal, malaria epidemics are reported quite frequently, particularly in areas where developmental activities take place. These epidemics are very instrumental in the re-establishment of malaria endemicity in areas previously freed from malaria. From Fig. 12, a stepwise increase in incidence can be seen, which has been occurring during the last decade or so, with levels starting at 2500-3000 cases in the 1970s and rising to 12,000-16,000 during the 1980s.

In Sri Lanka, apart from local outbreaks, epidemics occur in cyclical periods at intervals of 5 to 6 years, chiefly in the intermediate zone. Table 11 reflects the data for the last 15 years. It will be seen that during the last malaria epidemic which occurred in 1986, it took nearly 4 years to bring down the malaria incidence. The other important thing was that, unlike the previous epidemic cycle, *P. falciparum* incidence increased sharply and it constituted almost 30 per cent of the total malaria.

Major epidemiological types of malaria

Rapid socio-economic development in the countries of the southeast Asia region for the last 2-3 decades has brought about considerable ecological change, which in turn has resulted in alteration of the epidemiological pattern of malaria. The various epidemiological types of malaria in the region are determined to a large extent by the type

Table 9. Status of resistance to various insecticides among malaria vectors in the countries of the southeast Asia region*

Anopheles spp.†	Bangladesh	Bhutan	India	Indonesia	Maldives	Myanmar	Nepal	Sri Lanka	Thailand
aconitus	– D	–	–	+ + DH	–	+	+ DH	–	+ D
annularis	– D	–	+ + DH	– H	–	+ + D	+ + DH	–	– DH
barbirostris	– DHO	–	–	+ + D	–	– D	–	– O	– DH
campestris	–	–	–	–	–	–	–	–	+
culicifacies	–	– DH	+ + DHO	–	–	+ DH	+ DH	+ + D	+ D
dirus	+ +	–	+ +	+ +	–	+ + D	–	–	+ +
fluviatilis	+	–	+ + DH	–	–	–	+ + H	–	+
hyrcanus	–	–	– DH	+ D	–	– DH	–	– DHO	– D
letifer	–	–	–	+	–	–	–	–	–
maculatus	–	+ +	+ D	+	–	+	+ +	–	+ +
minimus	+ +	–	+ +	+ +	–	+ +	+ +	–	+ + DH
punctulatus	–	–	–	+ + D	–	–	–	–	–
philippinensis	+ +	–	+ + D	–	–	+	–	–	+ D
sinensis	–	–	–	+	–	–	–	–	–
stephensi	–	–	+ + DHO	–	–	–	–	–	–
subpictus	– DH	–	– D	+ + DH	+ +	– D	– DH	– DHO	–
sundaicus	+ +	–	+ +	+ + DH	–	+ +	–	–	+ D
tessellatus	–	–	– D	+ +	+ +	+	–	–	–
varuna	–	–	+	–	–	–	– D	– D	–

* All sibling species or related forms of each group, if any, are treated as a single species as practical purposes; †Resistance to insecticides; D = DDT; H = HCH and Dieldrin; O = Organophosphates; (–) = not important vector; (+) = secondary vector; (+ +) = principal vector.
Source : WHO/SEARO, New Delhi, 1991.

Table 10. Malaria incidence in epidemic prone areas of India (1988-1990)

State/Union territory	Number of cases		
	1988	1989	1990
Punjab	33,342	32,146	29,141
Haryana	9,237	23,711	50,452
Gujarat	4,60,683	5,98,753	4,88,541
Rajasthan	1,04,109	1,12,316	85,864
Delhi	14,423	10,761	12,044
Uttar Pradesh	1,35,096	1,01,815	98,927
Tamil Nadu	75,953	90,478	1,17,428
India	18,54,830	20,22,809	17,77,253
Per cent of total cases	44.9	47.9	43.0

Source : NMEP, 1991.

of occupational activity of the population, exemplified by the data in Table 12, which shows the occupational distribution of malaria cases in various provinces of Thailand.

It is observed that the risk of aquiring malaria is considerably higher among mobile workers and among those exposed to mosquito bites in the open air on account of their occupation.

Since various occupational activities play an important role in the epidemiology of malaria, classification of the various epidemiological types of malaria in the countries of the region has been contemplated in order to help in the choice of malaria control measures (Table 13).

Of the various malaria epidemiological types related to occupational activity, forest-related malaria appears to be the most important one in the majority of endemic countries in the region. A review of the magnitude of the forest-related ma-

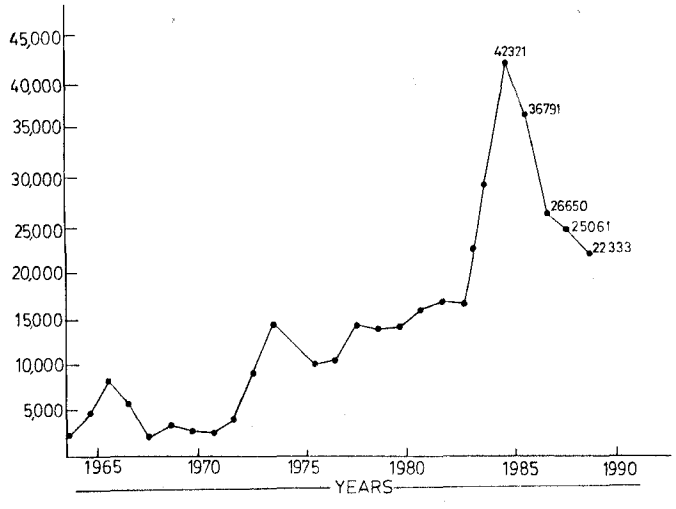

Fig. 12: Malaria cases in Nepal from 1965 to 1989.
Source : NMEO, 1991.

Table 11. Malaria profile in Sri Lanka (1975-1990)

Year	Positive	SPR	*P. falciparum*	*Pf%*
1975	4,00,777	28.96	63,853	15.93
1976	3,04,487	24.62	18,860	6.19
1977	2,62,460	27.49	10,760	4.10
1978	69,685	7.20	1,876	2.69
1979	48,004	4.79	1,368	2.85
1980	47,949	5.57	1,475	3.08
1981	47,383	5.31	1,240	2.62
1982	38,566	3.42	1,599	4.15
1983	1,27,264	12.06	4,495	3.53
1984	1,49,470	17.40	3,758	2.51
1985	1,17,816	10.11	13,057	11.08
1986	4,12,521	28.07	84,078	20.38
1987	6,76,569	38.84	1,83,092	27.06
1988	3,83,294	28.60	84,239	24.59
1989	2,58,727	23.00	66,640	25.76
1990	1,87,105	21.07	41,351	22.01

Source : Antimalaria Campaign, 1991.

laria problem in the region revealed that malaria incidence is very high in forested areas as compared to the rest of the country. Though the total forest cover is approximately 20% of the geographical area of eight endemic countries of SEAR, annually approximately 40% of total malaria cases and more than 60% of *P. falciparum* cases are reported from these areas (Table 14).

Table 12. Occupational distribution of malaria cases in three leading provinces of Thailand (Oct 1989 – Sept 1990)

Occupation	Yala (Southern)		Mae Hongsorn (Northern)		Chanthaburi (Eastern)	
	No. cases	(%)	No. cases	(%)	No. cases	(%)
Gem mining	6	0.13	15	0.65	4,114	25.32
Forest activities	123	2.65	823	35.60	593	3.65
Rubber tapping	2,146	46.29	0	0	385	2.37
Orchards	0	0	0	0	2,169	13.35
Children	1,955	42.17	143	6.19	2,246	13.82
Farming	4	0.09	361	15.61	4,176	25.71
Rice farming	6	0.13	173	7.48	132	0.81
Labour	76	1.64	3	0.13	295	1.82
Other	252	5.43	491	21.24	2,041	12.56
Unknown	68	1.47	303	13.10	95	0.59
Total	4,636	100	2,312	100	16,246	100

Source : Malaria Division, 1991.

Table 13. Classification of malaria epidemiological types in south and southeast Asia

I. Agriculture related malaria

1. Irrigated agriculture malaria
- Rice field
- Irrigation canal
- Tube-well
- Reservoir/Pond

2. Non-irrigated agriculture malaria
- Cotton plantation
- Sugarcane/Cassava plantation
- Tea gardens/Coffee plantation

3. Tree plantation malaria
- Rubber tree plantation
- Coconut tree plantation
- Fruit orchard

II. Industry related malaria

1. Coal/Ore mining

2. Development project malaria
- Road/Dam construction
- Plant construction
- Others

III. Urban malaria

1. Urban malaria

2. Peri-urban malaria
- Slums
- Industrial areas

IV. Forest economy related malaria

1. Deep forest malaria
- Gem/Gold/Ore mining
- Hunting/Food gathering

2. Forest fringe malaria
- Settled cultivation
- Shifting cultivation
- Resettlement
- Animal grazing
- Logging/firewood collection

V. Disturbed areas malaria

1. Refugee camps

2. Servicemen

VI. Transport related malaria

1. Land transport

2. Sea transport

VII. Pilgrim/tourist malaria

Table 14. Malaria cases in the countries of the region and their proportion in forests and forest-related areas (1990)

Countries	Malaria cases		Cases in forest		% cases in forest	
	Total +ve	*Pf* +ve	Total +ve	*Pf* +ve	Total +ve	*Pf* +ve
Bangladesh	50,738	35,780	44,365	35,237	87.43	98.73
Bhutan	20,585	9,660	NA	NA	> 95	> 99
India*	20,17,823	7,46,219	6,32,721	3,89,980	31.35	52.26
Indonesia	22,736	–	10,003	NA	43.99	> 50
Myanmar	1,35,194	1,16,267	NA	NA	68	87
Nepal	22,333	2,371	11,016	1,697	49.32	71.57
Sri Lanka	2,58,727	66,640	NA	NA	NA	NA
Thailand	2,99,137	–	1,25,638	NA	42.01	> 50

*All forest area have not been included; NA – Not available. Source : Sharma *et al.* (1991).[7]

Malaria transmission in forested areas is intense due to highly efficient vectors, multiple vector transmission, prolonged transmission due to ecological conditions favouring enhanced vector longevity and intensive population movement. Moreover, large areas in the forests remain inaccessible and *P. falciparum* infection dominates in the forest settlements, resulting in high morbidity and mortality. The bulk of malaria is concentrated in forested, hilly areas, commonly sharing a number of other characteristics: ethnic minorities; low population density; wide open houses; population mobility; inadequate health services, below national average; indoor and outdoor vector biting; resting outdoors; inaccessible breeding places; limited or questionable impact of residual spraying on transmission both in the villages and in the forests; and *P. falciparum* resistance to various antimalarials[6,7].

ANTIMALARIA ACTIVITIES

Existence of malaria control programmes

The dynamics and the scope of antimalaria activities undertaken in the countries of the region for the last few decades are presented in Table 15.

At present, malaria control programmes (MCP) exist in every malarious country of the region. The strategy of malaria control through the primary health care and integration approach has been actively pursued in the region. In view of the complexity of the technical and managerial problems, attempts have been made to adapt the malaria control strategy to the local epidemiological situation and the availability of resources as well as community interest and participation. Malaria control programmes have been fully integrated into the basic health services in Bangladesh, India, Indonesia, Maldives, Myanmar, Nepal and Sri Lanka at the intermediate and peripheral levels, while at the national level a core group of specialists is entrusted with the task of planning, technical support supervision, training, research and coordination. In Bhutan and Thailand, malaria control continues to be implemented as a special programme, although efforts are being made to involve basic health services personnel in the diagnosis and treatment of malaria cases and in referring severe and complicated cases to the appropriate institutions.

Objectives of malaria control programme

Some of the countries still have the achievement of malaria eradication in a very distant future as a long-term objective of the malaria programme. Therefore, some of the MCPs retain the name of National Malaria Eradication Programme (NMEP).

However, short-term general objectives of all countries of the region are much more realistic. The MCPs have the following general objectives:

- Prevention of malaria mortality
- Reduction of malaria morbidity

Table 15. Dynamics and scope of antimalaria activities in SEAR

Up to 1950	Up to 1953	Up to 1957	Up to 1970	After 1971
Limited malaria control activities in selected areas	Demonstration of malaria control projects in various parts of the country	Country - wide Malaria Control Programme (MCP)	National Malaria Eradication Programme (NMEP)	*Malaria Control Programme* — Integrated Health Services — Primary Health Care system — Tactical variants — Epidemiological approach — Disease management — *Global Malaria Control Strategy*

Table 16. The use of antimalarials in different type of areas in southeast Asia

Treatment	Type of area							
	1		2			3	4	5
	A	B	A	B	C			
Presumptive by ACD PCD DDC FTD	1st line drugs	In single dose		2nd line drug		As in 1	As in 1 & 2	Single dose of 1st line drugs or 2nd line drugs
Radical	Standard 5 or 14 day treatment with 4-aminoquinoline and 8-aminoquinolines	1500 mg chloroquine + 30 mg primaquine (once × 8 weeks)	Standard treatment	2nd line drugs or 3rd line drugs		As in 1	As in 1 & 2	Standard treatment with 1st, 2nd or 3rd line drugs
Suppressive	–	–	–	–	–	–	–	4-aminoquinolines once a week
Mass drug administration	Single dose of 4-aminoquinoline and 8-aminoquinolines	Single dose of 4-aminoquinolines only	Single dose	1st line drugs		–	–	As in 2

Type of area: 1 = Area with an overwhelming prevalenee of *P. vivax* malaria; A = Local population is free of G-6-PD deficiency; B = Local population is affected by different levels of G-6-PD deficiency; 2 = Area with an overwhelming prevalence of *P. falciparum* malaria; A = *P. falciparum* sensitive to antimalarials; B = *P. falciparum* resistant at RI and RII levels; C = *P. falciparum* resistant to RIII level; 3 = Area with a prevalence of *P. malariae* malaria; 4 = Area with a more or less equal distribution of different malaria species; 5 = Area with a large migrant population.

ACD = Active case detection; PCD = Passive case detection; DDC = Drug distribution centres; FTD = Fever treatment depots.
Source : Kondrashin (1987)[8].

