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Note: The editor assumes no responsibility for the statements and opinions expressed by the contributors.

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Epidemic of Malaria in Barmer District (Thar Desert) of Rajasthan during 1990

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Barmer district of Rajasthan in Thar desert and hitherto a hypoendemic area for malaria came in the grip of a severe malaria epidemic during 1990. The epidemic occurred as an aftermath of floods, preceded by normal rains during 1988 and 1989 after a prolonged drought phase. The epidemic was spread over the whole district including Barmer town. Annual Parasite Incidence (API) and Annual falciparum Incidence (AFI) for the district touched record figures of 17.20 and 5.83 respectively while for the Barmer town they were 36.5 and 14.0 respectively. Out of the eight PHCs, Baitu PHC was the worst affected where the two indices touched all-time high figures of 55.3 and 19.6 per cent respectively. A total of 122 infants were reported positive for malaria, of which 103 were contributed by Baitu PHC alone. Eighty per cent infant positivity was spread over September and November, indicating a high rate of transmission. A total of 47 deaths due to cerebral malaria were reported. However, in view of the high infectivity among infants and paediatric groups there was a strong possibility of deaths among these groups which could not be verified.

Entomological findings revealed that a sudden increase in *An. culicifacies* densities due to extensive breeding potential, as a sequel to floods, activated the transmission, which was maintained at a low level by *An. stephensi*, predominant in this desert region. Other factors which contributed to the intensity and extent of epidemic were the return of drought-migrated population from malarious areas, low cattle density, malaria non-immune population, inadequate and poor spray coverage and delayed radical treatment. Insecticide adult susceptibility tests revealed a high degree of resistance in *An. stephensi* against DDT and dieldrin.

INTRODUCTION

Barmer district, a western desert area of Rajasthan, was classified as hypoendemic at the time of inception of NMEP in the state[1]. Because of the typical desert physiography and climate, non-conducive to malaria transmission, the disease has never been a problem in the area in spite of poor surveillance. However, the year 1990 witnessed an epidemic of malaria as a sequel to floods in the region, causing 47 deaths. The eco-epidemiological and entomological factors responsible for the current malaria epidemic in Barmer were investigated and the findings are presented in this paper.

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Study area

The district lies between 24° 4 and 26° 32′ north latitude and 70° 5′ and 72° 52 east longitude and covers 28,318 km². It is a tropical dry sandy desert region. There is no forest, except sparse scrub vegetation. There are three rivers, viz. Gurrha, Pachpadra and Luni, which are mainly seasonal and remain dry most of the time. Barmer on its western side has an interstate border of 50 km with Tharad of Banaskantha region of Gujarat and about 70 km international boundary with Pakistan. The adjoining areas of Pakistan are mainly dry and desert tracts of Thar and Bahawalpur, while the bordering region of Gujarat is semi-desert.

The population of the district[2] is 11,18,892. It is a sparsely populated area comprising eight *panchayat samities*, each with a block PHC, with a population ranging from 10.2 (Baitu) to 19.4 lakhs (Ramsar). Barmer and Balotra are the two townships with 0.6 and 0.3 lakh populations. The population density is about 36/km² ranging between 15 and 59/km².

Extremes of climate are a very characteristic feature of this area. The average temperature is around 26°C, whereas the maximum temperature ranges between 42°C and 48°C during summer. Minimum temperature ranges between 3°C and 10°C during winter. The average relative humidity is 53-60 per cent.

The annual normal rainfall is 27.75 cm. July and August are the rainy months. Minimum rainfall recorded was 10.65 cm during 1987. The period 1985-87 was one of severe drought. However, 1988 and 1989 had normal rainfall.

Flood

The year 1990 had the century's highest rain fall (79.0 cm), which was 185 per cent more than the normal rainfall of the area and caused unprecedented floods. Thirteen villages of the five block PHCs, viz. Baitu, Samdari, Ramsar, Sindhari and Gudamalani, were completely submerged.

Drinking water sources

Water is a rare commodity. Rain-water stored in the underground cement tanks/reservoirs is the only source of drinking water. The total number of such tanks in the district is around 11,000. However, their functional status varies with rainfall and need.

MATERIALS AND METHODS

Epidemiological

Epidemiological data for the last 14 years were reviewed to ascertain the past and current trends of malaria incidence in the district. Mass blood sample survey was carried out in the worst affected villages of PHC-Baitu to compare the actual trend in field with that recorded in the government hospital/PHCs. Comparative malaria incidence in different PHCs and Barmer town was also studied during the inter-epidemic and epidemic phases. Various eco-epidemiological factors, viz. climatic changes, drought, population migration, and man-animal ratio, were also studied in conjunction with the incidence of the disease in the area. Malaria intervention measures, i.e. spray coverage, case detection and treatment, were also assessed.

Entomological

Density estimates of adults and immature stages of malaria vector(s) and other associated mosquito species were made by standard methods in the selected rural and urban locations of Barmer. Susceptibility of *An. stephensi* (adults and larvae) and *An. culicifacies* (adults) to various larvicides and adulticides were determined in accordance with the WHO test methods[3].

OBSERVATIONS

Epidemiological

The malaria incidence trend of the district since 1977 in relation to annual rainfall is given in Table 1 and Fig. 1. Rise in malaria incidence was found to be associated with increase in the rainfall. During 1977, annual rainfall was 13% above the normal, the Annual Parasite Incidence (API) showed a conspicuous rise at 5.3 and Annual falciparum Incidence (AFI) was 0.6. While during the dry spell the incidence was on a very low profile, API ranged between 0.2 and 1.5, and AFI between 0.03 and 0.1. However, 1985 was a year of exception, when there was a noticeable increase in cases, particularly *Pf*, despite low rainfall. The increase could be attributed to only migratory cases, as the cases did not proliferate locally, due to non-conducive transmission conditions. This was substantiated by a significant decline in the incidence in the next 2-3 years. Further, the Annual Blood Examination Rate (ABER) during 1983-1988 had been consistently low (10%)

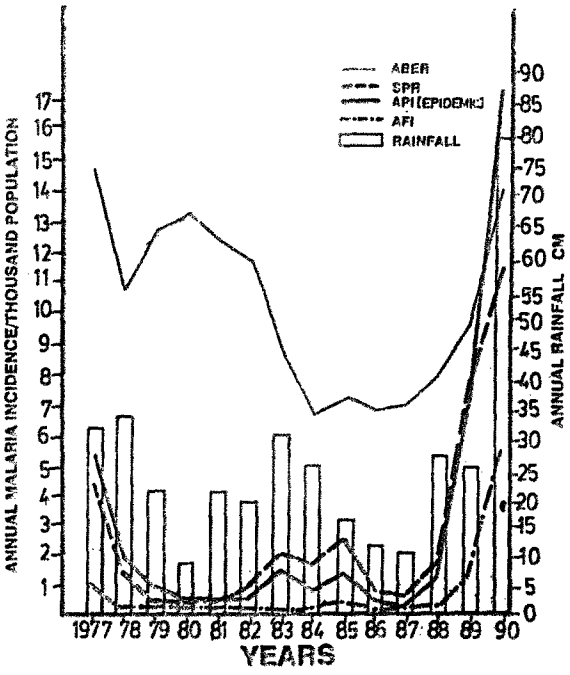

Fig. 1: Malaria incidence in relation to annual rainfall in Barmer

Table 1. Annual rainfall and incidence of malaria in Barmer district (1977-1990)

Year	API	ABER	SPR	AFI	Rainfall* (cm)	Deviation (%)
1977	5.3	14.6	4.1	0.6	31.4	+13.0
1978	1.8	10.7	1.6	0.14	33.62	+21.0
1979	0.5	12.7	0.3	0.005	20.57	–26.0
1980	0.3	13.3	0.2	0.02	18.9	–19.0
1981	0.4	12.4	0.3	0.04	21.1	–24.0
1982	0.6	11.7	0.5	0.07	19.26	–31.0
1983	1.6	8.7	1.8	0.13	30.8	+11.0
1984	0.9	6.8	0.1	0.09	25.7	–7.3
1985	1.5	7.4	2.4	0.28	16.3	–41.0
1986	0.5	7.0	0.7	0.05	11.87	–57.0
1987	0.18	7.2	0.25	0.03	10.65	–62.0
1988	1.52	8.07	1.89	0.17	27.54	–1.0
1989	7.26	9.8	7.34	2.06	25.71	–7.0
1990	17.2	14.2	12.1	5.87	79.01	+185.0

*Normal rainfall: 27.75 cm

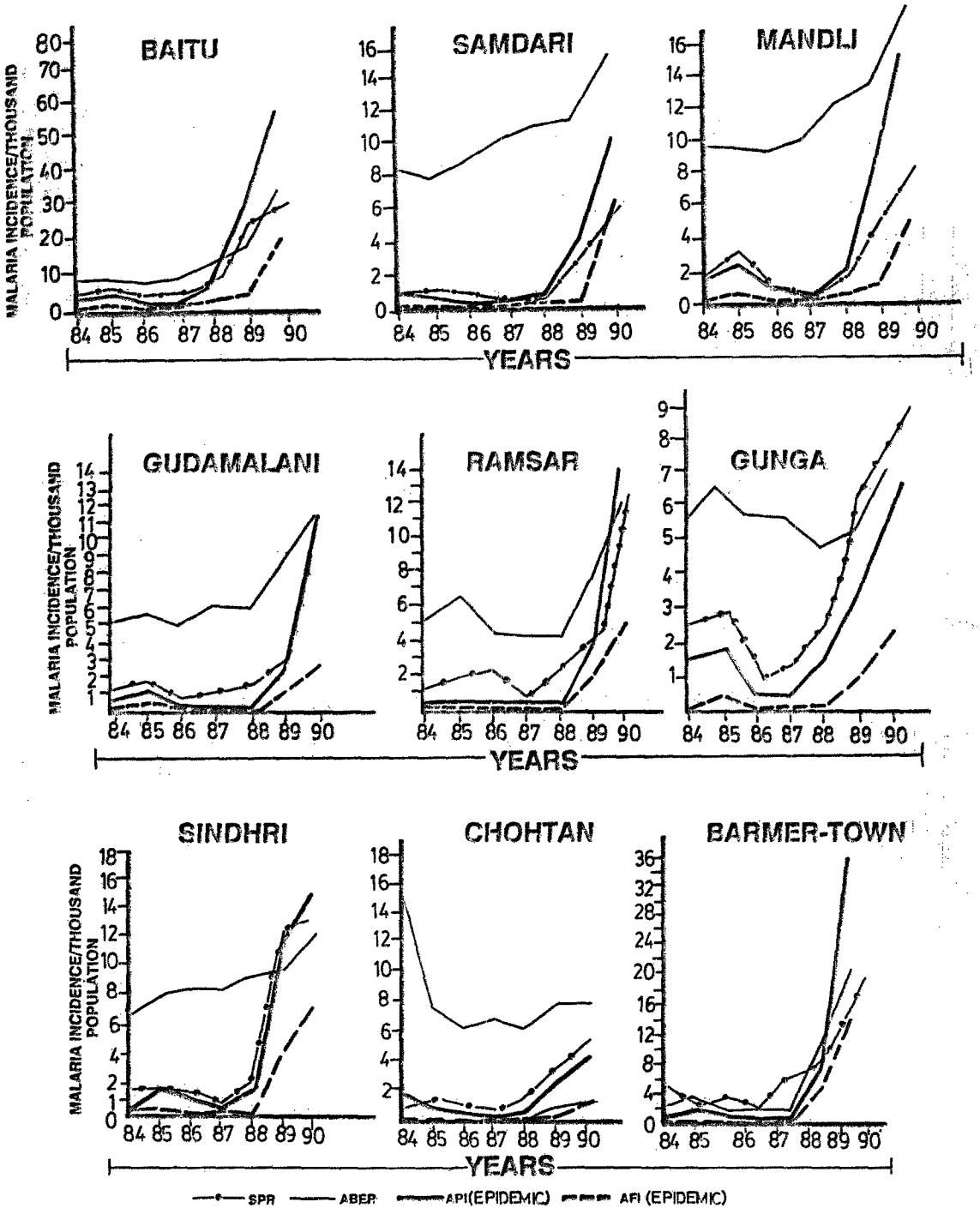

Fig. 2: Malaria epidemic 1990 in Barmer; PHC-wise incidence.

with noticeable fluctuations due to irregular surveillance. The variation in incidence during the period had been proportionate to the rise or fall in the blood smear examination rate. This signifies that API during the period does not depict a true picture of incidence. The linear depictions of SPR hence provide a real trend of the build-up of cases in the pre-epidemic phase.