- Control of malaria transmission, wherever possible
- Control of malaria epidemics
- Maintenance of gains achieved

In addition to the general objectives, every MCP has its specific objectives, relevant to that particular country only.

Antimalaria drug practices and policy

The role and place of antimalarials in the countries of the WHO southeast Asia region are determined by the general objective of preventing malaria mortality and reducing morbidity. The extent to which antimalarials are used in each programme may vary widely depending upon local epidemiological conditions, as well as on the operational capabilities of the programme and the financial resources of the country. The selection of particular antimalarial drug or of the drug regimen to be applied will also depend upon the prevailing epidemiological conditions in each country. Of particular importance in this respect are certain special situations which may be encountered in southeast Asian countries as can be seen from Table 16.

Anti-parasite measures, consisting of single-dose, presumptive and radical treatment, chemoprophylaxis and mass drug administration (MDA), are implemented in all malarious countries to various extents. Presumptive and radical treatment, until recently, were given in all areas irrespective of the malaria transmission level. Chemoprophylaxis was used exclusively for some high-risk groups of population. MDA is practised in focal outbreaks. Various antimalarials are used for the above purposes.

Single dose/presumptive treatment consists in some countries of chloroquine only, while in other countries primaquine may be added. Radical treatment of sensitive strains of malaria parasites consists of chloroquine and primaquine. In areas where *P. falciparum* resistance to the 4-aminoquinolines is well established, the second line (Sulfadoxine/Pyrimethamine or Sulfalene/ Pyrimethamine), or even third line drugs (Mefloquine) are used. Parenteral use of quinine alone or in combination with other drugs is done under hospital conditions. Community involvement in a single dose drug treatment and in referring severe cases to nearby health centres/health institutions has been progressing in many countries.

In view of the limitation of vector control measures and due to the fact that a control programme does not aim at the elimination of the malaria parasite or the disease vectors from the community, increasing emphasis is now being placed by the countries of the region on prompt diagnosis and treatment of cases. Establishment of "malaria clinics" at the periphery and "mobile malaria clinics" in inaccessible areas, particularly in areas with the problem of drug resistance, has facilitated quick microscopic diagnosis and treatment with appropriate drugs in Thailand. This approach is being gradually adopted in Myanmar and Nepal. There are, however, a few limiting factors operating in many countries of the region in relation to anti-parasite measures. The indiscriminate collection of blood slides, particularly through the ACD mechanism to meet a given target, unnecessarily overloads laboratory services and results in delay in radical treatment of cases. The time-lag between taking the blood slide and examination has increased considerably, resulting in delayed and incomplete treatment of patients, thus making the benefits of presumptive treatment questionable. In the case of *P. falciparum* infection, the single-dose treatment, when distributed in areas where organized malaria control programmes do not operate through the drug distribution centres, is likely to hasten development of resistance by way of selection through inadequate treatment. Supervised administration of drugs is not generally practised in MCPs and in many cases self-administration leads to incomplete treatment. Although it is claimed by the programmes that the dosage and regimens followed in their respective

countries are effective in achieving radical cure, there has been no general agreement that this is in fact the case.

Essential antimalarials distributed to populations in the countries of SEAR through various institutions of the MCPs are free of charge. However, a drug market exists in practically every country of the region where antimalarials of choice are available, though some of the drugs such as mefloquine in Thailand and primaquine in India are available only through MCPs.

Vector control practices and policy

The major emphasis in all the programmes continues to be integrated vector control, for which residual spraying is the main strategy. DDT, HCH, malathion and a few other insecticides are being widely used (Table 17). The criteria for insecticide application, the dosage used and the cycles of application vary from country to country and largely depend on the status of vector susceptibility and/or availability of insecticides. Focal spraying is practised to control epidemic outbreaks.

Larvicidal control is limited to certain selected localities except in India, where petroleum-based or pyrethrum-based oil, paris green, fenthion, temephos and a few others are extensively used in major cities for control of urban malaria vectors.

Bioenvironmental measures, particularly the use of larvivorous fishes, is mostly limited to trial areas in India[9], Myanmar, Nepal and Thailand. Mosquito nets, both impregnated and non-impregnated, coils and repellents[10,11], although have increasingly attracted the attention of national authorities for use on a large scale, nevertheless have limited use at the present time.

Attempts have been made recently by some of the programmes to reduce the dependence on re-

Table 17. Insecticide usage in malaria control programme in SEAR countries (1986-1990)

Country	Insecticide (in metric ton)	1986	1987	1988	1989	1990
Bangladesh	DDT (75% wdp)	1,102	941	479	533	644
India	DDT (75% wdp)	11,700	11,600	11,600	8,400	11,200
	HCH (50% wdp)	14,800	13,800	10,200	7,200	7,600
	Malathion (25% wdp)	7,400	5,200	5,900	2,600	2,200
Indonesia	DDT (75% wdp)	NA	301.6	150.7	643.8 *	784.4 **
	Fenitrothion (40% wdp)	NA	60.8	49.6	214.2 *	426.8 **
Maldives	DDT (75% wdp)	0.78	–	–	2.41	2.21
Myanmar	DDT (75% wdp)	205.0	238.0	145.0	196.0	200.0
	DDT (75% wdp)	109.2	48.1	8.9	0.64	0.0
	Malathion (50% wdp)	72.2	54.0	240.7	245.2	77.7
Nepal	Bendiocarb (FICAM) (80% wdp)	24.0	7.9	12.2	6.8	6.1
	Pirimiphos methyl (50% EC)	0.0	39.9	37.6	0.11	59.0
	ICON (10% wdp)	0.0	0.0	0.23	0.63	0.2
	Demon (10% wdp)	0.0	0.0	0.46	0.02	0.0
Sri Lanka	Malathion (50% wdp)	1,523.6	2,603.8	2,119.4	2,739.4	1,797.1
Thailand	DDT (75% wdp)	540.9	580.2	520.2	552.1	656.5

*1989/1990; **1990/1991; NA – Not available. Source : WHO/SEARO, New Delhi, 1991.

sidual insecticides to the extent possible under the prevailing epidemiological conditions[12], while earmarking savings from the purchase of insecticides for development and operational use of bioenvironmental and personal protection measures. This is particularly so since, at present, insecticide spraying is not as effective as it used to be in the past due to various factors[13]:

- Vector resistance/avoidance to commonly used insecticides, seen in some species (Table 9).
- The spraying coverage of houses is poor due to non-cooperation of the population.
- Ever-increasing operational costs causing difficulty in securing funds.
- Increased population movement and increase in temporary dwellings/shelters.
- Diversion of insecticides for use in agricultural operations.
- The high cost of insecticide and a shift of emphasis by some donor agencies have resulted in difficulties in procuring the required insecticides (Table 18).

Health education on malaria

The health education component has been made an integral part of all programmes in the region through the creation of a separate Health Education Unit. There is a network of Health Education staff at the State/Region/Division etc. levels, who assist the MCP at the periphery in securing community involvement for anti-malaria activities. At the district level this network operates through Village Development Committees, which appoint Village Health Workers (VHW) and health related workers who are trained in various health education techniques.

Health education activities aim at the individual as well as at the community. The target groups for these activities include the population under malaria risk with special reference to pregnant women, women and children and the mobile population. Health personnel of General Health Services are also targeted for health education, including Community Health Volunteers. Personnel of Health Education Units are also engaged in the development and distribution of health education materials, such as brochures, folders, posters, movie films, video-films etc. The media are being used widely for health education of communities.

Nevertheless, at present, information and communication do not emerge as a planned activity either within the health care system or by extension between health workers and the people they are to serve.

Table 18. Total expenditure of malaria control (for insecticides plus operational costs)

Country	Year	US$
Thailand	1986	4,000,000
	1987	4,920,000
	1988	5,000,000
	1989	5,080,000
	1990	6,360,000
Bangladesh	1986	2,736,465
	1987	2,314,142
	1988	1,362,173
	1989	1,435,255
	1990	1,996,008
Nepal	1986	2,385,915
	1987	1,490,408
	1988	2,336,691
	1989	2,231,433
Sri Lanka	1987	5,992,706
	1988	8,417,549
	1989	8,555,049
India	1986	127,519,000
	1987	131,448,000
	1988	129,783,000
	1989	118,139,000

Source : WHO/SEARO, New Delhi, 1991.

Budgets and resources for malaria control programmes (including training)

National malaria control budget: The (NMC) budget in the countries of the region presents a very mosaic picture, the only common feature for all countries being the constant allocation of money by the concerned government to control malaria, reflecting their political commitment (Table 19).

There is a very wide variation in these budgets as far as the percentage of the malaria budget to the total health budget is concerned, the highest being in India and Nepal and the lowest being in the Maldives, Myanmar and Bangladesh. That malaria is considered to be the disease of highest priority by the health authorities themselves is exemplified by the high percentage of the malaria budget in relation to the total budget for the control of communicable diseases.

Variations in actual expenditure to protect populations at risk (per capita) are even more wide and indicate the acute need to augment country efforts to control malaria by donor agencies.

WHO/country budget: WHO/SEARO collaborated with member countries in the prevention and control of malaria since the time of inception of National Malaria Control (Eradication) Programmes. The WHO country and intercountry budget to control malaria for 1990-91 was US$ 2,981,800. WHO support at the country level provided for the strengthening of national epidemiological capabilities to develop and implement malaria control strategies based on the prevailing malaria situations. WHO also assisted in the development of applied research capabilities to facilitate malaria control operations as part of PHC, and in the training of various categories of PHC workers in the diagnosis and treatment of malaria.

Other budget: International bilateral and multilateral agencies, such as the Swedish International Development Agency (SIDA) in India, the Canadian International Development Authority (CIDA) in Myanmar, USAID in Sri Lanka and Nepal, ODA (UK) in Nepal and Bangladesh, and the World Bank and JICA (Japan) in Indonesia are actively collaborating with the countries concerned in their malaria activities. UNDP provides funds for conducting training courses and seminars on *in-vitro* microtechniques and for providing test materials. It has also provided support for manpower development and carrying out field studies in Bangladesh.

Table 19. National malaria control budget in SEAR countries (1990)

Country	Population at risk (million)	% of total national budget	% of total health budget	% of total budget for CDC	Per capita expenditure (US $)
Bangladesh	92.5	0.50	1.98	NA	0.01
Bhutan	0.35	NA	4.0	50.13	NA
India	815.0	0.40	21.54	NA	0.07
Indonesia	4.2	0.031	3.30	12.58	1.21
Maldives	0.22	0.13	1.60	9.4	+
Myanmar	24.8	NA	1.80	NA	NA
Nepal	10.8	0.39	10.3	NA	0.29
Sri Lanka	4.0	NA	7.9	49.0	NA
Thailand	19.0	0.12	2.7	28.0	0.89

CDC – Communicable Disease Centre; + = No indigenous transmission; NA – Not available.
Source : WHO/SEARO, New Delhi, 1991.

Table 20. VBDC budget and resources for malaria control in Myanmar (1982/83 to 1988/89 FY)

Year	Expenditure on VBDC (million kyats)	External sources (% of VBDC budget)			
		UNICEF	CIDA	Netherlands	WHO
1982/83	5.7	5.6	–	–	–
1983/84	5.9	–	90.0	12.7	61.3
1984/85	6.3	–	119.1	15.8	55.7
1985/86	6.6	–	54.2	–	5.1
1986/87	6.6	–	92.5	–	45.0
1987/88	6.8	–	96.2	–	43.5
1988/89	7.1	–	62.8	–	36.9

VBDC – Vector borne disease control. Source : Country Report, 1991.

The importance of international and bilateral support to control malaria in the malarious countries is reflected in Table 20, which gives an example of such collaboration.

Training

Though all the countries of the region have some capacity and facilities for training manpower at low and intermediate levels of the PHC system, and some can also train Medical Officers in Basic Malariology, only very few countries conduct specialized or regular courses on malaria control. No significant efforts have been made to modify the training curricula and course contents to match the training with the needs of a malaria control programme being carried out through the PHC system. The current training programmes have not yet succeeded in bringing about the necessary orientation amongst health workers and decision-makers at different levels.

However, efforts are being made to improve training manpower at all levels of the country programmes, using for this purpose either WHO country budget or the assistance of bilateral and international agencies. Fellowships have been provided by WHO/SEARO for regional as well as extra-regional training courses and study tours on malaria. A number of health services staff at the peripheral level in most of the countries have been given training in malaria and malaria control activities. Thailand/Italy courses on malaria and planning of antimalaria activities have been attended by more than 30 middle-level staff engaged in the implementation of malaria control programmes. A series of seminars on the management of acute and complicated malaria have been conducted in all the countries of the region for both health services staff and private practitioners. In some countries, e.g. in India, seminars were organized for architects and engineers engaged in the construction of water-resource development projects to prevent the creation of mosquitogenic potential.

Intersectoral activities

At present, all countries are at the stage of establishing and strengthening linkages with various departments both within the Health System and outside, as various sectors concerned with the developmental activities have never been seriously involved or concerned with malaria situation created by them. An example of such linkage is given in Table 21, which shows joint activities by MCP and different departments in Indonesia.

In India, a large number of workshops have been organized for engineers working in different departments, to appraise them on their involvement in malaria control. Travelling workshops

Table 21. Intersectoral coordination in Indonesia

Sector	Joint Activities/Measures
Transmigration Department	Selection of suitable areas, proper housing, proper location of agricultural fields with proper drainage system
Local Authorities and Agriculture	Synchronization of rice planting, crop rotation and pesticiding
Irrigation	Intermittent watering
Fishery	Water management to regulate water salinity
Forestry	Mangrove planting, deforestration, afforestation
Tourism	Maintenance of malaria free status of tourist sites

Source : Country Report, 1991.

have been organized for technical and administrative heads of related sectors of the economy in some states. The objective was to appraise them of the feasibility of various types of bioenvironmental malaria control methods and their active participation.

Coordination within the Health Sector has been established in many countries at various administrative levels. There are linkages with other government departments such as Red Cross, Nurse and Midwife Associations, Voluntary and Missionary hospitals, clinics, indigenous medicine practitioners, maternal and child welfare societies, religious organizations, parent/teacher associations and other social organizations. In some countries, like Thailand, coordination has been established with universities for research and training.

Management of epidemics

Capacity to forecast malaria epidemic: By and large, identification of epidemics comes through rather slowly processed surveillance data. Very often, first reports of malaria epidemics come from the media or from the communities themselves, or from sources other than the health sector.

However, many programmes in the region have already initiated the development of some elements of Epidemiological Early Warning System (EEWS). Thus, in India, NMEP has started monitoring reports on rainfall by the Meteorology Department. In Myanmar and Nepal, attempts have been made to develop an EEWS throughout the country based on reports from township hospitals and health centres, which have regularly reported malaria morbidity and mortality data. By monitoring these data, abnormal increases in clinical malaria cases will be detected as early as possible. In Thailand, there is an attempt to establish an EEWS through the country-wide introduction of early warning morbidity graphs. In addition, all countries of the region monitor drug and vector resistance and population movement.

Capability to combat/contain epidemics: Due to late receipt of information on malaria epidemics/outbreaks, remedial measures are often delayed. Control of epidemics usually embraces mass fever treatment combined with indoor residual spraying. These capacities exist in every MCP of the region. Some countries (e.g. Bangladesh) have recently embarked on the development of a disaster preparedness programme through intensification of training of field personnel through practical training on containment of epidemics in epidemic-prone areas.

CAPACITY OF MANAGEMENT OF MCP AT THE COUNTRY AND REGIONAL LEVELS

Management capacity at the country level

Management of malaria control programmes at country level is carried out by a special technical unit (Directorate, Division, etc.) within the

Department of Public Health. This unit is responsible for technical guidance of the programme and development of policies on various administrative and technical issues, which thereafter are sent to the periphery (region/state/district, etc.). Peripheral units are responsible for implementation of the programme at their levels as well as for the development of local policies, since they have some flexibility in running their own programme.

Some of the programmes are assisted by High Power Boards and Technical Advisory Committees in framing technical guidelines and policies. In addition, all the malaria control programmes in the region are frequently evaluated by international and national experts and the recommendations of such assessments usually serve as a basis for the development of malaria control strategies for the next few years.

There has been a trend in many countries of the region over the last few years to assign responsibilities for carrying out measures against other vector-borne diseases, such as kala azar, filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever to Malaria Technical Units.

Recent external assessments of the majority of the MCP in the region revealed that they continue to be cost-effective and technically efficient organizations. However, there was also a feeling that the MCPs could be much more operationally and technically efficient if the information system established by them functioned more effectively, either with a surveillance mechanism or a computerized system. Currently, much of the essential epidemiological information is often lost in the process of communication from the lowest observation unit to the district, region or national headquarters.

Management capacity at the regional (intercountry) level

Intercountry collaboration in malaria control activities is particularly remarkable in the exchange of epidemiological information related to border areas. Previously supported by WHO, border meetings between neighbouring countries have now become a regular feature of intercountry cooperation in the field of malaria control. Collaboration with WHO/SEARO is a continuous process aimed at the smooth implementation of malaria control activities and the solving of both technical and operational problems. Support is provided to the programmes in staff collaboration, manpower development, procurement of essential supplies and equipment and in conducting programme-oriented field studies. Initially supported by WHO/SEARO, regional collaborative studies on drug resistance proved to be extremely useful for all participating countries, particularly for the development of National Drug Policy.

Exchange of scientific information, exchange visits by eminent scientists, use of research facilities and a few other related activities continue between countries of the region.

Research capabilities

Following the recommendations by the 18th Expert Committee that plans to control or modify existing MCPs should be based on the best available information and experience and that in most instances this would require acquired complementary knowledge and well-documented learning-by-doing experience to be fed back into the management process, the countries of the region have adopted the concept of "research and development". Practically all MCPs have established a special research unit within the programme. Though their research capabilities are rather limited, nevertheless the countries have managed to contemplate a few operational research projects. These units have facilities, manpower and equipment for conducting drug trials, *in-vivo* and *in-vitro* screening, insecticide susceptibility, new insecticide trials and vector bionomics studies. An example of a knowledge generating mechanism established in the region is the monitoring of chloroquine resistance in *P.*

falciparum. This has helped the countries to delimit the areas under the influence of drug resistance and has constituted the basis for adoption of a rational drug policy in the countries. Similarly, a change of insecticide is usually associated with monitoring resistance levels, which Malaria Control Programmes also are trying to do.

There is a shortage, however, in many instances, of appropriate resources and personnel to conduct other operational research on epidemiology and control activities. Therefore, to fill the gap, these programmes are trying to initiate collaboration with specialized research groups within other government departments (e.g. Department of Medical Research in Myanmar; Indian Council of Medical Research in India), in research institutions and universities (e.g. Mahidol and Chulalongkorn Universities in Thailand; Tribhuvan University in Nepal). In addition, some countries have secured funding for essential research from outside sources such as UNDP, the World Bank, and WHO TDR. It is anticipated that the targeting of research to operational problems would lead to the development of new and appropriate technologies.

One of the major problems being encountered by countries that are attempting to upgrade their training capabilities is the scarcity of people who can provide the requisite training. Therefore, some countries attempted teaching through research as an important resource where studies of research projects are valuable tools for training operations and research personnel. In Thailand, for example, one study looked at the cost and performance of different measures in the malaria control programmes. As a result of the findings, not only was there some reallocation of resources, but personnel involved in the studies are also now being used for training purposes.

CONSTRAINTS AND NEEDS

The long list of constraints and needs experienced by the malarious countries of the region can be grouped into 4 categories related to technical, administrative, operational and logistics problems.

Technical constraints

- Rapidly changing epidemiological pattern of malaria
- Diversity of epidemiological types of malaria
- Resistance of malaria parasites to various antimalarials and resistance of malaria vectors to different insecticides.

Administrative constraints

- Budget inadequacy
- Problems of finding donors
- Fluctuations in budget allocation
- Inadequacies of epidemiological set-up and response capabilities
- Acute shortage of trained personnel at all levels and vacancy of many posts for long periods, compounded by a high turnover of personnel posted at key positions.
- Inadequacies in training, facilities and research personnel.

Operational constraints

- Difficult terrain and accessibility of many malaria prone areas.
- Uncontrolled large-scale population movement in various forms.
- Reduced cooperation of population in implementation of certain antimalaria measures.
- Lack of intersectoral collaboration and community participation.
- Excessive delay in reporting from periphery to the centre and irregular feedback from the centre to the periphery.

Logistics

Needs

- Enforcement of the political commitment to malaria control.
- Re-orientation of the health services to meet the objectives of malaria control.
- Rational use of manpower and its strengthening through training and operational research.
- Community awareness in regard to malaria risk, consequences, methods of prevention and treatment.
- Identification, quantification and prioritization of risk areas and population groups through malariogenic stratification.
- Measures to prevent mosquitogenic conditions through intersectoral cooperation.
- Flexibility in field operations.
- Development of comprehensive information systems.
- Development of the scientifically based concept of malaria control strategy at present.

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Selection of Permethrin Resistance in the Malaria Vector *Anopheles stephensi*

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The laboratory strain of *Anopheles stephensi*, a well-known urban malaria vector, was selected with permethrin, a synthetic pyrethroid at LD_{90} level up to five generations. The selection resulted in the development of resistance in F_5 generation to the tune of 13-fold to permethrin and cross-resistance to the tune of 7-fold to cypermethrin, 9-fold to alphamethrin, and 10-fold to deltamethrin. The development of cross-resistance to 4% DDT was also noticed. The susceptibility status against 5% malathion was maintained throughout the five generations. The synergistic effect of piperonyl butoxide with permethrin did not overcome the development of resistance. The development of resistance showed a significant relationship between hatchability and different generations.

INTRODUCTION

Insecticide resistance is a highly practical problem as well as a fascinating case of evolution in action. Each new insecticide produces a selection for one or more mechanisms of resistance and each mechanism selected results in cross-resistance to related insecticides. The development of resistance in mosquito vector species to different insecticides has been studied by many workers[1-5]. The spread of gene for cross- and multiple-resistance among insect pests has rendered most of our present insecticides obsolete and very few novel insecticides are under development as substitutes.

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The present study is therefore aimed at finding the speed of selection for resistance to permethrin in the adults of *Anopheles stephensi* and at evaluating the extent of cross-resistance to other pyrethroids and DDT and multiple-resistance, if any, to an organophosphorus insecticide, malathion.

MATERIALS AND METHODS

The standard procedure for rearing anophelines was followed. Pupae of *An. stephensi* of Pondicherry strain were obtained from the cyclic colony maintained in the laboratory. Larvae were allowed to breed at a water temperature of $28 \pm 2^{\circ}C$ and were fed on the superannuated water of the larval food (mixture of dog biscuits and yeast ground together) during the I and II instar stages while the III and IV instars were provided with the larval food as such. Adult males

Table 1. Adult susceptibility status of parent strain of *An. stephensi* against different synthetic pyrethroids

Pyrethroid	LD_{50}	LD_{90}	$\chi 2$	b	Fiducial limits	
	($\mu g/cm^2$)				LCL	UCL
					LD_{50}	
Permethrin	0.68	1.38	16.31	1.84	0.64	0.73
Cypermethrin	0.68	1.15	27.98	2.47	0.64	0.73
Deltamethrin	0.47	0.94	1.83	1.83	0.42	0.52
Alphamethrin	1.00	2.66	2.30	1.30	0.89	1.12

b—Slope; LCL—Lower confidential limit; UCL—Upper confidential limit.

had access to 20% glucose solution which was provided with the help of soaked cotton pads, and soaked raisins/monacca were provided on alternate days. As most of the females were reluctant to feed on rabbits, feeding was mostly done on human blood.

Technical grades of DDT, permethrin, cypermethrin and alphamethrin of more than 90% purity were obtained through World Health Organization, Geneva; technical-grade malathion of more than 90% purity was obtained through American Cynamid Co., New Jersey, USA, and technical-grade deltamethrin of more than 99% purity through Roussel Uclaf, Bombay, India. Technical-grade piperonyl butoxide of 90-95% purity (Fluka make) was used as the synergist.

The WHO's standard technique for determining the susceptibility status of adult mosquitoes was followed[6]. Insecticide-impregnated papers of different concentrations of the pyrethroids, 4% DDT and 5% malathion, were prepared according to the need with technical-grade insecticides in acetone with olive oil base using Whatman No.1 filter paper[7]. The papers were dried at room temperature for 24 h, packed in aluminium foils and kept airtight at 27 ± 2°C.

In determining the susceptibility of the vector mosquitoes to different insecticide-impregnated papers, the observed mortality was corrected using Abbott's formula[8] and LD_{50} and LD_{90} values were calculated using dosage-mortality regression by Probit analysis.

A selection line was established using permethrin. Mosquitoes surviving at LD_{90} level were selected to bring about the next progeny (F_1) and the same process was followed up to the fifth progeny (F_5), thus eliminating all susceptible ones to bring up a batch of mosquitoes resistant to permethrin. The development of resistance in different generations of *An. stephensi* selected with permethrin was monitored by determining the LD_{50} values against permethrin. The LD_{50} values of the parent strain and those of F_5 generation to other pyrethroids were determined to study the development of cross-resistance. 4% DDT and 5% malathion papers were used as the diagnostic concentrations to study the cross-resistance pattern.

Table 2. Susceptibility status of *An. stephensi* of different selected strains against DDT and malathion

Insecticide	Diagnostic dose %	Percentage mortality					
		F_0	F_1	F_2	F_3	F_4	F_5
DDT	4	92.5	90.5	65	25	0	0
Malathion	5	86.25	87.5	100	100	95	95

The impact of the development of resistance on the hatchability and immature development period was monitored with the eggs laid by 10 gravid females of *An. stephensi* from each generation. Chi-square test was used to test the significance in hatchability.

RESULTS AND DISCUSSION

The susceptibility of the parent strain (F_0) of *An. stephensi* to the different pyrethroids, viz. permethrin, cypermethrin, deltamethrin and alphamethrin was studied and the results are given in Table 1. *An. stephensi* was highly susceptible to deltamethrin with an LD_{50} value of 0.47 $\mu g/cm^2$ followed by 0.68, 0.68 and 1.00 $\mu g/cm^2$ against permethrin, cypermethrin and alphamethrin respectively.

The susceptibility status of the parent strain against DDT and malathion is given in Table 2. *An. stephensi* was susceptible to DDT and malathion with the percentage of mortality being 92.5 and 86.25 respectively.

The results of the susceptibility status of different generations against permethrin are given in Table 3. The selection of resistance in *An. stephensi* has resulted in a 13-fold increase in tolerance in F_5 generation (LD_{50} = 13.5 $\mu g/cm^2$) when compared to the parent strain (LD_{50} = 0.68 $\mu g/cm^2$). A similar record on the rapid development of knock-down resistance was reported in *An. stephensi* against fenvalerate, another synthetic pyrethroid[9]. Our results are also comparable to those made in the continuous larval selection of *Culex quinquefasciatus* for 40 generations with the synthetic pyrethroid deltamethrin, which resulted in 1449-fold resistance to deltamethrin[10].

The susceptibility status of F_5 generation of *An. stephensi* to different pyrethroids with the values of LD_{50}, chi-square and slope is given in Table 4. The development of resistance in *An. stephensi* with permethrin also resulted in a 7-fold, 9-fold and 10-fold cross-resistance to cypermethrin, alphamethrin and deltamethrin respectively.

The resistance in *An. stephensi* selected, with permethrin was also found to induce F_4 and F_5 generations resistant to DDT (Table 2). This may be due to the similar mode of action of organochlorines and pyrethroids. In the case of malathion there was no marked difference in the susceptibility status of different generations (Table 2). Since the mode of action of organophosphorus compounds is different from that of both organochlorines and pyrethroids, the development of resistance in *An. stephensi* selected with permethrin could not induce any cross-resistance to malathion.

The impact of resistance acquired by the vector on the hatchability of their eggs and the immature

Table 3. Susceptibility status of different generations of *An. stephensi* to permethrin

Generation	LD_{50}	LD_{90}	$\chi 2$	b	Fiducial limits	
	($\mu g/cm^2$)				LCL	UCL
					LD_{50}	
F_0	0.68	1.38	16.31	1.84	0.64	0.73
F_1	0.70	1.50	32.42	1.67	0.65	0.75
F_2	1.62	2.73	0.73	2.44	1.50	1.73
F_3	4.87	8.32	2.85	2.39	4.35	5.45
F_4	5.76	10.44	3.80	2.15	5.12	6.47
F_5	13.58	22.97	15.56	2.44	12.47	14.70

b—Slope; LCL—Lower confidential limit; UCL—Upper confidential limit.