Pre-epidemic phase (1988-89)

1988 and 1989 were the years of normal rainfall, which brought about favourable changes in the atmospheric humidity, vector prevalence, behaviour, man-animal ratio, parasite load and malaria immunity pattern in the population. This led to rapid multiplication of cases during 1989, a five-fold increase in total annual parasite incidence and ten-fold increase in the AFI.

Epidemic phase (1990)

1990's unprecedented floods gave a further boost to the ascending trend by way of favourable changes in the vectorial potential and resulted in the severe epidemic. The eco-epidemiological and entomological findings of the epidemic are discussed in the following paragraphs :

(a) Population migration : The normal population migration among adult males (>20 yrs) for employment was found to be 2.45% of the total adult population[4], whereas during 1985-87, due to severe drought, 40 per cent of the adult males left and settled largely in the adjoining Banaskantha region of Gujarat. About 1% adult males also migrated to Saudi Arabia.

(b) Animal population and man-animal ratio : The total loss of livestock population during 1984-87 drought phase in Barmer was found to be 50.87 per cent, as is evident from the figures of the 1983 and 1988 animal census[5] (Table 2).

The 1990 flood further resulted in the loss of 6037 animal lives in five of the eight worst affected

Table 2. Effect of drought on livestock population vis-a-vis man-animal ratio in Barmer

Year	Human population	Livestock population	Man/animal population ratio
1983	1119000	3184462	1 : 2.9
1988	1454700	1620000	1 : 1.1

panchayat samities. Thus it significantly altered the man-animal ratio and consequently disturbed the natural zoo-prophylaxis pattern of malaria in the region with the increase in the frequency of man-vector contact.

(c) Extent and intensity of epidemic : PHC-wise incidence data of malaria since 1984 in Barmer rural and urban is shown in Table 3 and Fig. 2. It is evident from the table that both the rural and urban areas were equally and simultaneously affected by the epidemic. Though all the eight PHCs presented a high-rising trend, the PHC-Baitu was the worst affected with API touching a peak of 55.3 and AFI 19.6. Highest increases in AFI were in Mandli (× 4.5), Gudamalani (× 4), Ramsar (× 2.8), Gunga (× 2.3), Sindhari (× 1.75), and Chohtan (× 1.6). The Barmer town also recorded a 4-fold rise in API and 3-fold rise in AFI. The district overall API and AFI recorded 2.5- and 3-fold increases respectively.

(d) Ratio of active and passive case detection in Barmer (urban and rural) : A significant change in the ratio of Active Case Detection (ACD) and Passive Case Detection (PCD) was found during the pre-epidemic and epidemic phases in both rural and urban areas, as is evident from Table 4.

(e) Age and sex-wise distribution : The results of mass blood survey carried out in six affected villages of PHC-Baitu are summarized in Table 5.

Both adults and children, males and females and all age groups were equally affected. Slide

Table 3. PHC-wise epidemiological data for Barmer (1984-1990)

Year	PHC : Baitu (1,02,229)*				PHC: Samdari (1,32,738)*				PHC: Mandli (1,76,625)*			
	ABER	API	SPR	AFI	ABER	API	SPR	AFI	ABER	API	SPR	AFI
1984	6.5	1.72	2.64	0.41	8.7	0.42	0.48	0.02	9.74	1.36	1.4	0.02
1985	7.7	4.45	5.77	0.93	8.2	0.49	0.6	0.037	9.63	2.36	2.45	0.36
1986	6.6	1.0	1.42	0.17	9.2	0.1	0.33	0.015	9.3	0.7	0.7	0.07
1987	7.5	0.4	0.49	0.1	10.5	0.1	0.07	0.038	9.8	0.2	0.19	0.02
1988	11.6	6.2	5.34	0.84	11.4	0.28	0.2	0.07	12.0	2.1	1.7	0.2
1989	15.5	26.9	17.3	3.3	11.9	4.2	3.52	0.57	13.4	7.6	5.67	1.1
1990	27.0	55.3	20.48	19.6	17.7	10.6	5.98	6.8	19.0	15.0	7.89	4.9
	PHC: Gudamalani (1,34,222)*				PHC: Ramsar (1,94,236)*				PHC: Gunga (1,01,198)*			
1984	5.16	0.67	1.29	0.07	5.27	0.27	0.76	0.05	5.4	1.37	2.54	0.16
1985	5.57	0.1	0.17	0.16	6.6	0.9	1.36	0.15	6.6	1.89	2.8	0.36
1986	5.0	0.1	0.2	0.0	4.4	0.3	1.63	0.005	5.7	0.5	0.9	0.04
1987	6.1	0.21	0.35	0.01	4.2	0.1	0.2	0.005	5.06	0.54	0.96	0.03
1988	5.9	0.5	0.8	0.02	4.3	0.15	1.63	0.15	4.9	1.6	3.3	0.15
1989	8.6	2.6	3.02	1.0	7.4	3.9	5.27	1.7	5.4	3.4	6.29	1.0
1990	11.6	10.6	9.13	2.9	12.0	14.0	11.66	4.8	7.0	6.8	9.71	2.3
	PHC: Sindhari (1,36,843)*				PHC: Chohtan (1,40,801)*				Barmer town (55,554)*			
1984	6.42	0.88	1.4	0.01	14.3	1.1	0.77	0.07	1.88	0.83	4.4	0.02
1985	8.29	1.4	1.7	0.45	7.7	0.8	0.98	0.0	3.59	1.05	2.9	0.2
1986	8.4	1.0	1.16	0.15	6.1	0.3	0.5	0.14	1.73	0.58	3.3	0.0
1987	8.4	0.02	0.024	0.0	6.5	0.14	0.21	0.15	1.4	0.18	1.3	0.0
1988	8.5	1.5	1.7	0.07	6.3	0.5	0.8	0.05	1.8	0.83	4.6	0.14
1989	9.4	11.91	12.6	4.2	7.9	2.8	3.54	0.4	10.2	9.38	9.2	4.83
1990	12.0	15.0	12.5	7.34	8.0	4.5	5.6	1.4	20.4	36.47	17.8	14.0

*Population figures of 1990.

Table 4. Ratio of malaria cases by ACD and PCD

	1984	1985	1986	1987	1988	1989	1990
	ACD : PCD	ACD : PCD	ACD : PCD	ACD : PCD	ACD : PCD	ACD : PCD	ACD : PCD
Barmer distt. (overall)	85 : 15	80 : 20	64 : 36	79 : 21	69 : 31	57 : 43	54 : 46
Barmer town	30 : 70	20 : 80	12 : 88	30 : 70	12 : 88	5 : 95	7 : 93

Table 5. Mass blood survey : Age and sex-wise distribution of cases in Baitu PHC

Age group (year)	No. examined			Malaria positives				Species-wise		
	M	F	Total	Sex-wise		Total (M+F)	Age-wise %	Pv	Pf	Mix
				M	F					
up to 1	1	4	5	1	1	2	40.0	1	1	—
1 — 5 yrs	38	35	73	19	25	44	60.3	8	35	1
6 — 15 yrs	202	100	302	116	68	184	60.9	7	173	4
16 — 35 yrs	132	103	235	96	64	160	68.0	5	153	2
36 — 50 yrs	81	58	139	42	28	70	50.4	—	70	—
Total	454	300	754	274	186	460		21	432	7
precentage	(60.2)	(39.8)	—	(59.6)	(40.4)	(61)		(4.6)	(93.9)	(1.5)

Positivity Rate was 61%. More than 93% had *Pf* infection. As per district epidemiological records no significant difference in the sex ratio of malaria positives was found in the overall annual trend when compared with that in the epidemic phase. A total of 122 infants during 1990 were found positive for malaria of which less than 30% had *Pf* infection. 103 infant positives were from PHC-Baitu. Eighty-eight per cent of the positive infants were found during September to November.

Comparative incidence in infants in the district and PHC-Baitu is shown in Table 6.

(f) Seasonal variation in Pv and Pf infections : As per 1989 and 1990 monthly incidence records, transmission period started in the month of July, with the onset of monsoon. However, high incidence of *Pv* was recorded during August to December with a peak during October, whereas in case of *Pf*, active transmission commenced from September to December with a peak in December. Build-up of an epidemic wave in the case of *vivax* was found to be much sharp whereas in the case of *Pf* it was relatively slow but persistent with increased mortality.

(g) Mortality due to malaria : The intensity of disease manifestations in *Pf* cases was quite severe and symptoms of convulsions, neck rigidity, and anemia were quite common. A total of 47 deaths were reported during September to November with sudden and temporary rise in the mortality rate in all the PHCs. Eighty-seven per cent of deaths occurred in the age group 16 and above. The highest number of deaths (13) was recorded each from Baitu and Ramsar, followed by 6 from Sindhari, 4 each from Mandli and Samdhari, 3 from Gunga and 2 each from Chohatan and Gudamalani. Maximum number of deaths (21) was recorded during October while mortality figures in September and November were 12 and 14 respectively. There is a distinct possibility of deaths

Table 6. Comparative month-wise incidence of malaria in infants in Barmer (1990)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
PHC-Baitu	2	—	—	2	6	—	—	—	37	32	22	2	103
Barmer (overall)	2	—	—	2	6	—	1	1	46	38	23	3	122

among infants and in paediatric group in view of equal infection among age groups up to 15, and 16 and above (Table 3). However, these deaths could not be verified.

Entomological

(a) Vector prevalence : An. culicifacies in rural areas and *An. stephensi* in urban towns were found to be the predominant species among the malaria vectors. A most significant change in vector prevalence/density was found during 1990 as a result of floods in the rural areas, in comparison with the 1989 figures. Changes in malaria vectors prevalence observed during the normal rainfall and excessive rainfall years are highlighted in Table 7.

An. culicifacies, which was recorded in insignificant numbers during 1989, dominated the scene in the post-flood survey of 1990. *An. stephensi* adult density recorded during 1989 survey was between 5 and 15 per man-hour in the villages of PHC-Baitu and Gunga; it was found to be low during the current survey in the rural areas, while a sharp rise in *An. culicifacies* density was mainly due to an enormous increase in groundwater/breeding potential. On the contrary, *An. stephensi* recorded a decline in its adult density, as a result of floods. The flood water flushed off the breeding in the 'tankas' due to overflow of water in the flood-bound areas.

(b) Larval survey : Breeding of *An. stephensi* during the current survey was most commonly encountered in the residential overhead tanks (40% +ve), ground cement tanks (38% +ve), and 'tankas' (8% +ve) in the town, while in the rural areas domestic and peridomestic 'tankas' were found to be the most preferable breeding sites during the 1989 sample survey in PHC-Baitu and Gunga.

(c) Vector susceptibility to insecticides : The predominant *An. stephensi* species in the desert area of Barmer was tested for its insecticidal susceptibility at larval and adult stages. The species were collected from the town. LC50 values for larvae against different compounds computed as per Finney's probit analysis are given in Table 8.

A high degree of resistance in adults against DDT and dieldrin/HCH is evident in the species. Low survivors at the discriminating dosage of malathion need to be verified. In the case of cyfluthrin, 100 per cent mortality was obtained at 1 h exposure to 0.05% concentration. Mortalities at 15 min and 30 min exposure were 60 and 80% respectively. *An. culicifacies* is known to be highly resistant to DDT and HCH in this region[6].

Table 7. Anopheline species encountered with per man-hour density (PMD) and parity rates (PR)

Year and duration of survey	Species				
	An. stephensi	*An. culicifacies*	*An. subpictus*	*An. vagus*	*An. annularis*
1989 (Normal rainfall) during 2nd week of Nov.					
Per man-hour density (PMD)	5-15	1	0.5-1	—	—
Parity rate (PR)	(50%)	(0-1%)	(10%)	—	—
1990 (Excessive rainfall) during 2nd week of Oct.					
Per man-hour density (PMD)	1-3	18-25	20-30	5-10	1-3
Parity rate (PR)	(75%)	(44%)	(50%)	(25%)	(30-35%)

Table 8. Insecticidal susceptibility of *An. stephensi* at larval and adult stages

(a) Larval test results

Insecticides	DDT	HCH	DLN	Malathion	Temephos	Deltamethrin
Larval LC50 (in ppm)	0.207	0.16	0.46	0.107	0.013	0.000047

Insecticides	Fenthion	Fenitrothion	Chloropyrifos	Bromophos
Larval LC50	0.006	0.0007	0.0014	0.0095

(b) Adult test results (Laboratory-reared blood-fed females)

Per cent mortality at the discriminating dosage

Insecticides	DDT	DLN	Cyfluthrin	Malathion
	3.8(53)*	1.7(57)*	100(80)*	96(100)*

* No. of mosquitoes exposed.