Table 4. Susceptibility of F_5 generation of *An. stephensi* to pyrethroids

Pyrethroid	LD_{50}	LD_{90}	χ^2	b	Fiducial limits	
	($\mu g/cm^2$)				LCL	UCL
					LD_{50}	
Permethrin	13.58	22.97	15.56	2.44	12.86	14.18
Cypermethrin	7.94	12.62	1.44	2.76	7.56	8.34
Deltamethrin	10.80	19.50	0.58	2.17	10.18	11.46
Alphamethrin	10.28	23.96	1.76	1.51	9.53	11.08

b—Slope; LCL—Lower confidential limit; UCL—Upper confidential limit.

development period, i.e., egg-adult emergence, were monitored and the results are given in Table 5. The immature development period has been shortened from 14 days in the parent strain to 11 days in F_5, which indicates that the development was faster in the resistant strain. There was a significant relationship ($\chi 2 = 54.96$; $p < 0.01$) between the hatchability and the different generations.

Owing to the marked development of resistance in *An. stephensi* in F_5 it is of interest to study the synergistic effect of piperonyl butoxide (PB) with permethrin. It has been found that the concentration of permethrin, 10 $\mu g/cm^2$, which produced only 17.5% mortality against the F_5 generation of *An. stephensi*, could produce only 37.5% mortality when PB at 5 $\mu g/cm^2$ was added to the insecticide-impregnated paper of permethrin at 10 $\mu g/cm^2$. This is in accord with the observation of Omer *et al.*[5] that none of the selected strains of *An. stephensi* was significantly synergised by the metabolic inhibitors like PB.

Table 5. Effect on development period and hatchability of different generations of *An. stephensi*

Generation	Development period (days)	Hatchability (%)
F_0	14	93
F_1	13	90
F_2	13	91
F_3	12	85
F_4	12	76
F_5	11	60

The study shows that the high level of resistance to permethrin also results in a significant cross-resistance to other pyrethroids and DDT. Even a combination of synergist PB could only show a slight increase in susceptibility. As we have observed that the development of resistance selected with permethrin in *An. stephensi* is faster, we suggest that pyrethroid insecticides should be judiciously applied in vector control programmes at the recommended application rates. Since the susceptibility status against malathion is maintained throughout the generations, we recommend change of insecticides with different modes of action in situations where the vector mosquitoes develop resistance against pyrethroids or organochlorine insecticides.

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Hepatic Changes in *P. falciparum* Malaria

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Liver function tests were performed in 165 hospitalized patients suffering from *P. falciparum* malaria with complications. Serum bilirubin was found increased in 33 patients, and 22 of them had unconjugated hyperbilirubinaemia. Serum alanine aminotransferase was increased in 5 patients, but only to mild to moderate levels. Serum alkaline phosphatase was increased in 11 patients, gamma-glutamyl transpeptidase in 3 patients. Serum total protein and albumin were significantly decreased but these were considered more as indicator of acute phase response. Liver cell necrosis was observed in one patient, and oedema and mononuclear cell infiltration in two patients. Though hepatomegaly and mild elevation of enzymes can be observed in a significant proportion of patients, involvement of liver leading to acute hepatitis or liver cell necrosis is a relatively uncommon complication in *P. falciparum* malaria.

INTRODUCTION

Impairment of liver function in acute *P. falciparum* infections has been reported since the early twentieth century by Sinton and Hughes[1]. Kern and Norris[2] observed bromsulphthalcin retention in all types of malaria. Evidence of liver dysfunction during both *P. falciparum* and *P. vivax* infections was reported by Mirsky *et al.*[3] After the availability of serum enzymes as markers of liver disease, Sadun *et al.*[4] reported a significant increase in serum level of alanine aminotransferase (AlAT/SGPT) but not aspartate aminotransferase (AsAT/SGOT) with a marginal decrease of alkaline phosphatase (AIP) in infected patients. Liver biopsy examinations in *P. falciparum* malaria patients showed Kupffer cell hyperplasia and mononuclear cell infiltration associated with increased serum aminotransferases and liver tenderness but liver cell necrosis was not noticed[5]. Hepatomegaly is reported to be a common association in acute primary malaria infection[6]. A significant number of children with *P. vivax* malaria were found to have increased serum amino transferases which correlated well with hepatomegaly and a concomitant increase in alkaline phosphatase[7]. Cases of malarial hepatitis are also reported from other Indian studies[8,9]. Though hepatomegaly and jaundice are frequently encountered in falciparum malaria, the extent

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of structural and functional impairment of liver is still controversial. Reports from West Africa[10] and Thailand[11] could not demonstrate convincing signs of liver cell failure in cerebral malaria patients. *P. falciparum* malaria is widely prevalent around Rourkela. Many of the patients with multiple complications get admitted to the Ispat General Hospital. Hence we studied the nature of hepatic changes in the context of *P. falciparum* infection in this area.

SUBJECTS AND METHODS

Adults with *P. falciparum* malaria admitted to the medical wards of the Ispat General hospital during 1988-1990 were the subjects. A detailed history and clinical examination was done immediately after admission of the patient. Five ml blood was drawn by venepuncture for identification of the parasite, estimation of its count and haemoglobin and biochemical assessment of liver function, renal function, glycaemic status and electrolytes.

Investigations for liver disease

Among the enzyme markers of liver disease, alanine aminotransferase (SGPT), alkaline phosphatase (AIP) and gamma-glutamyl transferase (γGT) were estimated. All the three enzymes were measured by kinetic methods using Boehringer Mannheim test kits on a Hitachi-705 autoanalyser; SGPT was estimated by measuring the disappearance of NADH, AIP by using *p*-nitrophenyl phosphate as substrate in diethanolamine buffer, and γGT by using L-γ-glutamyl-*p*-nitroanilide and glycylglycine as substrate.

Total protein, albumin and bilirubin (both total and conjugated) were measured in all the patients. Total protein was measured by biuret method and albumin by bromocresol green dye binding method in the autoanalyser. Post-mortem necropsy specimens of liver, collected wherever informed consent could be obtained, were subjected to histopathological examination.

RESULTS

The complications in 165 patients of *P. falciparum* malaria admitted to the hospital during the study period are given in Table 1. The age of the patients ranged between 13 and 60 with a median of 23. There were 125 males and 40 females, and 147 patients recovered completely while 18 patients (11%) died owing to various complications.

Hepatomegaly was encountered in 44 patients (27%). However, there was no correlation between hepatomegaly, severity of the disease or the height of the icterus. Alanine aminotransferase (SGPT), the single most important indicator of liver cell involvement, was raised beyond 3 times the upper limit of normal in 5 patients. The reference range of SGPT is 12-40 u/l but because of the sensitivity of the kinetic method and mild elevation noticed in many nonspecific infections, 3 times the higher limit of the reference range, i.e. more than 120 u/l, was considered to be an indicator of liver disease. The clinical and biochemical profiles of these patients are given in Table 2. In another 30 patients, SGPT was found to be marginally raised (range, 40-120). Bilirubin

Table 1. Complications of severe *P. falciparum* malaria in 165 hospital patients

Complications	Male ($n=125$)	Female ($n=40$)
Cerebral malaria	20	11
Renal failure	22	8
Hypoglycaemia	6	3
Jaundice	27	6
Respiratory problem	4	2
Multiple complications	33	8
Others	13	2
Total	125	40

Table 2. Clinical and biochemical details of patients with hepatic involvement

Clinical details	Names of the patients				
	RL	JS	B	SA	MZ
Age/Sex	49M	23M	38M	28F	25M
Bilirubin (mg/dl) (total)	20	3.1	8.5	1.9	1.6
(conjugated)	13	1.8	6.0		
SGPT (u/l)	210	301	126	226	248
γGT (u/l)	23	76	8	22	17
AIP (u/l)	300	604	200	240	216
Protein (g/dl)	5.1	6.4	5.2	3.5	6.0
Albumin (g/dl)	2.2	3.6	2.5	1.2	2.0
Urea (mg/dl)	350	41	37	145	60
Creatine (mg/dl)	6.9	0.9	0.6	3.1	1
Plasma Glucose (mg/dl)	101	75	65	44	114
Hb (g/dl)	8	8	8.5	9	3.5
Complications	C	C	C	C&P	C
Outcome	D	R	R	D	R

RL, JS, B, SA, MZ—Abbreviated names of the patients; C—Cerebral; P—Pregnancy; D—Death; R—Recovered.

was raised above 2 mg% in 33 cases (range 2-20 mg/dl, median 3.2 mg/dl). Jaundice was diagnosed when total bilirubin exceeded 2 mg/dl. Unconjugated hyperbilirubinaemia was noticed in 22 patients (unconjugated fraction > 75% of total), while conjugated hyperbilirubinaemia (conjugated fraction > 50% of total) was encountered in 5 patients only. Of the latter, 3 had an associated increase in SGPT, two of whom also had raised AIP. The highest bilirubin level encountered was 20 mg/dl, with a conjugated fraction of 13 mg/dl.

Serum alkaline phosphatase was found raised in 17 patients (range, 307-624 u/l; median 361 u/l; reference range, 100-300 u/l in adults). One of them was pregnant and 5 were in adolescent age group, in whom serum AIP is known to be raised up to 3 times the normal. Gamma-GT was raised in 13 patients (range, 55-130 u/l; reference range, 11-50 u/l). Seven of them were chronic alcoholics, three of whom had consumed alcohol during the previous 2-3 days. Associated increase in AIP was noticed in 3 patients, suggesting biochemical cholestasis. Serum total protein and albumin were decreased significantly, the values being 5.73 ± 0.91 g/dl and 2.863 ± 0.651 g/dl respectively.

Needle necropsy specimens from liver were examined in 14 cases. In 11 patients, specimens showed only Kupffer cell hyperplasia with pigment deposition and without any evidence of hepatitis. In three cases there was heavy infiltration from the portal areas of inflammatory cells (predominantly mononuclear cells) and parenchymal oedema. In one patient (RL), patchy areas of lysis and necrosis of the hepatocytes were observed.

During the course of hospitalisation two patients with raised SGPT died, one of them, RL (49 M), was admitted with cerebral malaria, renal failure and jaundice. He was a known alcoholic. The patient was treated with quinine, blood transfusion, peritoneal dialysis and other supportive measures. His sensorium improved but renal condition and jaundice worsened. He died ultimately

on the 12th day of hospitalisation. The necropsy specimen of liver showed necrosis of the hepatocytes. The second case, SA (28 F), was a multigravida in the 2nd trimester. When admitted she was suffering from confusion and renal failure. She had pretreatment demonstrable hypoglycaemia which persisted in spite of infusion of 50% dextrose (50 ml) and maintenance therapy with 10% dextrose. (Plasma glucose was 14, 44 and 54 mg/dl). She aborted on the third day of hospitalisation and subsequently became comatose. She died on the 6th day owing to cerebral malaria.

DISCUSSION

Liver is supposed to be the first organ to be involved in malaria infection as parasite reproduction following the infective mosquito bite takes place in it. However, acute involvement of liver in our patients was comparatively low. Out of 165 patients, only 5 showed increased SGPT; the increase was only mild to moderate. *Vis-a-vis* acute viral hepatitis the extent of hepatic damage seemed to be extremely low. Hepatic involvement in a significant proportion of malaria patients reported earlier was based on nonspecific diagnostic tests like hepatomegaly, jaundice, levulose tolerance test, etc.[6,12,13]. Jaundice so commonly associated can be due to haemolysis frequently encountered in malaria. Unconjugated hyperbilirubinaemia was found in 22 of the 33 of our patients having jaundice. Similarly, AIP increases with age and in pregnancy. In fact, when AIP level is corrected for these conditions, only 11 of our patients had mild to moderate increase and in a large number of patients, increase of γGT level was associated with chronic alcohol intake.

Total protein and albumin were significantly reduced in our patients. Reduced albumin level is an indicator of chronic liver disease. Since albumin is one of the serum proteins with longest half-life, reduction in its blood level due to defective synthesis in the liver disease cannot be expected to take place until after several weeks. Therefore, there ought to be other reasons for decreased albumin levels in acute malaria. Extravasation of albumin from the vascular compartment is observed in infection and injury as an acute phase response[14]. In fact, transcapillary escape rate was found to be increased in malaria infection[15]. Hence decreased albumin levels encountered in our patients could have been due to negative acute phase response but not liver cell failure.

The histological lesions encountered in the liver necropsies are no different from those reported in earlier studies. Whereas Kupffer cell hyperplasia, pigment deposition and patchy parenchymal necrosis have been explained to a large extent, the mechanisms leading to mononuclear cell infiltration and intercellular oedema are not clear and need further elucidation.

In conclusion, hepatic involvement is not rare in falciparum malaria, but hepatic dysfunction is uncommon and the severity is also less in comparison to viral hepatitis. Acute hepatic necrosis appears to be a relatively uncommon complication of *P. falciparum* malaria.

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A Longitudinal Study to Monitor Chloroquine-Resistant *P. falciparum* Malaria in Bokajan and Manja PHC Areas of Karbi Anglong District, Assam

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In-vivo as well as *in-vitro* studies were carried out at regular intervals from 1979 to 1988 to monitor chloroquine resistance in *P. falciparum* in Bokajan and Manja PHC of Karbi Anglong district, Assam. The results showed no significant change in resistance status in the area. Intensified antivector measures, withdrawal of chloroquine pressure and prompt detection and treatment of *P. falciparum* cases with alternative drugs helped bring down the higher grades of resistant parasites by 1984, which however could not be maintained. The results of *in-vitro* tests correlated well with those of *in-vivo* tests. The Karbis seemed to have better immunity against the strain of *P. falciparum* than the non-Karbis residing in the area. However, there was no difference in the rate of sensitive and resistant cases detected amongst the two groups.