(d) Assessment of intervention measures

Insecticide spray and its impacts : During 1990 spray operations, only 40% of the population under malaria risk was sprayed mainly with DDT and HCH in the bordering villages. Further, extremely low room coverage (8 to 50%) had hardly any perceptible impact on vector density vis-a-vis incidence.

Case detection and treatment : Poor surveillance, inordinate delay in the blood slide examination and radical treatment also adversely influenced the epidemic.

DISCUSSION

Christophers[7] has described this type of epidemic as fulminating or "regional epidemic", which is confined to dry tracts of north-west India. Such an epidemic most often had been found to be closely associated with flooding and referred to be diluvial in consequence. Bruce-Chwatt[8] referred to regional epidemic when the incidence of malaria increases sharply over a vast geographical area, often severe with a high mortality due to unusual climatic factors. Similar regional malaria epidemics in the Thar desert of Rajasthan had occurred during 1975 and 1976 in the Jaisalmer and Jodhpur districts bordering Barmer as an aftermath of floods. API and AFI had touched all-time peaks of 38.94 and 4.44 in Jaisalmer and 38.52 and 9.17 respectively in Jodhpur[9]. Though high mortality was recorded, it could not be documented due to lack of investigation. Thus the 1990's malaria epidemic in Barmer matched in its features with what has been described above as a regional epidemic. The events which led to build-up of epidemic potential were favourable ecological and climatological changes as a consequence of floods. Vast stretches of flood water provided extensive breeding potential for *An. culicifacies*, and resulted in its emergence in high densities. Altered man-animal ratio brought this primarily zoophilic species in frequent contact with human population and consequently accelerated the transmission in the presence of high parasite load in the community. *An. stephensi*, which is known to maintain low to moderate transmission in this desert region[10], along with *An. culicifacies*, compounded the transmission potential and caused one of the severest

epidemics in this desert area. Ineffective insecticide spray coverage,and inordinate delay in case detection and treatment further worsened the situation. All-out efforts are warranted to arrest the persistent transmission trend.

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Gujarat Model of Health Management Information System with Reference to Malaria

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The Gujarat model of health management and information system has been developed with the objective of reducing the work load of health workers at all levels pertaining to record keeping and report preparation for enhancing the quality of health services, as well as for better implementation and timely monitoring of health programmes. Pro formas currently in use under various health programmes have been reorganized and updated. For monitoring malaria, the total number of registers has been reduced from seven to one, reporting forms from 23 to six and the number of columns in reporting forms from 493 to 59. The model is expected to save the working time of laboratory technicians (15%) and multi-purpose health supervisors (5%) at PHC level and of all workers (100%) engaged in report preparation at district, state and national levels. The data generated by using this model are expected to be of high quality and accuracy and should lead to more rational planning.

INTRODUCTION

The national malaria control programme (later malaria eradication programme) in India was a show-piece for the world until mid-sixties. Its information and assessment system was so well planned that it was a near perfect system. However, since 1977 the system slowly and steadily eroded under Multi-Purpose Worker (MPW) scheme of primary health-care system. A sound health management information system is very important for monitoring the achievements of health programmes, perhaps as important as the programmes themselves. The Government of India realised the need to review the working of the existing information system in the country and to devise a suitable system which would be workable not only at central but more importantly in states at various levels. Four states, namely Gujarat, Maharashtra, Rajasthan and Haryana, were selected for developing a suitable health monitoring system with the help of World Health Organization. New sets of pro formas were developed for record keeping and reporting at all levels with the objective of rationalizing the existing system of recording information and streamlining the reporting system, utilizing data at each level of collection and timely feedback. In Gujarat, a test run of newly developed health information system has been in operation in Gandhinagar district since 1988.

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New MF 1: Daily Record of Houses Visited by MPHW

Name of MPHW : _____ Code : _____

Unit/PHC code : _____ Month : _____ Year : _____

Date	Houses visited		
	From	To	Total
1	2	3	4

Village-wise summary of houses visited Signature : _____

Village	Number of houses visited

Date : _____

New MF 2: Surveillance for Malaria Cases

Name of worker : _____ Date of collection : _____

Worker's code : _____ Type of collection: _____

Unit/Institute's code : _____

Village	House No/ location/ areas	Guest or mobile	Name of the head of the family	Patient's full name	Age	Sex
1	2	3	4	5	6	7

contd...

New MF 2: Surveillance for Malaria Cases (*contd...*)

Date of onset of fever	Presumptive treatment		BS No.	Results (Pv/Pf/ Mixed with stage)	For clinics/ laboratories	
	Chloro-quine	Para-cetamol			R.T. date	Reason for no R.T.
8	9	10	11	12	13	14

Technician's name _____ Code : _____ Worker's signature : _____

Signature : _____ Date : _____ Date : _____

In 1987, a committee appointed by the Government of India for in-depth evaluation of integrated vector control of malaria project in Kheda distt. of Gujarat recommended implementation of project strategies under primary health-care system, which was approved by the Central Council of Health in 1988 and accepted by the Government of Gujarat in 1989. Efforts were made to develop a computer-based model of health management information system covering all health programmes in operation under the primary health care system including provision for incorporation of any new health programme(s) in future. Intensive efforts led to the development of an innovative model of health management information system which has been christened Gujarat model because it has been developed in this state with the assistance and cooperation of a large number of workers and officials of the department of health of the Government of Gujarat. The model has been developed with the following objectives :

(i) to reduce the work-load of the health workers in maintenance of records and reporting so that more time could be devoted to provide the services; *(ii)* to reorganise and update the pro formas currently in use so as to avoid repetition of the same information columns; *(iii)* to simplify pro formas for easy understanding, prompt collection of information and quick reporting, analysis and feedback for better implementation of the programme. The Gujarat model of health management information system with particular reference to malaria is described in this paper.

MATERIALS AND METHODS

All pro formas of the existing system for collection of information and reporting were studied. The proposed six input forms (New MF 1 to 6), three report forms for PHC and five report forms for the district were discussed with the workers at all levels and officials of the department of health of the Government of Gujarat and Government of India and were finalised by incorporating the suggestions given by them. Information on monthly time spent by laboratory technicians and multipurpose health supervisors at PHC level, and laboratory technicians at district level in maintenance/updating of registers and reporting for malaria was also collected. One PC/XT with 20MB hard disc and dBase III software was used in the development of the model.

New MF 3: Daily Record of OPD/Indoor Cases and Fever Cases

(For clinics/laboratories/dispensaries)

Unit's name : _____ Month : _____

Code : _____ Year : _____

Date	Total Cases†	Fever cases
1	2	3

Total for whole month :

Signature : _____ Date : _____

†Total of all cases like new, old, indoor and call cases.

New MF 4: Weekly Report of Radical Treatment of Malaria Cases

(for supervisors)

Supervisor's code : _____ Year : _____

Unit's code : _____ Week _____ Month : _____

BS No.	Date of BS collection	Date of receipt of R.T. information	Date of R.T.	R.T. given by (code)	Reason for delayed R.T. (72 h after R.T. information)	Reason for no R.T.
1	2	3	4	5	6	7

Signature : _____ Date : _____

New MF 5: Daily Work Record of Laboratory Technician

Technician's code : _____ Unit's code : _____ Week : _____

Date	Previous backlog	BS received	Total (2+3)	BS examined	Backlog (4-5)	Please give reasons if column No. 5 is nil	Remark of MO for column 7
1	2	3	4	5	6	7	8
Total							

No. of working days : _____ No. of days on leave : _____ Technician's signature : _____

No. of days worked : _____ Average BS examined/day : _____ Date : _____

New MF 6: Monthly Stock Position Report

Units's code : _____ Month : _____ Year : _____

Item	Opening balance	Received during the month	Total (2+3)	Used during the month	Waste/ breakage	Balance at the end of the month		
						Pipeline	Headquarter	Total
1	2	3	4	5	6	7	8	9

Signature : _____ Date : _____

PHC Malaria Report 1: Agency-wise Blood Smear Collection and Malaria Cases

PHC : _____ Month : _____ Year : _____

Agency	Area code	Popu-lation	% pop. covered	BS collection						
				<1	1-4	5-14	15+	Male	Female	Total
1	2	3	4	5	6	7	8	9	10	11

contd...

PHC Malaria Report 1: Agency-wise Blood Smear Collection and Malaria Cases (*contd...*)

Positive								
<1	1-4	5-14	15+	Male	Female	Total	Pf	BER
12	13	14	15	16	17	18	19	20

PHC Malaria Report 2: Village-wise BS Collection and Malaria Cases

PHC : _____ Month : _____ Year : _____

Village	Popu-lation	% pop. covered	BS collection			Positive		
			Active	Passive	Mass	Active	Passive	Mass
1	2	3	4	5	6	7	8	9

contd...

PHC Malaria Report 2: Village-wise BS Collection and Malaria Cases (*contd...*)

Pf			Total			% Pf	BER	SPR	SFR	PI
Active	Passive	Mass	BSC	Pos.	Pf					
10	11	12	13	14	15	16	17	18	19	20

PHC *Malaria* Report 3: *Monthly* R.T. Pending Report

PHC: _____ Year: _____

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1. No. of positive cases												
2. R.T. given A. Current month												
B. Previous month												
3. R.T. pending												
4. R.T. pending without valid reason												
5. R.T. pending due to valid reason												
A. Infant												
B. ANC												
C. Old age												
D. Weakness												
E. Refusal												
F. Migrated/Referred												
G. Not traceable												
H. Drug reaction												
I. Death												
J. Discrepancy												
6. Progressive No. of positive cases												
7. Progressive No. of R.T. given												
8. Progressive R.T. pending												
9. Progressive R.T. pending without valid reason												
10. Progressive R.T. pending due to valid reason												
11. Time lag same day												
1-3 days												
4-7 days												
1-2 weeks												
2-3 weeks												
3-4 weeks												
> 4 weeks												

District *Malaria* Report 1 : Taluka, Area & PHC- wise Epidemiological Data

Month: _____ Year: _____

Unit	Pop.	% pop. covered	BS collection	Positive	Pf	% Pf	BER	SPR
1	2	3	4	5	6	7	8	9

contd...

District *Malaria* Report 1 : Taluka, Area & PHC-wise Epidemiological Data (*contd...*)

SFR	PI	Progressive							
		BSC	Pos.	Pf	% Pf	BER	SPR	SFR	PI
10	11	12	13	14	15	16	17	18	19

District Malaria Report 2: PHC- wise BS Collection and Positive Cases

Month : _____ Year : _____

Unit	Pop.	BS collection									
		Active	Passive	Mass	<1	1-4	5-14	15+	Male	Female	Total
1	2	3	4	5	6	7	8	9	10	11	12

contd...

District Malaria Report 2: PHC-wise BS Collection and Positive Cases (*contd...*)

Positive										
Active	Passive	Mass	<1	1-4	5-14	15+	Male	Female	Total	Pf
13	14	15	16	17	18	19	20	21	22	23

District Malaria Report 3: Report of Laboratory Technicians

Month : _____ Year : _____

Name of Technician	Unit/ Institute	Previous backlog	BS received	Total (3+4)	BS examined	Backlog at month end	No. of working days	No. of days on leave	No. of days worked	Average BS examined per day
1	2	3	4	5	6	7	8	9	10	11

District Malaria Report 4: Radical Treatment Report

Month : _____ Year : _____

Unit/ Institute	No. of +ve	RT given		R.T. pending		R.T. pending due to valid reasons	R.T. pending without valid reasons		R.T. pending without valid reasons for >1 week
		Current month	Previous months	Current month	Previous months		Current month	Previous months	
1	2	3	4	5	6	7	8	9	10

District Malaria Report & Stock Position

Item: _____ Month : _____ Year : _____

Unit/ Institute	Opening balance	Received during the month	Total	Used during the month	Wastage/ breakage	Balance at the end of the month		
						Headquarter	Pipeline	Total
1	2	3	4	5	6	7	8	9

RESULTS AND DISCUSSION

The flow chart of the existing system along with that of Gujarat model are given in Fig. 1. Under the existing system, a multi-purpose health worker (MPHW) collects blood smears from fever cases and records information pertaining to the patients in a form. The MPHW also records the same information in a register. The form is then sent to the laboratory technician who examines the blood smears and records the findings on the same form. The laboratory technician maintains four registers and sends four reports to the district headquarter through the medical officer of primary health centre (PHC). A multi-purpose health supervisor (MPHS) maintains two registers and prepares five reports on the basis of information collected from various sources to be sent to the district headquarter through the medical officer of PHC. At district headquarter the reports received from PHCs are compiled and 13 reports are generated for onward transmission to the state headquarter.