INTRODUCTION

Chloroquine-resistant *P. falciparum* malaria was first detected in the Bokajan PHC under Karbi Anglong district of Assam during 1973[1]. The Regional Office for Health and Family Welfare, Shillong, first conducted a study in 1979 covering areas of both Bokajan and Manja PHC of the district. Subsequently *in-vivo* studies to monitor the chloroquine sensitivity status were carried out in the same areas during 1982, 1984, 1986 and 1988. *In-vitro* microtests were also carried out in 1986 and 1988 in the same study areas. The observations made during these studies are presented in this paper.

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Study Area

The study area comes under the Manja and Bokajan PHC of Karbi Anglong district, Assam (Fig. 1). The area is hilly and inhabited by the tribal population group known as Karbi. About 30% of the population in the study area are Nepalese, who have settled here and work for the local people in the cultivation of rice, maize, etc. To and fro movement of the Nepali population between India and Nepal is very common. Other people living in the area are Assamese, Bengalis,

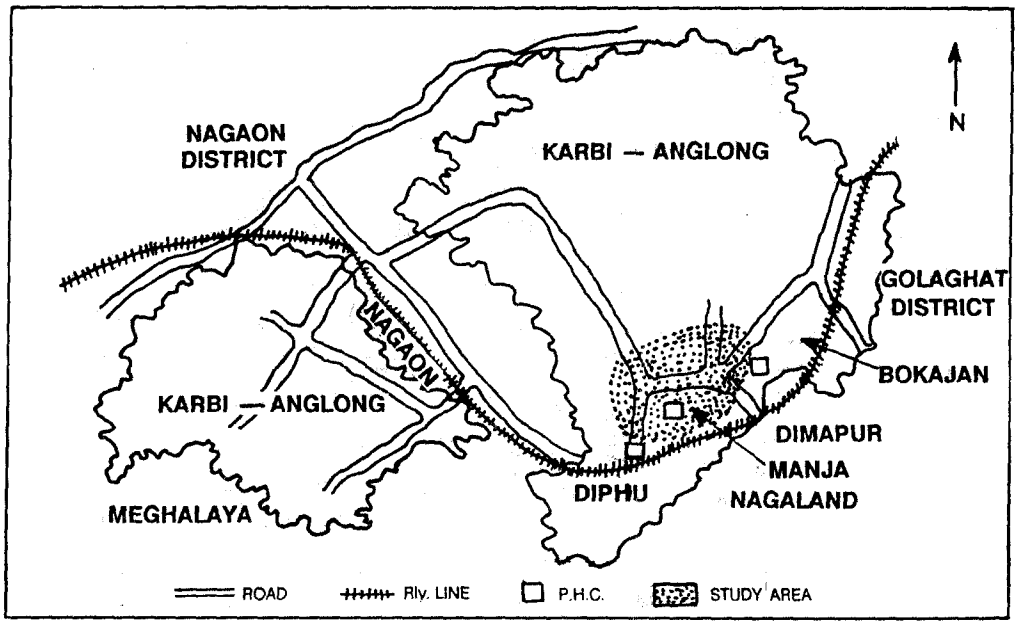

Fig. 1: Map of Karbi-Anglong district showing study areas under Manja and Bokajan PHCs.

Khasis, Garos, etc. Details of the study population are given in Table 1.

MATERIALS AND METHODS

In-vivo extended field tests in accordance with the WHO procedure[2] were conducted during 1979 (May-September), 1982 (April-July), 1984 (April-July), 1986 (June-September) and 1988 (August-September). The number of cases studied year-wise is as follows: 145 (1979), 43 (1982), 34 (1984), 104 (1986) and 29 (1988).

In-vitro microtests[3] were performed in a number of selected cases during 1986 and 1988, but only 8 and 3 tests respectively were successful.

The methodology for case selection and treatment were the same in all the years. Mass blood survey was carried out in the study villages, and the blood smears were quickly stained with JSB stain and examined. All the *P. falciparum* cases with an adequate number of asexual parasites were asked about the history of intake of antimalarial drugs. Urine was tested on Day 0 for

Table 1. Details of study population in Manja and Bokajan PHC

PHC	Population	Study villages	Population		
			Total	Karbis	Non-Karbis
Manja	34,884	17	5688	3472	2216
Bokajan	93,569	8	1516	911	605
Total	128,453	25	7204	4383 (60.84%)	2821 (39.16%)

Table 2. Results of mass blood survey in Manja and Bokajan PHC, Karbi Anglong district of Assam

Year	No. of blood smears collected	No. found positive				No. of cases selected	No. of cases with test completed
		Pf	*Pv*	Mixed	Total		
1979	2202	461	40	4	505	161	145
1982	2212	410	35	2	447	46	43
1984	1706	336	88	6	430	41	34
1986	1109	517	26	10	553	112	104
1988	673	207	18	5	230	31	29
Total	7902	1931	207	27	2165	391 (20.25%)	355 (18.38%)

chloroquine by Dill and Glazko test[4] and for sulfa drugs by lignin test[5]. All *P. falciparum* cases thus selected were taken up for study. Infants and pregnant women were not selected. The results of mass blood survey are given in Table 2.

The number of cases selected year-wise was 161 (1979), 46 (1982), 41 (1984), 112 (1986) and 31 (1988). These were put under treatment, but only 145, 43, 34, 104 and 29 cases in the respective years could be studied for a full period of 28 days.

Cases selected were administered chloroquine phosphate orally in the dosage of 25 mg/kg body weight on three successive days (Day 0 to Day 2). The drug was administered by the medical officer of the study team. Ingestion and retention of the drug were observed. Urine was again tested on Day 2 to determine excretion of chloroquine. The patients were observed daily from Day 0 for 7 days.

Duplicate blood smears were made immediately before drug administration on Day 0 and then daily up to Day 7. The cases were followed up for another 3 weeks during which blood smears were collected on Day 10, 14, 21 and 28. Follow-up blood smears were stained with Giemsa stain. One set of the blood smears was examined within 24 h and the other set sent to the reference laboratory, NMEP, Delhi, immediately for examination. The results received from the reference laboratory were taken as final.

Asexual parasite count was determined by counting the parasites against 300 WBC and multiplying by 25, the leucocyte count being arbitrarily taken as 7500/mm^3 of blood.

All the selected cases were epidemiologically investigated and it was found that the infection was of indigenous origin.

Both the local tribal (Karbis) and other population groups (non-Karbis) were studied as shown in Table 3.

The study areas were under two rounds of DDT spray since 1958. Since *P. falciparum* Containment Programme (PfCP) was first started in the latter part of 1977 in this district, spraying was done under better supervision. Even the flying squad stationed at Diphu was utilised to do mopping up wherever spray was not found adequate in the Manja and Bokajan PHC areas. A special round of spray in the study areas was given in the month of February 1981 to cut down transmission.

In the latter part of 1981, after the national drug policy was changed, Karbi Anglong district came under the long-acting sulfonamide and pyrimethamine therapy. Use of chloroquine was

Table 3. Number of Karbis and non-Karbis in the study population

Year	Total cases	Karbis	Non-Karbis
1979	145	38	107
1982	43	22	21
1984	34	9	25
1986	104	29	75
1988	29	13	16
Total	355	111 (31.27%)	244 (68.73%)

completely stopped. Presumptive treatment in domiciliary condition was given with amodiaquine. Presumptive treatment in hospitals was given with sulfapyrimethamine combination drugs. Radical treatment of the *P. falciparum* cases was given with sulfapyrimethamine combination drugs along with primaquine (45 mg) in a single dose.

Due to non-availability of required facilities, estimation of chloroquine concentration in blood of the persons under study could not be done.

No study so far has been done on the Karbi people to find the genetic factors, if any, responsible for malaria endemicity amongst them. This is necessary because though the tribal population in Assam is by and large of Mongolian origin, the origin of the Karbis is obscure[6]. It is not impossible that they are genuine autocthons.

For *in-vitro* micro test, blood from selected cases was put in the predosed chloroquine plates supplied by WHO. The plates were then sealed in a candle jar. The candle jar was then placed inside a portable incubator. The samples were incubated at 38°C for 30 h. Out of 46 (1986) and 12 (1988) samples put under incubation, only in 8 and 3 isolates respectively successful growth was observed.

Epidemiology

The incidence of malaria in the Manja and

Table 4. Year-wise incidence of malaria in Manja and Bokajan PHC

Year	B.S. collected/ examined	Positive	*Pf*	SPR	SFR
1979	38725	6804	4160	17.57	10.74
1980	18041	1600	1067	8.87	5.91
1981	23926	1934	1575	8.08	6.58
1982	26266	1793	1388	6.83	5.28
1983	20904	1389	1113	6.64	5.32
1984	29163	3093	2522	10.61	8.65
1985	28674	3705	2442	12.92	8.52
1986	37603	4370	2645	11.62	9.69
1987	24847	2345	2076	9.44	8.36
1988	33785	3378	3238	9.99	9.58

Bokajan PHC (combined) for the period from 1979 to 1988 is given in Table 4 as available from the Karbi Anglong district reports.

Total malaria as well as *P. falciparum* cases showed a gradual reduction from 1980 onwards till 1983. *P. falciparum* cases increased in 1984 by 126.6% over 1983 figures. *P. falciparum* cases further increased in 1986 by 49.26% over 1984 figures and maintained a high level up to 1988.

Entomology

Entomological studies in the Manja PHC areas were carried out by the zonal entomologist, Silchar, during 1982 to 1984. *An. philippinensis* was the main vector species found in the area. The vector was highly susceptible to both DDT and dieldrin with 100 % mortality at 4% DDT and 0.4% dieldrin concentrations. *An. minimus* and *An. balabacensis balabacensis* were not encountered. But these two vector species were found in the neighbouring areas of Dimapur in Nagaland[7], where *An. minimus* could be incriminated throughout the year.

RESULTS

The study population consisted of 60.84% local Karbis and 39.16% others. The breakup of the population into Karbis and non-Karbis is given in Table 3. 31.27% of Karbis and 68.73% of non-Karbis were among the test subjects. This suggests that more non-Karbis than the local tribal population suffer from malaria, which needs confirmation through further studies.

During the 5 studies, a total of 7902 blood smears were collected through mass survey. 2165 blood smears were found positive (SPR = 27.39), out of which 1931 were *P. falciparum* (SfR = 24.44). However, only 391 (20.25%) of the *P. falciparum* cases could be selected for *in-vivo* tests. Most of the remaining 79.75% of *P. falciparum* cases could not be selected because of prior antimalarial drug consumption. This shows easy availability and consumption of antimalarial drugs by the population as and when they suffer from fever. Another common reason for non-selection of cases was the parasite count in asymptomatic cases with less than 1000 asexual parasites per mm^3 of blood showing the presence of immunity in the population.

In-vivo Study

The resistance of asexual parasites to chloroquine was graded as per WHO[2]. The results are given in Table 5.

The results show that the sensitive cases varied from 31.03% in 1979 to 27.59% in 1988. There was

Table 5. Results of *in-vivo* studies in Manja and Bokajan PHC (1979 to 1988)

Year	Cases tested	Gradation of cases				Total resistant cases
		Sensitive	Resistant			
			RI	RII	RIII	
1979	145	45 (31.03)	76 (52.41)	13 (8.97)	11 (7.11)	100 (68.97)
1982	43	16 (37.20)	21 (48.84)	5 (11.63)	1 (2.33)	27 (62.80)
1984	34	10 (29.41)	24 (70.59)	—	—	24 (70.59)
1986	104	38 (36.53)	62 (59.62)	4 (3.85)	—	66 (63.47)
1988	29	8 (27.59)	16 (55.17)	1 (3.45)	4 (13.79)	21 (72.41)
Total	355	117 (32.96)	199 (56.06)	23 (6.48)	16 (4.50)	238 (67.04)

Note: Figures in parentheses indicate percentage.

Table 6. Group-wise classification of sensitive and resistant cases-population

Population group	Total cases tested	Sensitive	Resistant			Total
			RI	RII	RIII	
Karbis	111	36 (32.43)	65 (58.56)	6 (5.41)	4 (3.60)	75 (67.57)
Non-Karbis	244	79 (32.38)	136 (55.74)	17 (6.97)	12 (4.91)	165 (67.62)

Note : Figures in parentheses indicate percentage.

Table 7. MPCT and MPRT in the Manja and Bokajan PHC studies

Year	Sensitive and RI		RI	
	n	MPCT	n	MPRT
1979	121	2.95	76	16.55
1982	37	3.57	21	13.71
1984	34	3.70	24	12.04
1986	100	2.52	62	11.39
1988	24	2.53	16	15.13

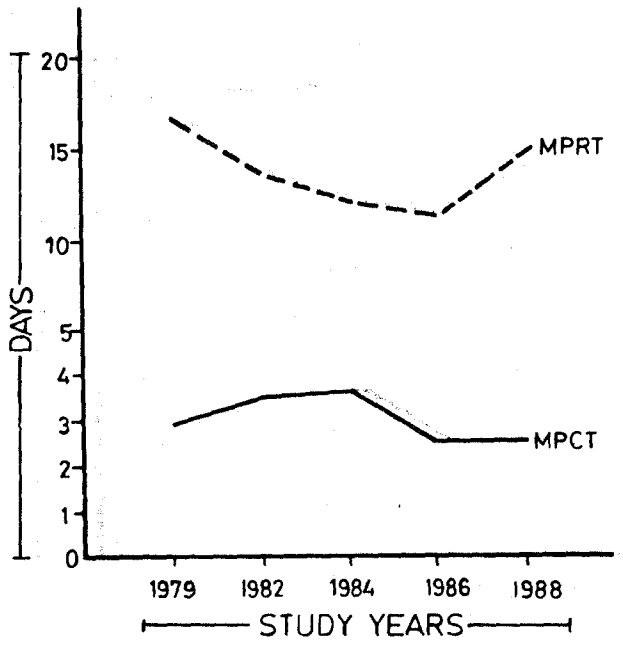

Fig. 2 : MPCT (S and RI cases) and MPRT (RI cases): 1979 to 1988.

an increase in the sensitive cases in 1982 (37.2%) and 1986 (36.53%). The variation in sensitive and resistant cases was statistically insignificant ($\chi 2 = 1.616$, $p > 0.05$, df 4).

Though the variation of sensitivity as a whole is insignificant, the increase of sensitivity by 17.76% in 1986 over 1979 figures is statistically significant at 1% level ($Z = 5.51$, $p < 0.01$, SEP = 0.9107).

The number of RII and RIII cases was reduced by 15.7% in 1982 over 1979 cases against the reduction of total resistant cases by 8.95%. However, in 1984, RII and RIII case were not found, though the total resistant cases increased by 12.4% over 1982 cases. In 1986, RII cases again reappeared, which increased by 347.79% in 1988 over 1986 cases, against 14.08% increase in total resistant cases. These variations in RII and RIII against RI were statistically very significant ($\chi 2 = 11.056$, $p < 0.02$, df 3).

An analysis of the sensitivity and resistance amongst the Karbis and the non-Karbis is given in Table 6. The table shows that there is practically no difference between the two groups so far as drug response was concerned.

Mean parasite clearance time for sensitive and RI cases (MPCT) and mean parasite recrudescence time (MPRT) of RI cases were worked out. These are given in Table 7.

The trend of MPCT and MPRT values is given in Fig. 2. As the RI level of resistance increases up to 1984, MPCT goes on increasing up to a maximum of 3.70. Simultaneously, MPRT goes on reducing and reaches a level of 12.04. However, in 1986, both MPCT and MPRT showed downward trends. In 1988 MPCT remained static but MPRT showed an upward trend. These variations confirm the instability in the resistance status in the area till now.

MPCT values for Karbis and other groups of population are as given in Table 8.

Table 8. MPCT values for Karbi and non-Karbis

Year	Karbis	Non-Karbis
1979	2.77	3.02
1982	3.43	3.63
1984	3.44	3.80
1986	2.22	2.63
1988	2.42	2.70

The MPCTs in the Karbis are slightly lower than in non-Karbis in all the years of study. This is probably due to a higher level of immunity in the Karbis than in the other population group amongst whom movement to and from outside is common.

In-vitro study

The results of *in-vitro* micro tests during 1986-88 are given in Table 9. In 1986, out of 8 isolates tested, 3 (37.5%) were sensitive and 5 (62.5%) were resistant. The percentage of sensitive and resistant cases found *in vitro* were similar to the *in-vivo* results of 36.53% sensitive and 63.47% resistant cases. In 1988, only 3 tests were successful, of which 2 (66.67%) were sensitive and 1 (33.33%) was resistant cases. In contrast, sensitive and resistant cases in *in-vivo* tests were 27.59% and 72.41% respectively.

All the 11 cases tested by *in-vitro* method were also tested for chloroquine sensitivity *in vivo*. Table 10 shows the comparative results of the tests.

In 8 (77.73%), out of the 11 isolates tested *in vitro*, the response correlated with that in the *in-vivo* study. This further brings confidence to the resistance level detected *in vivo*.

The results of *in-vitro* tests are shown in Fig. 3. The number of isolates successfully tested were very low. However, percentage of resistant cases has shown correlation with cut-off values.

Table 9. Results of *in-vitro* microtests (1986 and 1988)

Year	Test No.	Schizont count per test well of								Remarks
		K	1 pmol	2 pmol	4 pmol	5.7 pmol	8 pmol	16 pmol	32 pmol	
1986	1	31	23	17	8	2	0	0	0	R
	2	15	10	8	2	0	0	0	0	S
	3	28	20	12	3	0	0	0	0	S
	4	34	30	26	8	3	0	0	0	R
	5	27	22	17	9	5	0	0	0	R
	6	35	28	20	15	8	4	0	0	R
	7	20	16	12	6	0	0	0	0	S
	8	36	30	19	11	7	2	0	0	R
1988	1	139	86	61	28	0	0	0	0	S
	2	99	78	45	13	2	0	0	0	R
	3	92	86	79	18	0	0	0	0	S
Mean 1986		28	22	16	8	3	1	0	0	
Schizont count 1988		110	83	62	20	1	0	0	0	

R—Resistant; S—Sensitive.

Table 10. Comparative results of *in-vivo* and *in-vitro* study in 11 *P. falciparum* cases

Year	Sl. No.	Case No.	Age	Sex	Results	
					In vitro	*In vivo*
1986	1	VD-19	7	F	Resistant	Resistant (RI)
	2	VD-29	7	M	Sensitive	Sensitive
	3	VD-30	6	F	Sensitive	Sensitive
	4	VD-31	10	M	Resistant	Sensitive
	5	VD-32	10	F	Resistant	Resistant (RI)
	6	VD-34	12	F	Resistant	Resistant (RI)
	7	VD-35	6	M	Sensitive	Resistant (RI)
	8	VD-42	30	F	Resistant	Resistant (RI)
1988	9	VMC-2	25	M	Sensitive	Sensitive
	10	VMC-3	3	F	Resistant	Resistant (RI)
	11	VMC-5	5	M	Sensitive	Resistant (RI)

DISCUSSION

Monitoring of the extent and degree of resistance in *P. falciparum*-resistant areas at regular intervals is of great importance for (i) evolving adequate treatment schedules and (ii) taking active measures to prevent and contain the spread of resistance. In our study, monitoring was done at

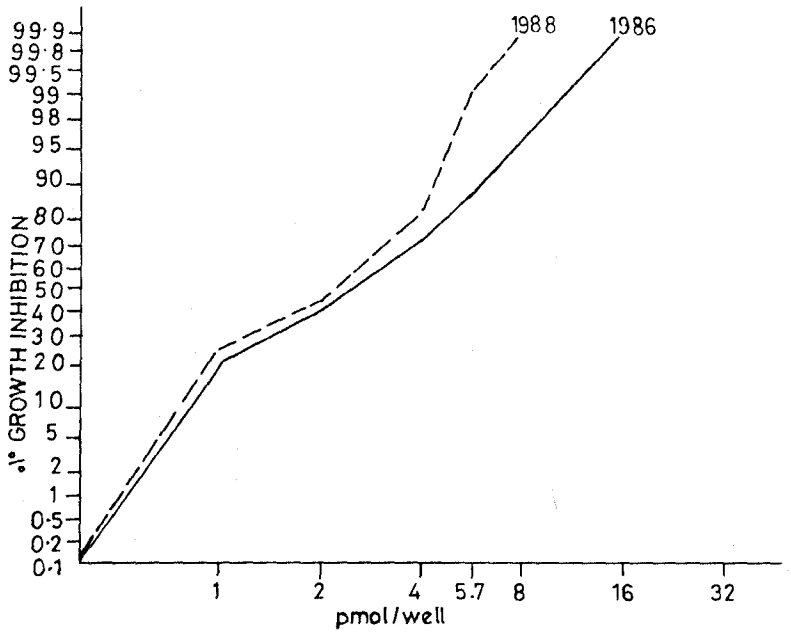

Fig. 3 : Percentage of inhibition in chloroquine *in-vitro* tests (1986 and 1988).

two-yearly intervals in 25 villages of Manja and Bokajan PHC, Karbi Anglong district, Assam, between 1979 and 1988, ensuring that the cases tested were of indigenous origin from the area.

The study population comprised both the local tribals (Karbis) and others (non-Karbis). It was observed that more persons amongst the non-Karbis residing in the area suffer from *P. falciparum* malaria infection. This is probably due to lower immunity level in the non-Karbis in comparison with the Karbis due to frequent movement of the former outside the area. Whether any genetic factor is also responsible for the lower incidence amongst the Karbis is not yet known. A large number of people in the northeastern region of India are vulnerable to the abnormalities of haemoglobin and G-6-PD[8]. Hbs is common among the migrant labour populations in tea-gardens, whereas HbE is prevalent among the local inhabitants. No such study was carried out amongst the Karbis.

In the mass surveys, slide positivity rate (SPR) and slide falciparum rate (SfR) were much higher than in the state reports. Many asymptomatic carriers with low parasite count were found in the mass surveys, which indicates a high level of immunity in the community.

Chloroquine was stopped in Karbi Anglong district since 1981 and amodiaquine was started for presumptive treatment by the field workers. These drugs are however available in the markets and many people use them as and when they have fever, because of poor communication and difficulty in reaching the health centres quickly.

During the nine years of monitoring, there was no significant variation in the resistant status of *P. falciparum* to chloroquine. Rosario *et al.*[9] showed that resistant parasites possess biological advantage over sensitive ones. So it is expected that continued chloroquine pressure associated with high malaria transmission will help in gradual in-

crease in the level and grade of resistance. Withdrawal of chloroquine from treatment schedule, intensive spray operation followed by case detection and prompt radical treatment with alternative drugs under strict supervision, helped in controlling the level of resistance. In fact, foci of RII and RIII levels of resistance were actually eliminated by 1984. However, the same situation could not be maintained because of various operational problems. The reduction in RII and RIII levels of resistance reappeared by 1988. These variations in levels of resistance were statistically very significant.

To explain the unstable nature of resistance levels, we need to have a better understanding of the parasite genetics mechanism. Walliker[10] has stated that the genetic basis of resistance to a given drug, and the ability of such resistant genes to recombine, have important implications. Widespread use of a low dose of a drug, such as chloroquine, is likely to select resistant forms, which may be due to recombination of different genes of different parasites. Mosquito transmission of mixed parasites could bring more than one gene together in a single parasite, thus producing a higher level of resistance. Conversely, if the drug pressure is released, hybridization between highly resistant and sensitive parasites is likely to occur, resulting in the segregation of different resistant genes from one another and thus lowering the resistance level.

Reappearance of the higher levels of resistance in the area was probably due to increased intensity of transmission on account of failure to maintain the highest degree of control measures. To contain chloroquine-resistant *P. falciparum*, once it is well established in an endemic area, the control measures should be constantly kept intensified even when the drug pressure is removed. In this area, the drug pressure was only partially removed as chloroquine was widely used by people due to free availability in the region.

The study showed lower incidence of *P. falciparum* infection amongst the Karbis than amongst the non-Karbis, but there was practically no difference in sensitivity and resistance status between the two groups of population.

Mean parasite clearance time (MPCT) depends upon the degree of sensitivity of the parasite to the drug and also the level of immunity in the host. As there is no difference in parasite sensitivit of chloroquine, a lower MPCT value amongst the Karbis indicates a higher level of immunity in the group as compared to the non-Karbis.

Mean parasite recrudescence time (MPRT), on the other hand, shows a degree of resistance. As the resistance increases, MPRT values go on reducing. Fluctuations in the MPRT values indicate instability in the resistant status in the area till now.

The similarity in the results of *in-vitro* and *in-vivo* studies during 1986 and also correlation of results in 72.3% of the 11 isolates tested *in vitro*, bring confidence to the resistance level detected in *in vivo*.

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Matrix Based Approach for Identification of Indian Anophelines

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Mosquitoes belong to class Insecta, order Diptera, suborder Nematocera, family Culicidae and subfamilies Anophelinae, Culicinae and Toxorhynchitinae. Genus *Anopheles* of subfamily Anophelinae and genera *Culex*, *Aedes* and *Mansonia* of subfamily Culicinae are medically important as they transmit important communicable diseases such as malaria, filaria, dengue, haemorrhagic fever, Japanese encephalitis, yellow fever and several viral diseases. Mosquitoes of subfamily Toxorhynchitinae do not transmit any disease as they do not suck blood. The discovery that anophelines transmit malaria by Sir Ronald Ross in 1898 created and immense interest among scientists for faunistic studies. Gradually the need for the identification of mosquito species was realized. The first key in the form of a wall-chart was published by Central Malaria Bureau, Kasauli in 1912. Subsequently several keys were published by Sinton and Covell (wall-chart, 1916), Strickland and Chowdhary[1]; Christophers *et al.*[2]; Christophers[3]; Puri[4,5]; Wattal and Kalra[6], and Das *et al.*[7] A monograph by Nagpal and Sharma[8], under publication, also deals with identification of Indian anophelines. Out of the above-mentioned published identification keys, only 2 are commonly used, namely the keys by Puri[5]; and Wattal and Kalra[6]. These keys deal with the identification of 40 mosquito species against the present fauna of 56 except the key by *Das et al.*[7], which describes 54 species. Also none of the keys gives any account of morphological variations. One problem in these couplet keys is that for each character the user has to jump over different pages and at any point a slight oversight may result in misidentification of the species.

We have developed a matrix based approach that can easily and unmistakably help in the identification of all the 56 species of Indian female anophelines including the reported variations. A matrix of size $m \times n$ is nothing but an arrangement of data into m rows and n columns. It represents a system in a compact manner, thus making the manipulations easier; it has found applications in almost all the fields. To quote a few, information theory, operational research, design of experiments, social sciences, medical sciences[9], renewable sources of energy[10], forest management[11], population studies[12,13] etc.

Two matrices, one of size 22 x 15 for subgenus *Anopheles* and another of 34 x 21 for subgenus *Cellia*, have been constructed. The user is required to fill up a pro forma and tally his code string with the matrix entries. Pro forma 1 is for subgenus *Anopheles* and pro forma 2 for subgenus

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Cellia (Annexure 1). To fill in the pro forma, the user should first see the number of pale spots on the costa, subcosta and vein R_1 of mosquito using a hand lens (x 25) or dissecting binoculars. If the total number of pale spots is less than 3 or if the wing is completely dark, the mosquito belongs to subgenus *Anopheles,* otherwise to subgenus *Cellia*. After selecting the form the user has to look for the identification features on the mosquito in the sequence as given in the pro forma and fill in the form accordingly. Each form is divided into 4 sections, namely Head, Wing, Leg and Abdomen, which have been sub-divided further to help in the identification. After the completion of form, the codes are assembled on the top page of the pro forma to form the code string. The user then has to go for sequential search of the coded string he has prepared, in the matrix given in Annexure 2.