In the proposed Gujarat model no major change is envisaged at MPHW level in the maintenance of register and filling up of the forms containing patient's information except that the form has been revised by deleting some columns and adding new ones for making it more informative without the loss of any information being collected in the existing system. However, a new form has been added to record daily house visits to monitor the population coverage. The laboratory technician is required to record the findings of blood smear examination as before in the form submitted by MPHW. He is also required to submit his work report as before but he is neither required to maintain/update any register nor to prepare any report. Similarly, the multi-purpose health supervisor is required to report in three forms only and there is no need to maintain any register or prepare any report. A total of six input forms (three by laboratory technician and three by MPHS) is required to be sent to the district computer centre, where three reports (PHC Malaria Report 1-3) will be generated for the use of the medical officer of each PHC for monitoring the work performance of the workers of his PHC. Reports will also enable the medical officer of each PHC to know the prevailng malaria situation in his area for any intervention measure to be taken. Similarly, five reports (District Malaria Report 1-5) will be generated for district and state level authorities. Reports will enable them to understand the comparative malaria situation in different PHC areas of the district. Report forms are absolutely flexible as they are computer-based and can be modified as per the need at any level.

Details of manual work required to be done under the existing system and the Gujarat model are given in Table 1. Under the existing system seven registers and 23 forms containing 493 columns need to be filled at different levels for monitoring the programme. In the Gujarat model only one register is to be maintained at MPHW level and there are only six input forms containing 59 columns. The data of a case study on monthly time

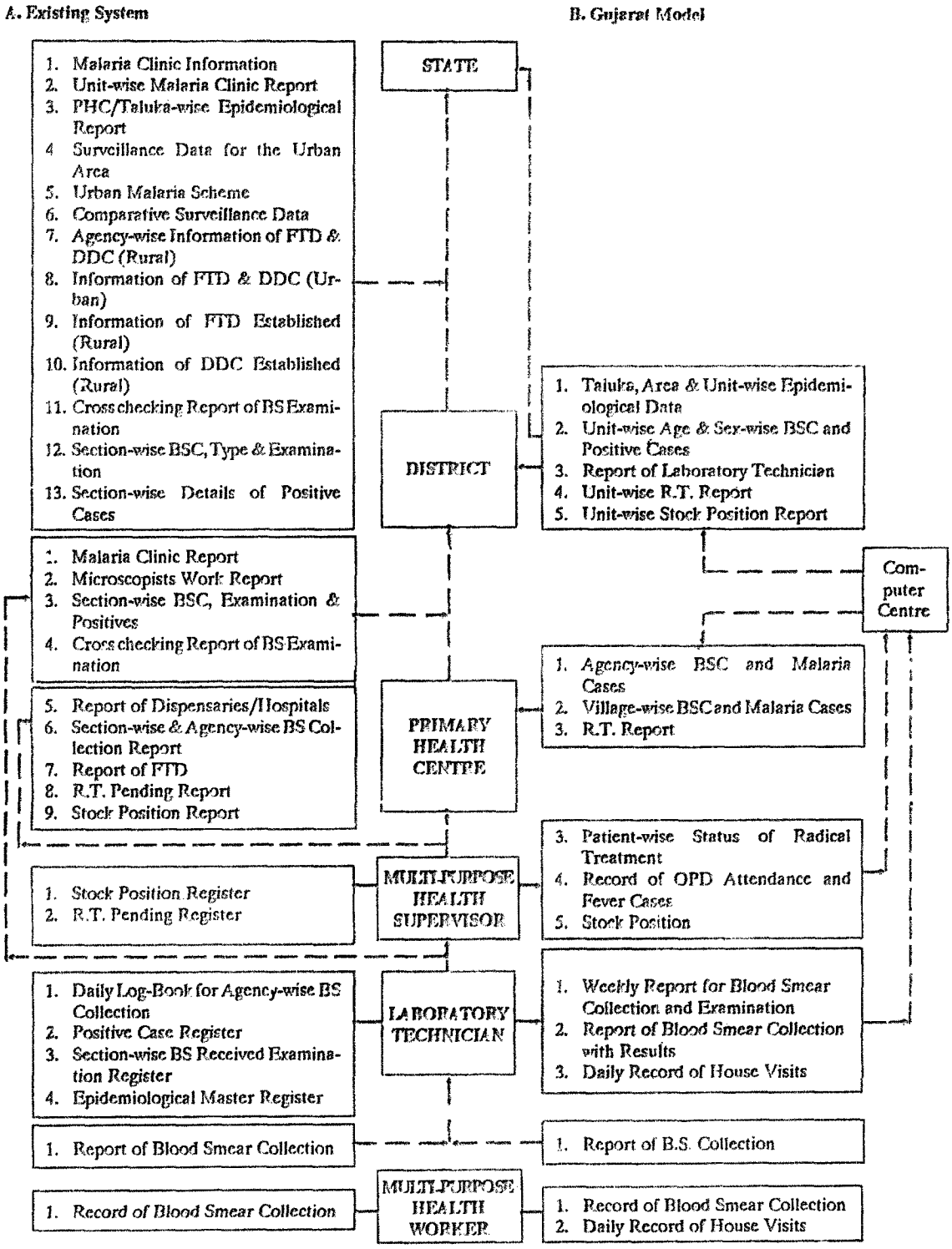

Fig 1: Flow chart of existing system and Gujarat model (new system).

Table 1. Comparison of registers and pro formas of existing system and new model

Level	Existing system			New model (New system)		
	Number of			Number of		
	Registers	Forms	Columns	Registers	Forms	Columns
1. MPHW	1	1	30	1	2	32
2. Lab. Tech.	4	4	190	—	1	8
3. MPH Supervisor	2	5	108	—	1	7
4. PHC	—	—	—	—	2	12
5. District	—	13	165	—	—	—
Total	7	23	493	1	6	59

spent on manual maintenance/updating of registers and reporting for malaria in Kheda distt. are given in Table 2. 95 laboratory technicians and 105 MPHS at PHC level, and three laboratory technicians at district level spend equivalent to 425 man-days of their time in maintenance/updating of registers and reporting, which is approximately equivalent to 19 workers involving an expenditure of about Rs. 3,42,000 every year @ Rs. 1500 per month per worker. Against this, in Gujarat model the time required is equivalent to seven workers only, which is likely to cost Rs. 1,50,000 per year, giving a net saving of time (man-days) equivalent to Rs. 1,92,000 as per the details given in Table 2.

Table 2. Monthly time spent in reporting and updating malaria in Kheda district

	Time spent in man-days/month	
	A. Existing system	B. New model
1. 95 Lab. Tech. @ one hour/day for 22 days in a month	299	1. R.O. 1 2. Programmer 1 3. Data entry operator 5
2. 105 MPHS @ 1 day per month	105	
3. 3 Lab. Tech. @ 7 day per month	21	
	425 = 19 workers	= 7 workers

Expenditure for one year

A.	19 workers	@ Rs. 1500/month for one year	= Rs. 3,42,000
B.	(i) 5 workers	@ Rs. 1500/month	= Rs. 90,000
	(ii) 1 worker	@ Rs. 2000/month	= Rs. 24,000
	(iii) 1 worker	@ Rs. 3000/month	= Rs. 36,000
		Sub-total	= Rs. 1,50,000
		Net saving/year	= Rs. 1,92,000

Saving in one year would alone be sufficient to finance five computers required for Kheda distt. (35 lakh population). Some small savings are expected on stationery as well, which have not been computed. Annual service charges, expected to be 10% of the cost of computers, can be financed from the savings in subsequent years. One may argue that there may not be any of financial gain because the time saved equivalent to 19 workers in Kheda distt. as estimated is unlikely to reduce the number of workers as such. But the time saved can be utilised in improving the quality of services which can be achieved under the existing system, only by hiring more workers. At present there exist a number of vacancies in the programme due to various constraints. By adopting the proposed system many of them may not be required to be filled.

In the existing system, though the parameters, e.g. Annual Blood Examination Rate (ABER), Annual Parasite Incidence (API), Slide Positivity Rate (SPR), Slide falciparum Rate (SfR) and per cent *P. falciparum* are calculated, only API is the main parameter currently used for stratification of the areas for the purpose of spraying of the residual insecticide in rural areas under the MPO[1,2]. For the regular monitoring the per cent increase or decrease in malaria cases in the corresponding month of the previous year, the previous month of the same year along with the progressive figures of the current year, with that of the corresponding period of the previous year, are also compared. However, it has been observed that such comparisons are almost always tentative owing to late and irregular receipt of reports at various levels, which defeats the very purpose of monitoring. For example, epidemics are usually detected when they have already occurred, specially due to near non-existence of an appropriate monitoring mechanism[3,4].

The Gujarat model envisages (i) elimination of maintenance of all registers except one at MPHW level and manual report preparation at all levels; (ii) timely flow of information in six input forms containing only 59 columns, which in turn is expected to help in generation of reports quickly; (iii) up-to-date data for timely intervention measures if required; and (iv) monitoring of the work performance of each worker as it envisages monitoring of the population coverage by individual worker in space and time for detection of malaria cases, blood smear examination rate, radical treatment to malaria patients, time-lag between blood smear collection and radical treatment and discrepancy rate in blood smear examination by the laboratory technicians for overall improvement of the programme by timely assessment and necessary inputs and also for forecasting of any brooding outbreak of malaria.

So far as the monitoring of malaria control programme is concerned no attempt has been made to improve the system specially by using a computer. Any attempt to improve the system by retaining manual mode of reporting without reducing the work-load of the workers in maintenance/updating of registers and reporting is likely to retain the problems of irregular and late reporting. Also, most of the information collected in the manual mode of information system goes waste in the absence of thorough and timely analysis of the data due to various limitations. Therefore, the computer-based Gujarat model of health management information system appears to be the answer to improving the malaria control programme for better service to the community under MPW scheme of primary health care system.

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Antigenic Diversity amongst Ten Geographic Isolates of *Plasmodium falciparum* Defined by Merozoite Invasion Inhibition Assay

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The extent to which human antibodies involved in functional immunity react with antigenic determinants varying between different isolates or strains of human malaria parasite *Plasmodium falciparum* will influence the design of vaccine against malaria. In this study, *in vitro* inhibition of merozoite invasion in erythrocytes by an immune human serum was used to define the antigenic differences in 10 isolates of *P. falciparum* from three endemic areas, i.e. Africa, South America and Southeast Asia. The serum inhibited the invasion of merozoites of all the strains but the extent of inhibition varied from low to moderate to high degree indicating antigenic differences amongst isolates of *P. falciparum*. The antigenic differences could not be correlated to the geographic origin of the parasite isolate.

INTRODUCTION

Antigenic diversity in parasite has an important application in the development of acquired immunity and in vaccine research[1]. The advent of *P. falciparum in vitro* culture technique[2,3] facilitated the development of *in vitro* growth inhibition and merozoite invasion inhibition assays which have been widely employed[4-13] to gain a better understanding of the acquired immune response to malaria infection as well as to study antigenic differences in various isolates of *P. falciparum*. It has been shown that due to antigenic variations the protective immunity against malaria parasite *P. falciparum* is strain-specific[12] and even isolate-specific[11,13]. Homologous strains are inhibited more than heterologous strains. In this study, antigenic diversity in asexual stages of *P. falciparum* was demonstrated by the varying effect of immune human serum on the invasion of erythrocytes by the merozoites of 10 isolates of *P. falciparum* from different malaria-endemic regions, i.e. Africa, South America and Southeast Asia. Attempts were made to correlate the antigenic diversity as defined by per cent inhibition of the invasion of merozoite and the geographic origin of the isolate.

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MATERIALS AND METHODS

Malaria parasite strains

Ten strains of *P. falciparum* from known malaria-endemic regions, i.e. Africa, South America and Southest Asia, were selected for the study. The strains and the countries of origin are given in Table 1.

Strains FCR 3, Itux 1 and CAMP were gifts of Walter Reed Army Institute of Research, Washington D.C. Other strains were isolated from human infections and adapted to *in vitro* culture in our laboratory.

Parasite culture

All the strains previously adapted to *in vitro* cultivation and stored by cryopreservation were thawed and cultured *in vitro* using RPMI medium, O^+ human red blood cells and 10% human serum of the group A^+, Rh^+ 1.

Immune human serum (IHS)

This was collected from a donor who was a nonnative resident of Madagascar and was recognised to be immune to *P. falciparum*. In previous studies in our laboratory his serum was found to be highly inhibitory to merozoite invasion (of erythrocytes) of FCR 3 strain[14]. Normal human serum was used as control. Both types of sera were heat-inactivated at 56°C for 40 min before use.

Synchronisation

Plasmodium falciparum cultures were synchronised by a sorbitol lysis procedure by the modified method of Lambros and Vandenberg[15] as described by Vernes *et al.*[12]. Briefly, the asynchronous cultures were centrifuged, the pellet was resuspended in D-sorbitol (Calbiochem), diluted with 0.1% glucose to give 5% D-sorbitol, vortexed, incubated again, vortexed and centrifuged, thereby destroying the more mature stages of the parasite but leaving the ring stages intact. The parasites were further cultured for another 27 h and the procedure was repeated once. The culture was then diluted with normal human erythrocytes to provide a 0.15% of parasitaemia. After another 72 h of culture and media changes at 48 and 64 h, 80-90% of the parasites were in the early schizont stage with a parasitaemia of about 1%. This was used to start the inhibition of invasion assay.

Table 1. Strains of *Plasmodium falciparum*, countries of origin and per cent merozoite invasion inhibition by an immune human serum at 1:5 dilution of the serum

Strain	Country of origin	Per cent invasion inhibition
FCR 3	The Gambia	72.2
ITUX 1	Brazil	65.2
BAR	French Guiana	79.9
DUF	French Guiana	45.0
DFS	Senegal	55.4
ABA	Gabon	55.2
ALB	Comoro Islands	79.0
TAB	Comoro Islands	57.3
CAMP	Malaysia	47.2
FCF 1	Combodia	45.0

Inhibition of merozoite invasion assay

The assay was carried out in accordance with the procedure of Vernes *et al.*[12]. Microcultures (100 µl, 1.5% v/v type O human erythrocytes 1% of which were parasitised with synchronised schizont stage parasite) in sextuplicate wells of 96 well flat bottom plates (Costar) were incubated in the presence of immune human serum or control serum. The sera were used at dilutions of 1:5, 1:10 and 1:20. The cultures were kept overnight for 18 h beginning at the schizont stage and ending after reinvasion. The microcultures were

washed twice and cultured with normal medium for another 8 h before triplicate wells were pulsed with 0.5 μci of ^{3}H hypoxanthine (Ameraham) in 25 μl medium for a final 16 h. Disintegration per minute of incorporated radioactivity as well as parasitaemias (per cent parasitaemia studied in Giemsa-stained smears) was determined. All incubations were at 37°C in a humidified atmosphere of 5% CO_2, 5% O_2, and 90% N_2.

The percentage of inhibition of invasion corresponding to the serum dilutions present in the culture medium at the time of reinvasion was calculated using the formula:

$$\text{Per cent inhibition} = 100 - \frac{\text{Parasitaemia or incorporation in immune human serum}}{\text{Parasitaemia or incorporation in control human serum}} \times 100$$

The per cent inhibition was graded into three degrees — low (30-50%), moderate (50-70%) and high (> 70%).

RESULTS

Preliminary experiments showed good correlation between the estimates of invasion obtained using incorporation of ^{3}H hypoxanthine or morphological evaluation (Fig. 1).

The per cent inhibition of merozoite invasion was maximum at 1:5 dilution of IHS (Fig. 2). All the isolates were inhibited by IHS at all the dilutions but the degree of inhibition varied. At 1:5 dilution of IHS the per cent merozoite invasion inhibition observed was between 40 and 80 by all the strains (Table 1). A high degree of inhibition (> 70%) was observed in three strains, FCR 3, BAR and ALB, belonging to the Gambia, French Guiana and Comoro Islands respectively.

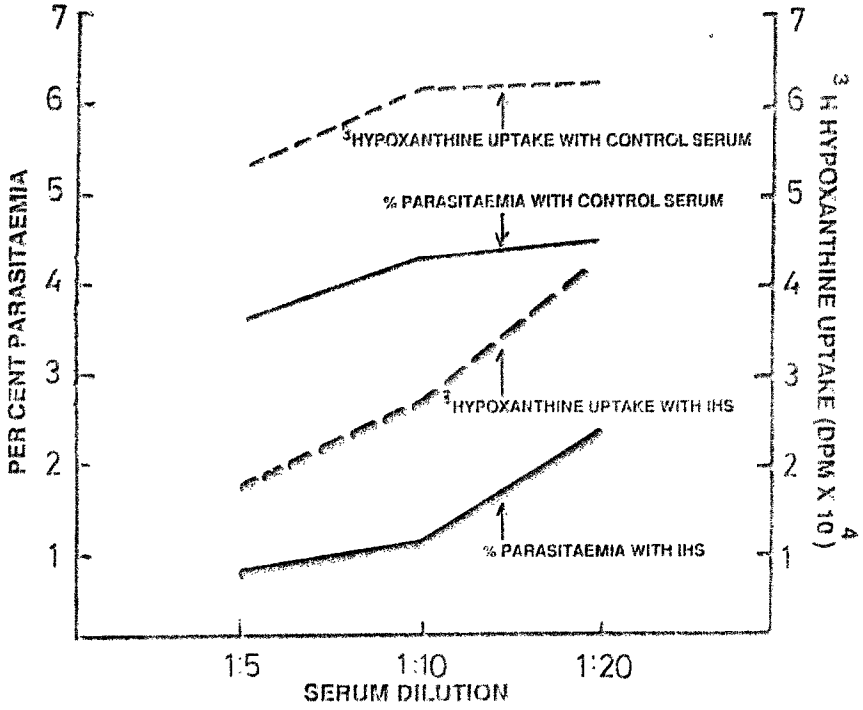

Fig. 1 : Merozoite invasion of FCR 3 strain of *P. falciparum* in the presence of different dilutions of control or immune human serum (IHS), estimations of invasion made by incorporation of ^{3}H hypoxanthine and morphological evaluation.

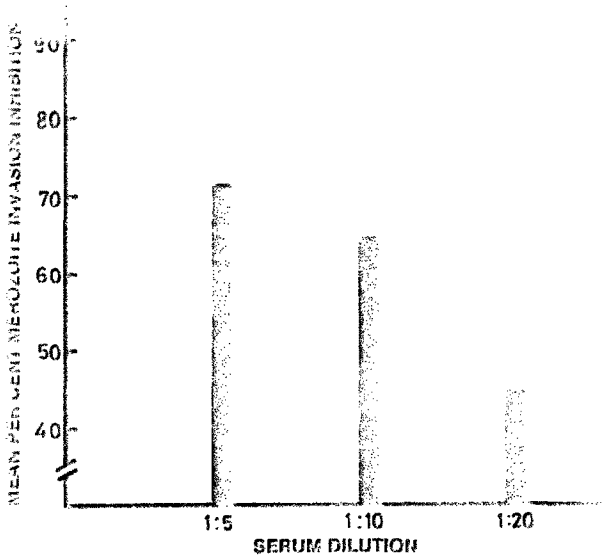

Fig. 1. Merozoite invasion inhibition of FCR 3 strain of *P. falciparum* at different dilutions of immune human serum (IHS).

Note : Per cent merozoite invasion inhibition represents a mean of values obtained by ^{3}H hypoxanthine incorporation and morphological evaluation.

DISCUSSION

Our results reveal that acquired anti-malarial antibody in adults living in malaria-endemic region can inhibit the *in vitro* invasion of *P. falciparum* merozoites in erythrocytes. The *in vitro* inhibition of parasite growth and merozoite invasion assays have been widely employed to study the role of protective antibodies. Merozoite invasion inhibition assay rather than intraerythrocytic parasite growth has been suggested to measure *in vitro* functional immunity[8,12] since it quantitates a decrease in viability of the parasite by an immune process which thus has an obvious function in protecting the host. In our study ten geographic isolates of *P. falciparum* were used as a target for an immune human serum. Broadly, these isolates could be divided into three malaria-endemic regions or geograhic areas, i.e. Africa, South America and Southeast Asia.

Our results reveal that merozoites of all the strains were inhibited from invasion in erythrocytes but the degree of inhibition varied. Moreover, the per cent merozoite invasion inhibition was not restricted to any particular geographic area isolates. The resultant inhibition in MII assay could be due to qualitative or quantitative antigenic differences or similarities in the parasite isolates and hence could be used as an index of antigenic differences or ubiquity. The inhibition of all the isolates by the same serum in our study reveals that *P. falciparum* isolates which had similar or different geographic origins do share some common antigens. Our studies corroborate the findings of Vernes *et al.*[12], and Chulay *et al.*[16], who found that inhibition of merozoites was not limited to any geographic origin of the parasite in their studies on Cambodian immigrants sera and FCR, and CAMP strain of the parasite; and strain-specific owl monkey sera and

various culture lines of parasites respectively. It is likely that most of the antigenic strains or serotypes of *P. falciparum* parasites to which functional immunity naturally develops are common or are becoming common to georgraphically diverse areas. Further, our results indicate variation in the degree of inhibition by various isolates. This could be due to either the lack of certain antigens or insufficient quantity of those antigens to which inhibitory antibodies were present in the serum.

Moreover, in our studies, isolates from the same geographic origin were not inhibited to the same extent, e.g. BAR and DUF from French Guiana or ALB and TAB from Comoro Islands. This shows that there could be antigenic differences (quantitative/qualitative) in the isolates of the same geographic origin also. These findings are quite similar to our previous observations made by using immunoprecipitation technique and monoclonal antibodies and the same *P. falciparum* isolates[17]. Similar lack in correlation of antigenic differences and geographic origin of the *P. falciparum* has been shown by McBride *et al.*[18]. On the other hand, studies with human sera samples and various isolates of *P. falciparum* have shown that homologous isolates are invariably inhibited more than heterologous strains indicating the restriction of some antigens in isolates of one particular geographic area[4,8].

Our results underline the importance of merozoite surface antigens and indicate that in isolates from various geographic origins there are some shared antigens and that antigenic differences could occur in the isolates of *P. falciparum* from the same region. Hence antigenic variation/differences/ubiquity must be taken into account while selecting or including strains and antigens for designing a malaria vaccine or for diagnosis.

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Observations on Malaria Patients Seeking Treatment in Hospitals in a Rural and an Urban Area of Sri Lanka

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Malaria in Sri Lanka is endemic in the dry zone and occurs during epidemics in the wet zone. A survey was carried out on malaria patients presenting at a hospital located in a predominantly rural area in the dry zone (Polonnaruwa) and an urban area in the wet zone (Ragama). Higher incidence of *Plasmodium falciparum* infections than reported nationally were observed in both locations. Of particular interest is the rapidity with which patients gained access to hospital treatment after the onset of malaria symptoms. The observed mode is 3-6 days. It is postulated that early treatment may impair the development of clinical immunity to malaria in the Sri Lankan population.

INTRODUCTION

Sri Lanka is an island with an area of 65,525 km^2 located between latitudes 5.55° and 9.50° north of the equator. The central hills of the island divide the surrounding plains into two distinct zones, viz. the wet zone and the dry zone. The wet zone, located in the central hills and the southwest of the country, receives rainfall mainly during the northeast monsoon in November–January and the southwest monsoon in May–July. Intermonsoonal rains also occur in the wet zone. The dry zone receives maximal rainfall during the northeast monsoon and little or no rain during the rest of the year. Malaria in Sri Lanka is endemic in the dry zone with a peak of transmission during the northeast monsoon season[1], and typically occurs during epidemics in the wet zone. *Plasmodium vivax* and *P. falciparum* are the causative organisms of malaria in Sri Lanka. In 1989, 198,651 cases of *P. vivax* malaria and 65,345 cases of *P. falciparum* malaria were reported in the country [2]. In Sri Lanka, malaria causes very little mortality, probably less than 100 per year[3]. In rural Africa where malaria is hyperendemic, there is high mortality, particularly amongst children, from malaria. For example in the Gambia, malaria causes 10.7 deaths per 1000 per year in children aged 1-4 years and 6.3 per 1000 per year in infants and is responsible for 25% and 4% respectively of all deaths in these age groups[4].