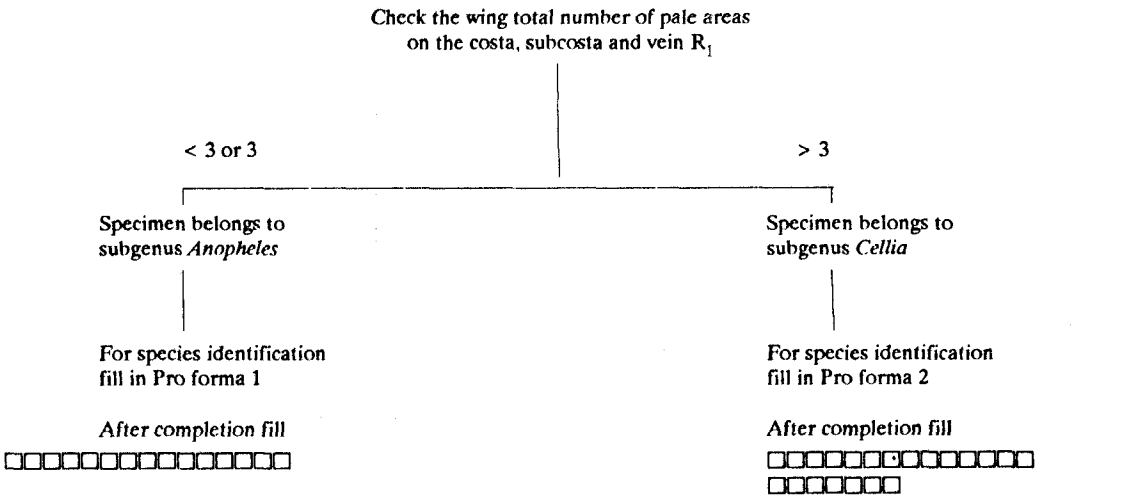
Suppose the mosquito to be identified belongs to subgenus *Cellia*. Mark the codes of the appropriate mosquito features in Pro forma 2 as seen in the microscope and assemble on the first page so as to construct the code string and match this with the strings given in Appendix 2 for species confirmation. Let the string of the codes thus formed be 511111014231322222233, matching this in Annexure 2, then the species identified is *An. aconitus*.

If the user has access to a computer, then this search could be done using the software developed for this purpose. Once the software is loaded it asks for the string and when the string is entered mosquito is identified. Wrong entry of data at any level i.e. either in the pro forma or in the computer, is also cautioned by the computer as 'Illegal Input'.

The added advantage with computer based identification is that if some of the features (up to 4) do not match with type form string, then it lists all possible mosquito species with the variations. If these variations are reported, then details of such variations are also displayed. This approach is new, has been field-tested and gives accurate and quick results. By using this approach the identification becomes very easy and the user is fully familiarized with all the characters of the species.

Annexure 1

Identification of female *Anopheles at the species level**



*Maxillary palpus long, slender and nearly equal to the length of proboscis.

Pro forma 1

For identification of species belonging to subgenus *Anopheles*

1. Head menu

(i)	Maxillary palpus	Completely dark	1	Completely dark but slightly shorter than proboscis	2	Palpus dark but with very small bands at the joints	3	Tip of the palpus pale (four-banded palpus)	4

2. Wing menu

(i)	Pale areas on wing veins	Present	1			Wing completely dark	2		
(ii)	Inner costa	Interrupted	1	Completely dark	2	With pale scales	3		
(iii)	Fringe spots	Present	1	Absent	2				
		On [ ]							
		(See Appendix)							
(iv)	Size of the basal dark area on vein CU (as compared to stem)	3/4 of the stem	1	1/2 of the stem	2	1/3 of the stem	3	1/4 of the stem	4
		1/6 of the stem	5	Stem completely dark	6	Stem with white and black scales	7		
(v)	Pale spots on the outer half of anal vein	Present	1	Absent	2				

3. Leg menu

(i)	Banding on the foreleg tarsomeres	Present	1	Absent	2
(ii)	Banding on hindleg tarsomeres	Present	1	Absent	2
(iii)	Pale band towards the end of femur and the termination of tibia	Present	1	Absent	2
(iv)	Pale bands on the hind femur	Present	1	Absent	2
(v)	Apex of femur with prominent tuft of white and black scales	Present	1	Absent	2

4. Abdomen menu

(i)	Scales on sternite segments 2 to 6	Present	1	Absent	2
(ii)	Tuft of dark scales on segment 7	Present	1	Absent	2

Pro forma 2

For identification of species belonging to subgenus *Cellia*

1. Head menu

(i) Maxillary palpus — Four banded [1] | Apical pale band nearly equal to the pre-apical dark band or Pre-apical dark band 1/4 or 1/5 of the apical pale band [2] [3] | Apical & subapical pale band separated by a dark band — Speckling on palpus — Present [4] / Absent [5] | Tip dark [6] | Any other [7]

(ii) Proboscis — (a) Size: Equal to the fore-femur [1] / Longer [2] / Shorter [3]

(b) Flavescent: Present [1] / Absent [2]

2. Wing menu

(i) Apex: Pale [1] / Dark [2] / With black areas [3]

(ii) Costa — (a) Base: Dark [1] / Pale [2]

(b) Inner costa: Interrupted [1] / Completely dark [2] / Completely pale [3]

(iii) Fringe spots present

On [ ]

(See Appendix)

(iv) Veins

Wing vein R_1

(a) Dark spots — If present then no. of spots. (b) Completely dark [5] (c) Completely pale [6]

One [1] Two [2] Three [3] More than three [4]

Wing vein R_{4+5}

(a) Dark spots — If present then no. of spots. (b) Completely dark [5] (c) Completely pale [6]

One [1] Two [2] Three [3] More than three [4]

Wing vein CU_1

(i) (a) Dark spots — If present then no. of spots. (b) Completely dark [5] (c) Completely pale [6]

One [1] Two [2] Three [3] More than three [4]

contd.

Pro forma 2 (*contd.*)

For identification of species belonging to subgenus *Cellia*

(ii) At the bifurcation of wing vein CU area is

(1) Dark [1]

(2) Pale [2]

Anal vein

(a) Dark spots [5] (b) Completely dark [6] (c) Completely pale

If present then no. of spots.

One [1] Two [2] Three [3] More than three [4]

3. Leg menu

(i) Speckling on tibia & femur — Present [1] Absent [2]

(ii) Banding on foreleg tarsomeres — Present [1] Absent [2]

(iii) Hind tarsomeres 5,4,3 — Banded [1] Completely dark [2] Small bands [3] Completely pale [4] Any other [7]

Pale band at the junction of tarsomeres 2 & 1

Present [5] Absent [6]

Size of the band

Small [4] Big [5]

(iv) A big white spot at the junction of femur and tibia of hind leg — Present [1] Absent [2]

4. Abdomen menu

(i) Tuft of pale scales on the dorsum — Present [1] Absent [2]

(ii) Tuft of black scales on the venter — Present [1] Absent [2]

(iii) Golden scales/hair on the dorsum — Present [1] Absent [2]

On all the segments [1] Restricted to last few segments [2]

(iv) Scattered pale scales on the venter — Present Absent [2]

On all the segments [1] On the apical segments only [2]

Appendix

Codes to be used for fringe spots

00	Absent on all veins
01	On all veins
02	On all veins except R_2 & R_3
03	On veins R_2, R_3, R_{4+5}, M_1, M_2, CU_1 & between CU_2 & Anal
04	On veins R_2, R_3, M_2 & CU_1
05	On vein R_2
06	On veins R_2, R_3, R_{4+5}, M_1, M_2, CU_1, CU_2 & Anal
07	On veins R_2, R_3, R_{4+5}, M_1, M_2, CU_1 & CU_2
08	On all veins except anal
09	On veins R_2, R_3, R_{4+5}, M_1, CU_1, CU_2 & Anal
10	On veins R_2, R_{4+5}, M_1, M_2, CU_1 & CU_2
11	On veins R_2, M_1, M_2, CU_1 & CU_2
12	On veins R_{4+5}, M_1, M_2, CU_1, CU_2 & Anal
13	On veins R_2, R_3, R_{4+5}, M_1, M_2, CU_1 & Anal
14	On veins R_3 & Anal
15	On veins R_1 & R_2
16	On veins CU_2 & Anal
17	On veins R_1, R_2, R_3, R_{4+5} & CU_2
18	On veins R_1, R_2, R_3, R_{4+5}
19	On veins R_{4+5} & CU_2
20	On veins R_{4+5}, M_1 & CU_2
21	On veins R_1 & R_{2+3}
22	On veins R_1, R_{2+3}, R_2 & R_{4+5}
23	On veins R_2, R_{4+5}, M_1 & CU_2
24	Present on veins .....

R_1 = vein 1; R_{2+3} = Vein 2; R_2 = vein 2.1; R_3 = vein 2.2; R_{4+5} = vein 3; M = vein 4; M_1 = vein 4.1; M_2 = vein 4.2; CU = vein 5; CU_1 = vein 5.1; CU_2 = vein 5.2; Anal = vein 6.

Annexure 2

Codes for mosquito identification

Subgenus *Anopheles*

(1) *An. umbrosus*	111121112222222
(2) *An. roperi*	111122412222222
(3) *An. lindesayi*	112115622221222
(4) *An. barbirostris*	113119711122211
(5) *An. ahomi*	113120711122221
(6) *An. barbumbrosus*	113123711122221
(7) *An. barianensis*	122200622212222
(8) *An. aitkenii*	122200622222222
(9) *An. bengalensis*	122200622222222
(10) *An. insulaeflorum*	122200622222222
(11) *An. pinjaurensis*	122200622222222
(12) *An. culiciformis*	222200622222222
(13) *An. sintoni*	222200622222222
(14) *An. annandalei*	311115622222122
(15) *An. interruptus*	311115622222122
(16) *An. gigas*	311216521121222
(17) *An. sinensis*	412117511122222
(18) *An. argyropus*	412118111122222
(19) *An. peditaeniatus*	412118311122222
(20) *An. crawfordi*	412118511122222
(21) *An. nigerrimus*	413117211122222
(22) *An. nitidus*	413117411122222

Subgenus *Cellia*

(23) *An. kochi*	111111014332311122133
(24) *An. tessellatus*	111111064441412222233
(25) *An. karwari*	112111014332221122233
(26) *An. pulcherrimus*	112121014332321521233
(27) *An. balabacensis* *	112211034442411112233
(28) *An. dirus**	112211034442411112233
(29) *An. elegans*	122111134442411112233
(30) *An. pseudojamesi*	212111014331311622233
(31) *An. subpictus*	212111014332221122233
(32) *An. philippinensis**	212111014332321422232
(33) *An. nivipes**	212111014332321422232
(34) *An. pallidus*	212111014332321622231
(35) *An. culicifacies*	212111044521222222233

contd.

Annexure 2 (*contd.*)

Codes for mosquito identification

Subgenus *Cellia*	
(36) *An. jeyporiensis*	21211106423132132233
(37) *An. sundaicus*	2121110643322111223 3
(38) *An. sergenti*	212111084521222222233
(39) *An. moghulensis*	21211208423132122223 3
(40) *An. jamesii*	2121210143323116222 13
(41) *An. annularis*	212211124331321522233
(42) *An. fluviatilis*	2122120742212222222 33
(43) *An. vagus*	312111014332221122233
(44) *An. splendidus*	412111014431311422233
(45) *An. stephensi*	4122210642323122222 33
(46) *An. aconitus*	511111014231322222233
(47) *An. varuna*	51111208432122222223 3
(48) *An. minimus*	511211104331222222233
(49) *An. willmorei*	51211101432221112221 3
(50) *An. maculatus*	512111014322211122223
(51) *An. theobaldi*	51211101432221112223 3
(52) *An. pseudowillmori*	51211101432221112223 3
(53) *An. majidi*	51211101433122112223 3
(54) *An. turkhudi*	612111114321222222233
(55) *An. dthali*	61211205455152222223 3
(56) *An. multicolor*	612112084232322222233

* Presector dark mark on vein R_1 not extending basally up to humeral dark mark

An. balabacensis
An. philippinensis

* Presector dark mark on vein R_1 basally extending up to humeral dark mark

An. dirus
An. nivipes

The software can be obtained free of cost on request by sending 5¼ or 3½ inch floppy to the Director, Malaria Research Centre, Delhi.

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SHORT NOTES

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Breeding Habits of *Anopheles stephensi* Liston in an Area of Calcutta

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Perennial transmission of malaria has become a common phenomenon in the city of Calcutta. Reports from the Calcutta Metropolitan Urban Health Organisation of the Government of West Bengal indicate that malaria strikes more than 15000 people of this metropolis every year. During July 1986 to June 1991, a total of 79,055 malaria cases were detected in Calcutta. The incidence of malaria cases was quite low during winter (November-February, with 2% in January); it started increasing (3.4%) with the onset of summer (March-June) and reached its highest peak (18.3%) in August during the rainy season (July-October). *Anopheles stephensi* is the incriminated vector[1-5]. Anti-vector measures by chemical insecticides through the agencies of the Government of India, Government of West Bengal and the Calcutta Municipal Corporation are not serving the purpose to a desirable extent. Antimalarial drug chloroquine is losing its effectiveness against *Plasmodium falciparum*.

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The need has arisen to adopt a long-term control strategy against the larval population of *An. stephensi* for effective containment of malaria in Calcutta for which detailed knowledge about the larval habitats of this vector species is very essential. Available reports on this aspect are quite old, neither are they adequately informative[6-11]. This paper deals with a year-long surveillance of *An. stephensi* larvae in and around a fixed number of human dwellings in an area of persistent malaria transmission in South Calcutta, called Chetla (area 6.5 km^2). The area was characterized by 1-3 storeyed brick-built houses, some of which were very old and some newly constructed. On the ground floor of almost all houses, 2-3 masonry tanks (*chowbachchas*) were found generally within roofed structures, but masonry tanks outside the rooms (within premises) were also not uncommon. Temporary hutments (*jhupries*) and cattlesheds were present here and there in patches.

Extensive search for *An. stephensi* larvae in Chetla area began quite a few months before the surveillance was started. *An. stephensi* larvae were detected in different types of domestic containers exclusively in and around brick-built houses (Unpublished report of the Vector Control Depart-

ment, CMC, 1990). Hence, we selected 100 brick-built houses in the area at random to study the breeding habits of this vector species throughout the year. All types of water containers in and around the selected houses were searched for *An. stephensi* larvae once a month from July 1990 to June 1991. Out of three methods, i.e. dipping, pipetting and netting as suggested by WHO[12], only the former two were used for collecting mosquito larvae.