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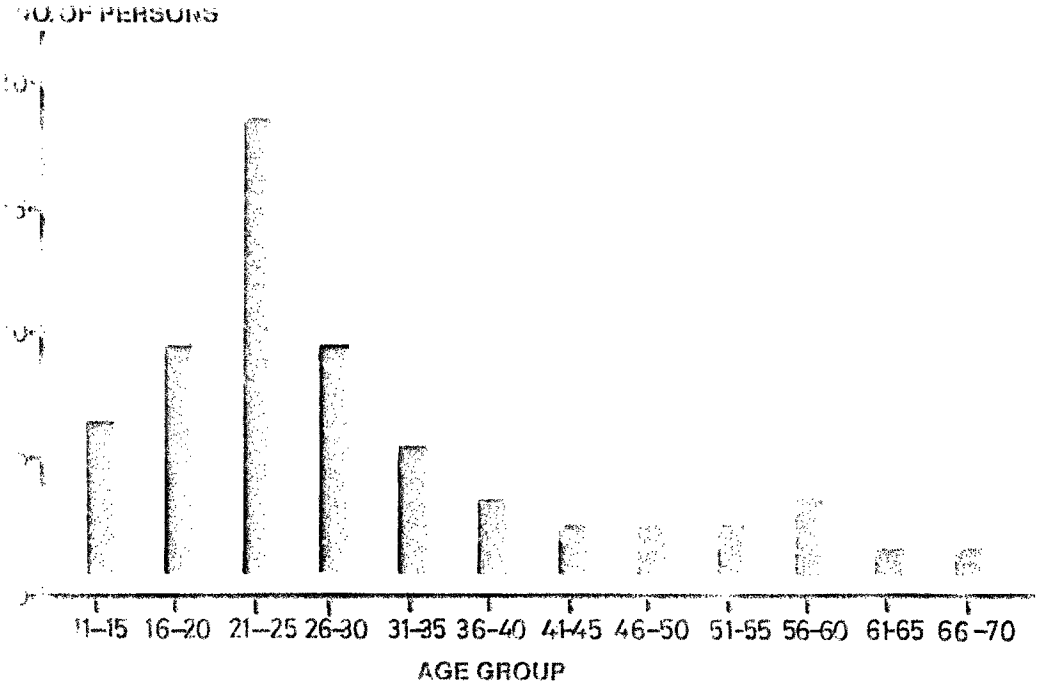

Fig. 1: Age distribution of patients at Polonnaruwa hospital (January–February 1991).

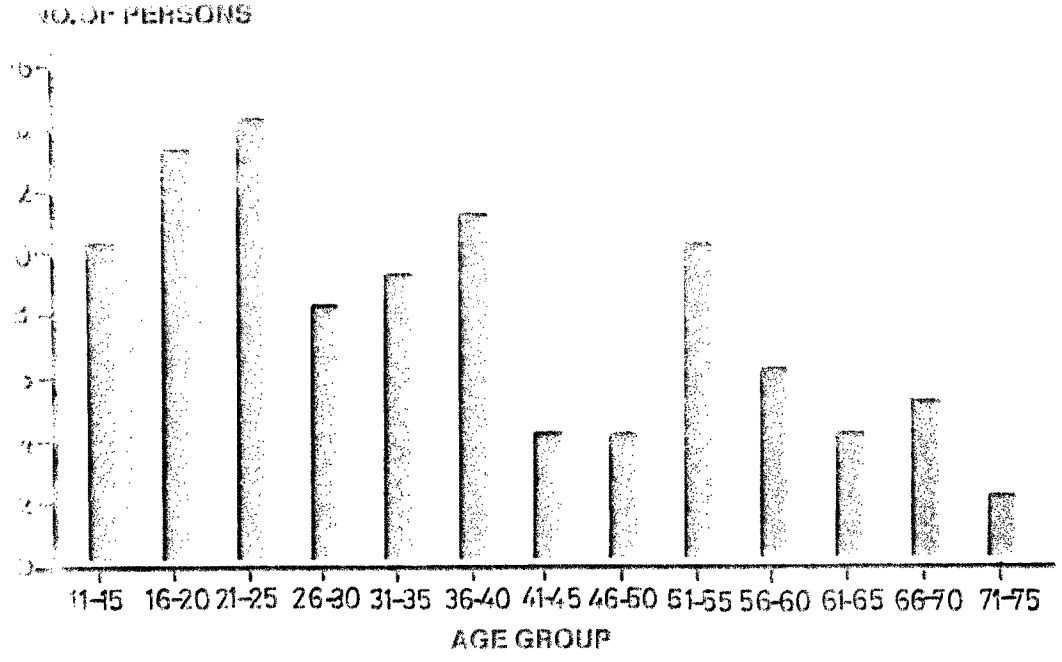

Fig. 2: Age distribution of patients at Ragama hospital (December 1990–January 1991).

In malaria hyperendemic areas of the world, e.g. rural Africa and Papua New Guinea, adults often develop a significant degree of clinical immunity to malaria. While this may occur to some extent in the endemic areas of Sri Lanka, it is also observed that recurrent attacks of malaria are not uncommon among many adults who have lived in such areas since birth[3]. Thus it is of interest to investigate factors that may possibly hinder the development of immunity in the Sri Lankan population in comparison with those in Africa and Papua New Guinea.

We carried out a survey on malaria patients reporting to the Polonnaruwa Base Hospital located in the malaria-endemic, north-central province in the dry zone of Sri Lanka. This was performed during the peak of the transmission season, which occurs soon after the advent of the northeast monsoon in November–February. At the same time an epidemic of malaria occurred in the northern outskirts of the city of Colombo, which is located in the wet zone on the west coast of the island. Malaria patients at the Colombo North General Hospital in Ragama were therefore surveyed for comparison.

MATERIALS AND METHODS

A close-ended questionnaire was designed and this was completed by physicians at the Colombo North General Hospital in December 1990 to January 1991 and in the Polonnaruwa Base Hospital in January 1991 to February 1991. Data were obtained on patients who were diagnosed to have malaria confirmed by positive Giemsa-stained blood films. The blood films were examined by standard procedures by microscopists attached to the hospitals from the Anti-Malaria Campaign, Ministry of Health.

The first 100 patients at the North Colombo Hospital and the first 63 patients (including a number of soldiers stationed in the area) at Polonnaruwa hospital who sought treatment during the time period provided data for the study.

RESULTS

At Polonnaruwa the distribution of the ages of persons seeking treatment for malaria showed a skewed normal distribution with a mode of 21-25 years (Fig. 1), reflecting the presence of soldiers in the sample. At Ragama, while the mode was again 21-25 years there was a much greater, even-age distribution among patients (Fig. 2)

The number of days that elapsed between the onset of symptoms and seeking of treatment was not very different in the two hospitals (Figs. 3 and 4). The mode was typically 3-4 days and 5-6 days in Polonnaruwa and Ragama respectively. The vast majority of patients sought treatment within 8 days in both hospitals.

A comparison of clinical, parasitological and other relevant features of malaria patients presenting at the two hospitals is given in Tables 1 and 2.

DISCUSSION

The observations made in Polonnaruwa and Ragama indicate that the ratio of *P. falciparum* to *P. vivax* infections is considerably higher than the 1 : 3 ratio of infections reported nationally[2] and may reflect an increased severity of symptoms associated with *P. falciparum* infections in Sri Lanka. Although a greater number of male patients were observed at both locations no conclusions regarding differential susceptibility to malaria can be drawn without more extensive studies. Most patients sought hospital treatment well within 8 days of the onset of symptoms and were usually accompanied by friends or relatives, showing that considerable family and community help was available to the patients. However more than 50% did not perceive their illness as malaria, which was surprising, particularly in a malaria-endemic area such as Polonnaruwa. This suggests the need for educating the public on malaria. More than half the patients had obtained other forms of treatment before coming to hospital. In the urban area of Ragama, 61% of

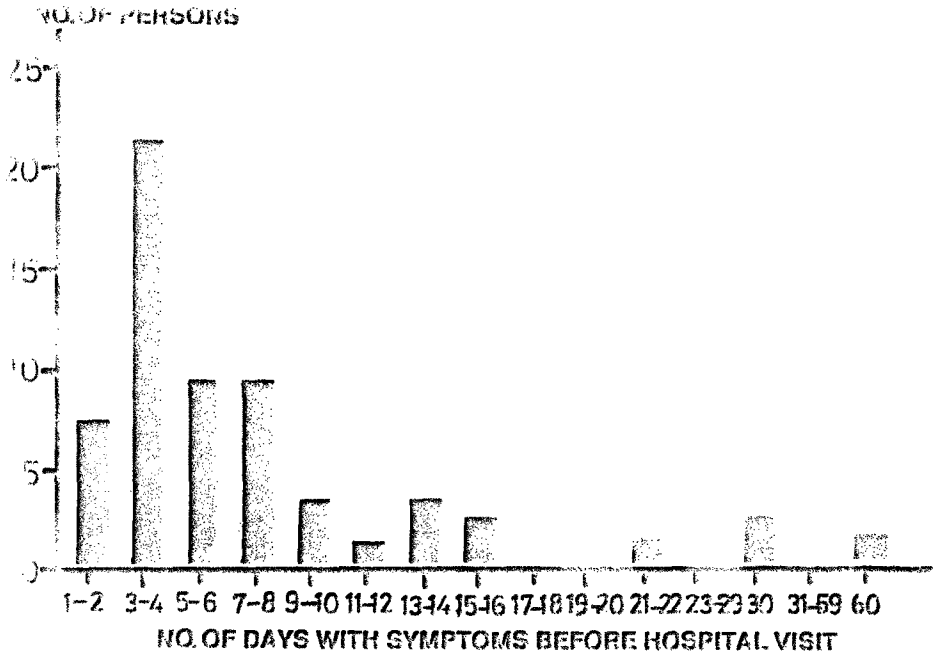

Fig. 6 Time between onset of symptoms and hospital visit at Polonnaruwa hospital (January–February 1991).

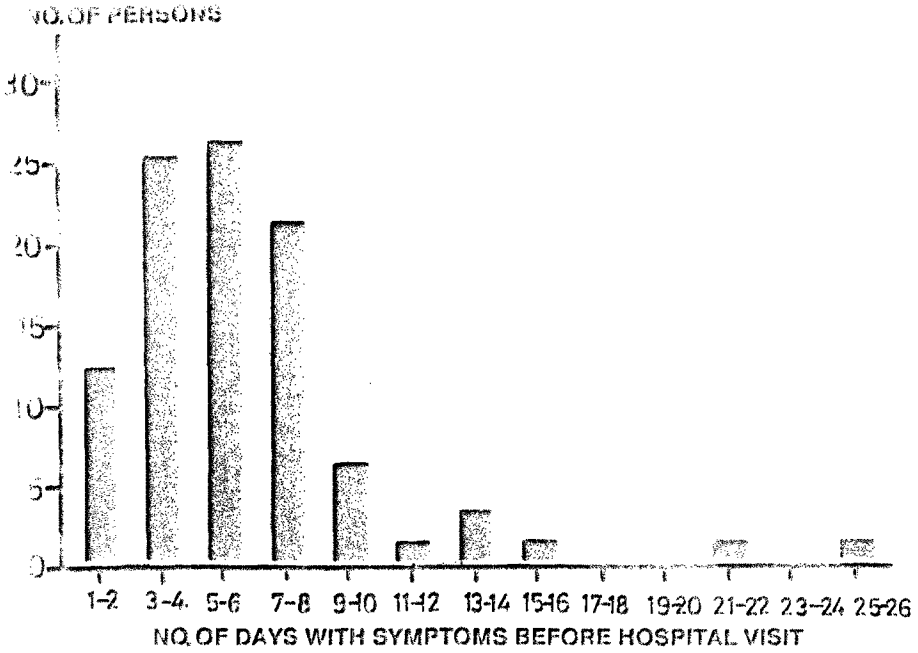

Fig. 7 Time between onset of symptoms and hospital visit at Ragama hospital (December 1990–January 1991).

Table 1. Comparison of some clinical and parasitological features of patients at Polonnaruwa and Ragama hospitals

	Polonnaruwa	Ragama
1. *Parasite*		
P. falciparum	65.1%	43%
P. vivax	34.9%	49%
Both	—	7%
Not determined	—	1%
2. *Stages of parasite observed*		
Asexual stages	96.8%	83%
Sexual stages	—	1%
Both asexual and sexual stages	3.2%	—
Not determined	—	16%
3. *Clinical features*		
Pallor or anaemia	3.2%	18%
Palpable liver	34.9%	88%
Palpable spleen	34.9%	76%
4. *Previous attacks of malaria in 5 yrs.*		
Yes	58.7%	10%
No	39.7%	86%
Not mentioned	1.6%	4%
5. *No. of previous malaria episodes in 5 yrs.*		
One	23.8%	1%
Two	11.1%	7%
> two	22.2%	2%
6. *Symptoms on presentation*		
Fever	96.8%	95%
Headache	95.2%	46%
Chills and rigors	85.7%	95%
Vomiting	44.4%	17%
Aching limbs	84.1%	18%
Others	19.0%	1%

the patients had consulted private medical practitioners, while in Polonnaruwa other forms of treatment, from ayurvedic to native physicians, were more popular. In another epidemiological study carried out at Kataragama in the extreme south of the country, it was observed that more than 50% of patients presented themselves for diagnosis and treatment within two days and more than 90% within four days of the onset of symptoms[5].

These observations suggest that access to medical treatment for malaria is both readily available and rapid in many, if not all, areas of Sri Lanka. A malaria infection with attendant clinical complications and mortality is therefore likely to be relatively rare and this situation is in contrast to that which prevails in much of rural Africa and Papua New Guinea. A consequence is that there may be insufficient time for an anti-parasite immune response to develop fully in a patient in Sri Lanka before the infection is cured. Detailed studies on the levels of immunity in Sri Lanka and other malaria endemic areas are needed to substantiate this hypothesis. However, such investigations are difficult to perform because immunological parameters that correlate with clinical immunity to malaria are not yet clearly identified[6].

From the epidemiological viewpoint rapid cure of malaria tends to reduce malaria transmission by

Table 2. Comparison of some sociological features of malaria patients at Polonnaruwa and Ragama hospitals

	Polonnaruwa	Ragama
1. *Sex*		
Male	88.9%	71%
Female	11.1%	29%
2. *Marital status*		
Married	39.7%	53%
Unmarried	57.1%	38%
Not determined	3.2%	9%
3. *Person accompanying to hospital*		
Parent	15.9%	34%
Spouse	7.9%	17%
Children	3.2%	25%
Other relatives	23.8%	14%
Friends	38.1%	7%
Unaccompanied	6.3%	3%
Not mentioned	4.8%	—
4. *Perception of illness as malaria*		
Yes	39.7%	29%
No	55.6%	57%
Don't know	4.8%	11%
Not determined	—	3%
5. *Other treatment before coming to hospital*		
Yes	61.9%	70%
No	36.5%	30%
Not determined	1.6%	—
6. *Source of previous treatment*		
Private medical practitioner	17.5%	61%
Others including traditional medicine	44.5%	9%

reducing the number of persons who are infective to mosquitoes. However, we speculate that it may cause the opposite effect by increasing the number of non-immune or malaria-susceptible individuals in the population. Delaying malaria treatment is ethically unacceptable, but the possible consequences of early treatment for malaria epidemiology need to be recognized.

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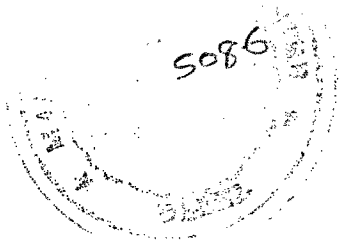

Breeding Habitats and their Contribution to *Anopheles stephensi* in Panaji

ASHWANI KUMAR* and D. THAVASELVAM*

A one-year longitudinal study conducted in 9 categories of breeding habitats in Panaji, Goa, showed that 1.1% of the 57,360 breeding sites contained *Anopheles stephensi* immatures and the overall positivity varied from 0.4 to 3.5% with a peak in June. The habitat-wise proportion of *An. stephensi* was : wells, 0-1.3%; fountains, 1.4-11.4%; masonry tanks, 0.8-6.1%; overhead tanks, 0.1-4.0%; curing water in construction sites, 0.6-9.0%; groundwater tanks, 0-1.4%; tyres, 0-8.9%; barrels and tins, 0-5.4%; and intradomestic containers, 0-1.9%. *An. stephensi* was breeding along with *An. subpictus*, *An. vagus*, *An. barbirostris*, *Culex quinquefasciatus*, *Cx. vishnui*, *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus*.

INTRODUCTION

Anopheles stephensi is an important vector of urban malaria in India. The species has been incriminated in different parts of the country and is capable of maintaining transmission at very low densities [1-4]. In urban areas, it plays, along with *An. culicifacies*, an important role in the periodic malaria epidemics[5]. *An. stephensi* recently established a strong foothold in Panaji, the capital city of Goa, resulting in an outbreak of malaria in 1986[6,7].

An. stephensi breeds in a variety of domestic and peridomestic habitats of permanent, semi-permanent and temporary nature. Most reports on the breeding of *An. stephensi* are based on spot larval surveys. Batra and Reuben[8], who conducted a longitudinal study on *An. stephensi* breeding in wells and cisterns in Salem (Tamil Nadu), found that wells were the main sites of vector breeding in that city. In Goa, *An. stephensi* could not be collected in adult catches [9,10] but breeding was detected in Panaji in a single spot survey in 29.6% out of 118 breeding sites[6]. Information on seasonal prevalence of *An. stephensi* in different habitats is lacking, which would be important in organising anti-larval measures. Hence, a longitudinal study on the breeding habitats of *An. stephensi* was conducted in Panaji in 1990. The results of this study are given in this paper.

MATERIALS AND METHODS

Study Area

Panaji is situated at 15° 31′N latitude and 73° 52′E longitude. The city has a population of about 43,000 and an area of 7.5 km² divided into

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15 municipal wards. Topographically the land is undulating and most of the inhabitation is on and around Altinho hillock. The river Mandovi runs along with northern expanse of the city. Weather conditions in Panaji are mild throughout the year with maximum temperature fluctuating between 30.8 and 36.2°C and minimum between 18 and 23.2°C, and the relative humidity varies from 75 to 95%. Rains start in May and continue up to November.

Sampling

A search for probable breeding sites of *An. stephensi* throughout the city revealed the presence of 593 operational wells, 32 abundant wells, 3004 overhead tanks, 789 groundwater cisterns, 191 masonry tanks, and 19 fountains. Besides, a large number of tyres, barrels, tins, bottles and a variety of other intradomestic containers were also surveyed on weekly basis. Five samples were drawn from each well using a galvanized iron bucket of 15-litre capacity. A similar number of samples were taken on each visit from fountains, overhead tanks, groundwater cisterns and masonry tanks with the help of dippers of 300 ml capacity and 12.5 cm dia. Plastic bowls of 300 ml capacity were used to draw samples from large tyres and barrels whereas, pasteur pipettes were used to collect immatures from narrow tyres, curing water and intradomestic containers. The immatures were transported to the laboratory in plastic containers with screw caps and reared at room temperature by providing a mixture of dog biscuit powder and yeast in 3:1 ratio. The adults emerged and dead larvae from each sample were identified using the keys of Christophers[11], Barraud[12], and Puri[13].

During the period of study, various control measures were undertaken following the detection of *An. stephensi* breeding in the city. These included weekly introduction of five species of larvivorous fishes, viz. *Aplocheilus blockii*, *Aplocheilus lineatus*, *Rasbora daniconius*, *Gambusia affinis* and *Poecilia reticulata*, at the rate of 5 fishes per m^2, in wells, tanks and fountains and wherever required. Thirty-two abundant wells were capped @85g/1000 cm^2 layer of expanded polystyrene beads[14]. The National Filaria Control Programme unit of Panaji used 1 ppm temephos (50% emulsifiable concentrate) to treat curing water and overhead tanks sparingly.

RESULTS AND DISCUSSION

Mosquito-breeding sites were searched on 67,360 occasions and 3010 (4.5%) were found positive for the mosquito immatures. Twenty-four species emerged from the samples. These were *Anopheles stephensi*, *An. culicifacies*, *An. nigerrimus*, *An. jamesi*, *An. barbirostris*, *An. theobaldi*, *An. varuna*, *An. tessellatus*, *An. splendidus*, *An. subpictus*, *An. vagus*, *Culex quinquefasciatus*, *Cx. tritaeniorhynchus*, *Cx. vishnui*, *Cx. nilgiricus*, *Cx. univittatus*, *Cx. (lutzia) fuscanus*, *Cx. gelidus*, *Cx. mimulus*, *Ae. aegypti*, *Ae. albopictus*, *Ae. vittatus*, *Armigeres subalbatus*, and *Toxorhynchites splendens*.

An. stephensi emerged from 747 samples, accounting for 1.1% of the total breeding habitats surveyed and 24.8% of the total positive sites. The positivity index of all the breeding sites and species pooled together varied from 2.2 to 9.8% in different months and peak positivity was observed in July (Table 1). On the other hand, *An. stephensi* positivity ranged between 0.4% in December and 3.5% in June.

The habitat-wise mosquito breeding in Panaji is given in Table 1. The data show that the overall positivity in wells declined from 8.3% in January to 1.6% in August. *An. stephensi* positivity was highest in June (1.3%) and there was no breeding from October till December. The low positivity of the wells can be attributed to the stocking of larvivorous fishes. Only occasionally I and II instar larvae were encountered in the wells. The positivity of *An. stephensi* fluctuated greatly in 19 ornamental fountains located in the gardens and bungalows. The highest positivity (11.4%) was detected in February and lowest (1.4%) in August.

Table 1. Habitat-wise mosquito breeding in Panaji, Goa

Months (1990)	Total breeding sites			Wells			Fountains			Masonry tanks			Curing water		
	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)
Jan	4107	170(4.1)	49(1.2)	980	81(8.3)	12(1.2)	32	4(12.5)	2(6.2)	244	15(6.1)	5(2.0)	86	7(8.1)	2(2.3)
Feb	3484	98(2.8)	38(1.1)	1036	27(2.6)	7(0.7)	35	6(17.1)	4(11.4)	171	11(6.4)	4(2.3)	52	9(17.3)	2(3.8)
Mar	5820	165(2.8)	32(0.5)	1617	68(4.2)	8(0.5)	25	2(8.0)	1(4.0)	420	28(6.7)	6(1.4)	76	8(10.5)	2(2.6)
Apr	5214	164(3.1)	49(0.9)	1427	52(3.6)	10(0.7)	36	4(11.1)	3(8.3)	314	24(7.6)	10(3.2)	194	14(7.2)	4(2.1)
May	5559	266(4.8)	102(1.8)	2039	56(2.7)	12(0.6)	40	3(7.5)	2(5.0)	475	45(9.5)	10(2.1)	242	27(11.1)	20(8.3)
Jun	5969	499(8.3)	208(3.5)	1581	63(4.0)	21(1.3)	46	4(8.7)	4(8.7)	688	111(16.1)	42(6.1)	356	57(16.0)	32(9.0)
Jul	4512	444(9.8)	87(1.9)	777	14(1.8)	5(0.6)	68	6(8.8)	4(5.9)	386	21(5.4)	7(1.8)	591	38(6.4)	22(3.7)
Aug	5192	205(3.9)	34(0.6)	746	12(1.6)	3(0.4)	73	3(4.1)	1(1.4)	356	17(4.8)	3(0.8)	643	19(2.9)	4(0.6)
Sep	5491	245(4.7)	38(0.7)	760	16(2.1)	3(0.4)	48	1(2.1)	1(2.1)	292	34(11.6)	7(2.4)	675	24(3.5)	6(0.9)
Oct	5464	220(4.0)	36(0.6)	676	10(1.5)	0	65	3(4.6)	3(4.6)	338	24(7.1)	5(1.5)	596	37(6.2)	8(1.3)
Nov	6461	311(4.8)	32(0.5)	1105	32(2.9)	0	66	5(7.6)	2(3.0)	516	38(7.4)	10(1.9)	697	43(6.2)	8(1.1)
Dec	10087	223(2.2)	42(0.4)	1598	31(1.9)	0	68	8(11.8)	2(2.9)	809	30(3.7)	11(1.3)	684	39(5.7)	9(1.3)
Total	67360	3010(4.5)	747(1.1)	14342	462(3.2)	81(0.6)	602	49(8.1)	29(4.8)	5009	398(7.9)	120(2.4)	4892	322(6.6)	119(2.4)

contd...

Table ... Intradomestic mosquito breeding in Delhi (contd.)