The larvae collected were brought to the laboratory for identification. Entomological indices, i.e. house index, container index, and 'Breteau index', commonly used for sampling larval population of *Aedes aegypti*, were also calculated for *An. stephensi* larvae every month, as both are stenotopic species and prefer to breed pre-dominantly in domestic containers[13]. The number and type of water containers searched and those found positive for *An. stephensi* larvae both indoors and outdoors were noted every month.

During July 1990 to June 1991, altogether 18 types of water containers were searched for *An. stephensi* larvae and all were found positive. Taken as a whole, 4.4% (341) of the containers contained *An. stephensi* larvae. Masonry tanks comprising 42.8% of the breeding containers were the major breeding source of this vector species. The percentage distribution of some other important categories of *An. stephensi* breeding containers were : earthen pitchers (14.3), tin drums (6.7), tin cans (6.7), plastic flower vases (6.1), earthen barrels (3.8), earthen flower pots (3.8), plastic buckets (3.5), overhead tanks (3.5), glass

Table 1. Larval habitats of *Anopheles stephensi* in and around fixed 100 houses in Chetla area in Calcutta (July 1990 to June 1991)

Type of breeding habitat	No. searched		No. positive			
	Indoor	Outdoor	Indoor		Outdoor	
			No.	%	No.	%
Masonry tank	2970	287	109	3.7	37	12.9
Earthen pitcher	425	130	20	4.7	29	22.3
Tin drum	453	125	10	2.2	13	10.4
Tin can	175	239	9	5.1	14	5.8
Flower vase (plastic)	429	–	21	4.9	–	–
Earthen barrel	315	95	4	1.2	9	9.5
Earthen flower pot	–	240	–	–	13	5.4
Plastic bucket	390	76	5	1.3	7	9.2
Overhead tank	–	366	–	–	12	3.3
Flower vase (glass)	207	–	12	5.8	–	–
Iron bucket	212	–	5	2.3	–	–
Bottle	–	110	–	–	3	2.7
Coconut shell	–	58	–	–	3	5.2
Unused tyre	–	134	–	–	2	1.5
Porcelain cup	–	17	–	–	1	5.9
Glass jar	–	144	–	–	2	1.4
Battery box	–	133	–	–	1	0.7
Total	5576	2154	195	3.5	146	6.8

Table 2. House index, container index and Breteau index of *Anopheles stephensi* in fixed 100 houses in Chetla area in Calcutta (July 1990 to June 1991)

Month	No. of containers searched	House index (%)	Container index (%)	Breteau index	Malaria incidence (%) during July 86 to June 91 (79,055)
Jul 1990	765	15	5.3	41	12.4
Aug	1139	14	5.0	58	18.3
Sep	618	16	5.1	32	15.1
Oct	575	11	4.1	24	12.4
Nov	666	12	2.7	18	8.9
Dec	396	14	3.7	15	3.8
Jan 1991	363	10	3.5	13	2.0
Feb	444	11	3.6	16	2.1
Mar	584	13	4.2	25	3.4
Apr	667	14	4.6	31	5.4
May	687	14	4.2	29	7.1
Jun	826	15	4.7	39	8.8

House index — % of houses and their premises with breeding containers; Container index — % of containers with larvae; Breteau index — No. of breeding containers per 100 houses.

flower vases (3.5), etc. The presence of *An. stephensi* larvae in a great variety of containers points to the versatility of the species in respect of its breeding habit. Some of the containers, i.e., earthen flower pots, overhead water reservoirs, bottles, coconut shells, unused tyres, broken glass jars, porcelain cups and empty battery boxes, were exclusively outdoor ones. Except for overhead water reservoirs, the containers became water-filled during monsoon downpour and a few of them supported *An. stephensi* breeding. Flower vases (both glass and plastic) and iron buckets were found only indoors with 5.2% (33 out of 636) and 2.3% (5 out of 212) positive respectively. Though the prev-alence of water containers, i.e. masonry tanks, earthen pitchers, tin drums, tin cans, earthen barrels, plastic buckets and iron frying pans, is considerably higher indoors, the proportion of each of them positive for *An. stephensi* larvae was significantly higher outdoors (Table 1), thereby indicating that the vector species had a greater preference for outdoor water bodies than indoor ones.

The percentage of houses with *An. stephensi* breeding containers (i.e. house index) varied from 10 in January to 16 in September. The average number of breeding containers per positive house was lowest (1.1) in December and highest (4.1) in August. House index remained more or less the same throughout the year owing to constant presence of some types of water containers, i.e. masonry tanks, earthen pitchers, flower vases, tin drums, tin cans, etc. which supported *An. stephensi* breeding throughout the year in varying proportion. Variation in container index (2.70-5.30) was also not very marked, which might be correlated with the multiplicity of domestic water containers. Thus, both house index and container index remained uniform throughout the year and failed to explain the fluctuation in the density of *An. stephensi* larval population. But Breteau index, i.e. number of breeding containers per 100 houses, showed a wide-range variation. The index was lowest (13) in January, started increasing (25) with the onset of summer (March) and reached its highest peak (58) in August (Table 2), the month

of highest malaria transmission in Calcutta. The Breteau index has been recognized as the most suitable sampling device for the larval population of *Ae. aegypti*[14]. Our study points out that this index can also be used as an effective sampling device for *An. stephensi* larval population.

That the water storage practice among the city dwellers is mainly responsible for *An. stephensi* in Calcutta has been well demonstrated, and this practice should be stopped by generating massive public awareness. Besides, a massive effort for the reduction of breeding sources of the vector species, preferably through the implementation of bye-law, is also very essential for bringing an effective solution to the age-old problem of malaria in this metropolis.

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Report of Three Cases of *P. falciparum* Showing Moderately High Parasitaemia

NEERU SINGH*, M.M. SHUKLA* and N. VALECHA†

Malaria continues to be a major threat to populations living in forested tribal belt of Madhya Pradesh despite implementation of vigorous control measures by NMEP. The state has a total population of 5.22 million, out of which 1.20 million are tribals, who are the main inhabitants of forests. The total forest cover is 75% of the geographical area of the state (4,42,841 sq km). The problems of forest villages are unique in nature and containment of malaria in such situations poses a great difficulty. Transmission is generally heavy in the presence of more than one vector species, a situation further compounded by the emergence of chloroquine resistance in *P. falciparum*. From January to December 1991, 1325 *P. falciparum* cases were examined and in 30, high *P. falciparum* infection was found during routine examination (parasitaemia was more than 10%). This paper reports the results of blood smear examination of two cases with high parasite density from Jabalpur and one case from Delhi.

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During routine epidemiological surveys undertaken in the forest of Mandla district, a very high density of *P. falciparum* parasite was found in two patients. Data on fever history, drug intake and movement outside the village were recorded. Thick and thin blood films were made from finger pricks; air-dried thin films were fixed with methanol and were stained with Giemsa for examination under microscope. Parasitaemia was counted in 100 thick films and multiple infections were studied in thin films among 5,000 RBC. *In-vivo* test for chloroquine resistance was carried out according to Rickman's simplified test[1]. According to this procedure, only two follow-up blood examinations are required and an infection can be declared resistant as early as the second day of drug administration. The patient was given chloroquine orally (25 mg/kg). The day of blood smear collection and drug administration was taken as Day 0 and asexual parasite density per mm^3 of blood recorded. Excretion of chloroquine in the urine to ensure absorption of chloroquine was monitored in the blood by Dill and Glazko test[2].

Case No. 1: A male Gond tribe patient, age 35 years, was a resident of Kukra village, situated in the deep forest 25 km from the Primary Health Centre. The village is in the foothills of teak and

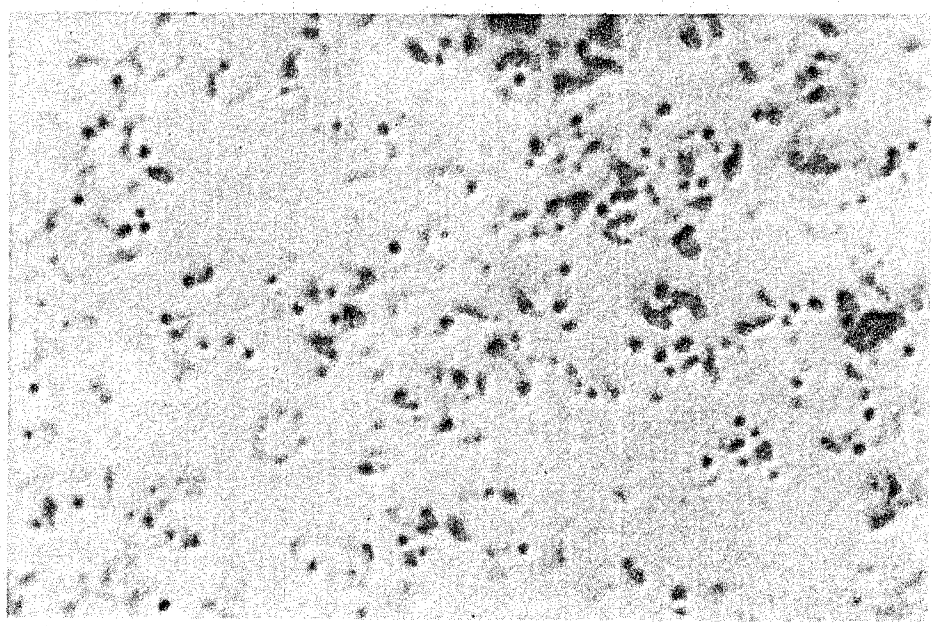

Fig. 1: Heavy density of *P. falciparum* parasites in thick smear (× 2000).

sal forests surrounded by a network of streams from the hills. On 16 July 1991 his blood smear was prepared and the information available was that he had high fever for three days with chills and rigor. He went to a village quack and was under his treatment for pneumonia. He did not take presumptive treatment. Because of heavy rains, the village became inaccessible and he could not be given antimalarials. He died 5 days later, on 21 July 1991. The blood smear showed only rings and young trophozoites of *Plasmodium falciparum*. Several erythrocytes were seen which contained 4-5 parasites and one red cell contained seven parasites. The parasitaemia was 12,86,100/mm^3 (Fig. 1) and rate of multiple infection was 7%. Several atypical forms were seen, i.e. in many ring forms there were four chromatin granules. One to three double chromatin rings in a single host cell were also observed. Marginal and tenue forms were scattered throughout the film. No other family member had malaria at that time.

Case No. 2: The patient (boy, 12 years) belonged to Gond tribe. The blood smear was made on 20 August 1991 from a peripheral village Ramtila. He had severe headache and fever (104°F) with shivering for the last 24 h. The parasitaemia was 7,84,791/mm^3. Maurer's dots were thicker, coarse and scattered in the host cell. Many red blood cells contained four, five or even six parasites. They were located at the periphery or arranged in a row in the host cell. Besides, 4 or more chromatin granules were observed in a line or arranged in a circle. Since he was not prepared for hospitalization, he was given oral chloroquine, 450 and 225 mg, on 22 and 23 August respectively by the medical officer and blood smear was made every day to observe the response. Hb was 10 g % and total RBC count was 4 million/mm^3 of blood. Spleen was palpable. *In-vivo* test showed that parasitaemia was 1,050/mm^3 after consumption of 900 mg of chloroquine on Day 2. The tendency of multiple infection of cells was much less after 24 h of chloroquine intake. Only two or three para-

sites in an erythrocyte were observed. Within 48 h of chloroquine consumption, this was also completely eliminated though blood smear still showed asexual stages (2nd day). Fever did occur (102°F) with severe headache. The presence of asexual parasitaemia on the 2nd day indicates that this was a case of chloroquine resistance. Metakelfin, 1.5 tablets (750 mg), was administered and subsequently blood film taken on the 7th day showed no parasite. Fever did not return and there was no feeling of weakness and headache. No other family member had fever in the house.

Case No. 3: The patient was a 25-year old Dutch lady who had visited Bihar 2 weeks prior to her illness. At the time of admission to the hospital on 18 October 1991 the patient was in shock, semiconscious, and had metabolic acidosis. Liver and spleen were enlarged. Blood urea, blood sugar and CSF were normal. Peripheral smear revealed 40% parasitaemia, multiple invasion and presence of mature forms of *P. falciparum*. Within two hours of admission the condition deteriorated and the patient died despite antimalarial treatment and supportive management. Interrogation from relatives revealed that the patient had fever for 7 days and was on homoeopathic treatment.

The phenomenon of multiple infections of the host RBC is common in *P. falciparum*[3]. In a fatal case, up to 8 young trophozoites were reported within a single RBC by Springall[4], where the parasitaemia was more then 44% and the rate of multiple infection was 37%. Field and Shute[5] also reported more than 38% parasitaemia with a rate of multiple infection of 24% and a maximum of 7 rings in a cell in a fatal case. In case no. 1, 19% erythrocytes were infected with 7% rate of multiple infection, and in case no. 2, 16% erythrocytes were infected and rate of multiple infection was 5%. In case no. 3, 40% erythrocytes were infected and up to 8 rings were present within a single RBC. Trophozoites and schizonts were also seen in peripheral smear. These cases exhibited the heaviest infection of *P. falciparum* that has been studied at MRC in recent years, with no comparable record of infection in the previous year.

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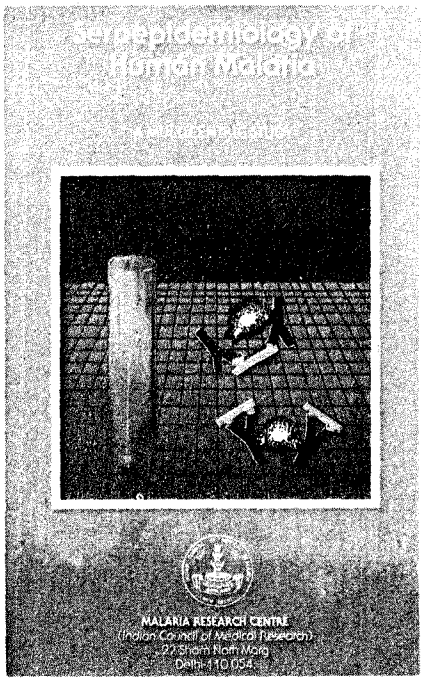

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