Months (1990)	Overhead tanks			Groundwater tanks			Tyres			Barrels and tins			Intradomestic containers		
	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)	No. surveyed	No. +ve for breeding (%)	*An. stephensi* breeding (%)
Jan	1904	24(1.3)	23(1.2)	567	11(1.9)	5(0.9)	28	13(46.4)	0	156	11(7.0)	0	110	4(3.6)	0
Feb	1414	19(1.3)	18(1.3)	307	5(1.6)	3(1.0)	41	14(34.1)	0	296	4(1.3)	0	132	3(2.3)	0
Mar	1900	13(0.7)	10(0.5)	611	10(1.6)	4(0.6)	65	15(23.0)	1(1.5)	954	17(1.8)	0	152	4(2.6)	0
Apr	1734	18(1.0)	13(0.7)	304	9(2.9)	4(1.3)	87	18(20.7)	4(4.6)	935	19(2.0)	1(0.1)	183	6(3.3)	0
May	1386	49(3.5)	46(3.3)	277	7(2.5)	4(1.4)	126	41(32.5)	6(4.8)	773	26(3.4)	2(0.2)	201	12(6.0)	0
Jun	2039	119(5.8)	82(4.0)	813	21(2.6)	8(1.0)	157	56(35.7)	14(8.9)	150	52(34.6)	5(3.3)	139	16(11.5)	0
Jul	1488	54(3.6)	30(2.0)	666	9(1.3)	0	132	111(84.0)	6(4.5)	240	94(39.1)	13(5.4)	164	97(59.1)	0
Aug	2152	12(0.5)	8(0.4)	804	7(0.9)	0	69	42(60.8)	3(4.3)	211	53(25.1)	10(4.7)	138	40(28.9)	2(1.4)
Sep	2052	9(0.4)	2(0.1)	765	10(1.3)	2(0.3)	53	24(45.3)	2(3.8)	595	57(9.6)	12(2.0)	251	70(27.9)	3(1.2)
Oct	2290	23(1.0)	10(0.4)	625	15(2.4)	2(0.3)	55	36(65.4)	2(3.6)	654	37(5.6)	5(0.8)	165	35(21.2)	1(0.6)
Nov	2220	27(1.2)	2(0.1)	904	19(2.1)	3(0.3)	150	68(45.3)	2(1.3)	698	50(7.2)	3(0.4)	105	29(27.6)	2(1.9)
Dec	4306	23(0.5)	9(0.2)	1202	44(3.7)	9(0.7)	55	14(25.4)	0	1177	25(2.1)	2(0.2)	188	9(4.8)	0
Total	24885	390(1.6)	253(1.0)	7845	167(2.1)	44(0.5)	1018	452(44.4)	40(3.9)	6839	445(6.5)	53(0.8)	1928	325(16.8)	8(0.4)

In masonry tanks near construction sites, breeding was found throughout the year but peaked in July when 6.1% supported *An. stephensi* breeding. In 1990, 126 multistorey buildings were under construction. Water used for curing cement on each floor of these buildings was found to support good breeding of *An. stephensi*. The overall breeding in curing water ranged from 2.9 to 17.3%, whereas per cent positivity of *An. stephensi* varied from 0.6 to 9.0. A sudden spurt in *An. stephensi* breeding was observed in the construction area from May to July and this was associated with monsoon, owing to which additional water accumulated on the floors of these buildings.

Overhead tanks supported breeding of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* in that order of prevalence. On an average, 1.6% of the overhead tanks were found breeding, with the highest breeding encountered in June when 5.8% overhead tanks supported mosquito breeding and 4.0% *An. stephensi*, and breeding was observed in all the months. The underground water sumps constructed near multistorey buildings were also found breeding throughout the year. The positivity of these tanks was 2.1% and varied from 0.9% in August to 3.7% in December, whereas *An. stephensi* was found in 0.5% tanks and breeding was negligible from July onwards.

Interestingly, tyres in the backyards and on the terraces of the buildings were found positive for *An. stephensi* throughout the study period except in the months of January, February and December. Breeding in tyres was most common and varied from 20.7 to 84%. Although a majority of these were found with *Ae. aegypti* yet up to 8.9% also supported *An. stephensi* breeding. Barrels and tins, mostly in hotel premises and constructions, were found positive throughout the year. *An. stephensi* was not, however, encountered in them from January to March and its positivity remained low in April and May. It gradually peaked in July when 13 of them contained *An. stephensi* breeding and positivity declined after September. Intradomestic containers such as grinding stones, coconut shells, pots and bottles were also checked. Although mosquito breeding varied from 2.3 to 59.1%, limited breeding of *An. stephensi* was encountered from September to November.

An. stephensi was found breeding with 8 other mosquito species in 294 out of 747, i.e. 39.3% habitats (Table 2). Three anophelines associated were *An. subpictus* (2.27%), *An. vagus* (0.53%) and *An. barbirostris* (0.13%). It was also found breeding in one habitat with *An. subpictus* and *An. vagus*. In as many as 136 (18.2%) habitats, it was found breeding with *Cx. quinquefasciatus*, in 5 (0.66%) habitats with *Cx. vishnui* and in 9 (2.54%) habitats with *Cx. quinquefasciatus* and *Ae. aegypti*. It shared 19 habitats with *Ae. aegypti*, 12 with *Ae. albopictus*, 5 with *Ae. vittatus* and 4 with both *Ae. albopictus* and *Ae. aegypti*. Thus, *An. stephensi* showed a high degree of interspecific association.

If all habitats are pooled together, *An. stephensi* breeds throughout the year but positivity starts

Table 2. Association of *Anopheles stephensi* with other mosquito species

No. of breeding sites				No. of samples in which *An. stephensi* was found with										
Surveyed	+ve	*An. stephensi* + other species	*An. stephensi*	*An. subpictus*	*An. barbirostris*	*An. vagus*	*An. subpictus* + *An. vagus*	*Cx. quinquefasciatus*	*Cx. vishnui*	*Cx. quinquefasciatus* + *Ae. aegypti*	*Ae. aegypti*	*Ae. albopictus*	*Ae. albopictus* + *Ae. aegypti*	*Ae. vittatus*
67360	3010	747	453	17	1	4	1	136	5	19	90	12	4	5

increasing in April and the population of immatures builds up, peaking in June when on an average 3.5% of the breeding sites searched were found supporting *An. stephensi*. This is followed by a declining trend in July and August, in turn followed by a low grade breeding till March (0.5 to 1% habitats support *An. stephensi*). Breeding is associated with the rainfall as initially with the onset of rains additional breeding habitats become available for *An. stephensi* but continuous rains disrupt breeding potential as could be seen by a drop in breeding during the rainy season.

A variety of habitats supported *An. stephensi* breeding either alone or with other species. In most of the habitats *An. stephensi* breeding was detected throughout the year in variable proportions. The study also revealed that bioenvironmental control measures could substantially reduce or eliminate *An. stephensi* breeding sites[15]. Greater attention needs to be paid to construction sites as these constitute an important epidemiological niche to promote transmission due to labour huts ments occupied by immigrants from endemic areas.

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Intradomestic Mosquito Breeding Sources and their Management

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Seven mosquito species were found to breed in intradomestic breeding sources. *An. stephensi* and *An. subpictus* bred in almost all types of containers. Among the culicines, *Cx. quinquefasciatus* and *Ae. aegypti* were predominant. Larval breeding was controlled through source reduction or introduction of larvivorous fish *Poecilia reticulata*. Health education helped in acceptance of the programme by the community. Intradomestic breeding positivity was 2-6% and 16-31% in experimental and control areas respectively.

INTRODUCTION

Insecticidal spraying as a means to interrupt malaria transmission is beset with many well documented problems such as vector resistance to insecticides, their high cost, misuse, refusal, mud plastering, sericulture, bee-keeping, environmental contamination, etc. This multifaceted problem coupled with administrative and financial constraints has brought malaria control to crossroads. Therefore, an integrated approach for the malaria control through non-insecticidal methods of disease vector control was launched in Nadiad taluka of Kheda distt. of Gujarat, with emphasis on source reduction, community participation, health education and biological control[1].

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Mosquitoes breed in intradomestic containers, which are used for water storage for domestic purposes. Hence a systematic study to control the mosquito breeding in intradomestic water containers through source reduction and introduction of larvivorous fish, *Poecilia reticulata*, along with health education was undertaken in the entire rural Nadiad taluka of Kheda distt. of Gujarat. The results of the two-year study are reported in this paper.

MATERIALS AND METHODS

All types of intradomestic breeding sources in the 100 experimental villages of Nadiad taluka were surveyed for mosquito breeding. The containers were classified into four categories, viz. overhead tank (OHT), intradomestic tank, cistern and miscellaneous containers (Table 1).

Intradomestic breeding sources were surveyed on weekly and monthly basis during 1988 and 1989

Table 1. Classification of intradomestic breeding sources

Habitat	Type
A. Overhead tank (OHT)	Cement tank
B. Intradomestic tanks (IDT)	1. Inside tank (IST) 2. Outside tank (OST) 3. Underground tank (UGT) 4. Earthen (Mud) pot (MP)
C. Cisterns	1. Barrel 2. Drum
D. Miscellaneous containers	1. Battery box 2. Tyre 3. Broken pitcher 4. Waste water collections

respectively in all the villages of Nadiad taluka, whereas quarterly surveys were done in the villages belonging to neighbouring talukas for comparison. Villages of Kheda distt. are rural to semi-urban type, mostly with *pucca* houses and tapped drinking water supply. Larval samples from the breeding sites were collected with the help of a dipper (9.5 cm dia. and 300 ml capacity), dropper and plankton net (120 mesh size) and brought to the laboratory for rearing and adult emergence. Adults that emerged were anaesthetized and identified using the keys of Christophers[2] and Barraud[3].

Intradomestic breeding in the experimental area was controlled by introducing the larvivorous fish *Poecilia reticulata* @ 5-10 fishes per container depending upon the container's size, or through emptying out the water at weekly intervals. Fishes were introduced in all the containers, except miscellaneous containers which were usually emptied out on a regular basis. Introduction of fishes was done both by the MRC workers and by the villagers themselves as and when required. In almost all the villages a small pond was used for rearing and collection of fishes. *Poecilia reticulata* was found most suitable because of its small size and high multiplication rate as well as its hardy nature in transportation[4]. Villagers were imparted health education about mosquitoes, their breeding habits and importance of their control for prevention of malaria[5], to create an awakening among the community and to enlist their cooperation in the programme. For this, live demonstrations were given to individuals and in group meetings.

RESULTS AND DISCUSSION

A survey of intradomestic breeding sources revealed the breeding of seven mosquito species,

Table 2. Species - specific breeding sources

Species	Type						
	OHT	UGT	OST	IST	MP	Cisterns	Misc. Cont.
An. culicifacies	+		+	+	+		
An. stephensi	+	+	+	+	+	+	+
An. annularis		+					
An. subpictus	+	+	+	+	+	+	+
An. barbirostris			+			+	
Cx. quinquefasciatus	+		+		+	+	+
Ae. aegypti	+	+			+	+	+
Total	5	4	5	3	5	5	4

+ denotes presence of the species; OHT — Overhead tank; UGT — Underground tank; OST — Outside tank; IST — Inside tank; MP — Earthen mud pot; Misc. cont. — Miscellaneous container.

viz. *An. culicifacies*, *An. stephensi*, *An. annularis*, *An. subpictus*, *An. barbirostris*, *Cx. quinquefasciatus* and *Ae. aegypti* (Table 2). *An. subpictus* and *An. stephensi* were found breeding in almost all types of containers. However, *An. culicifacies* preferred overhead tanks, outside tanks, inside tanks and earthen pots. *An. annularis* was found to breed only in underground tanks. Both *An. culicifacies* and *An. stephensi* were observed breeding in intradomestic containers regularly in spite of free access to peripheral breeding sources. The role played by the two vectors breeding in these sources in malaria transmission was however not quantified.

Habitat-wise per cent composition of different mosquitoes has been summarized in Fig. 1. Overhead tanks supported the maximum anopheline breeding, whereas cisterns, intradomestic tanks and miscellaneous containers supported mostly *Culex* breeding. In overhead tanks, *Culex* was the second predominant mosquito and *Aedes* was the second in cisterns.

Table 3 shows positivity of intradomestic breeding sources in experimental and control areas. Highest positivity was observed in miscellaneous containers, followed by cisterns, overhead and intradomestic tanks. During 1988 only 3.4% intradomestic containers were found positive in experimental area, whereas in control, their positivity was as high as 25%. Similarly, in 1989, only 4.5% intradomestic containers were found positive in comparison with 24.1% in control area. Fig. 2 shows that intradomestic positivity was kept under control throughout the study period. Control area showed high positivity and was always above 20% during corresponding period except for decline in June 1988 (16.5%). Statistical analysis also showed a significant difference between per cent breeding in control and experimental area (t = 11.17 and 26.65 respectively for both the years, $p < 0.01$). Table 4 shows the consolidated picture of the intervention measures taken for the control of mosquito breeding in intradomestic water collections. Breeding in 45,597 and 14,985 positive intradomestic breeding places (cumulative) was eliminated in 1988 and 1989 respectively and in 37,411 and 19,439 breeding sources (cumulative) larvivorous fish (*Poecilia reticulata*) was introduced during that period.

The study shows that through source reduction, health education and introduction of larvivorous

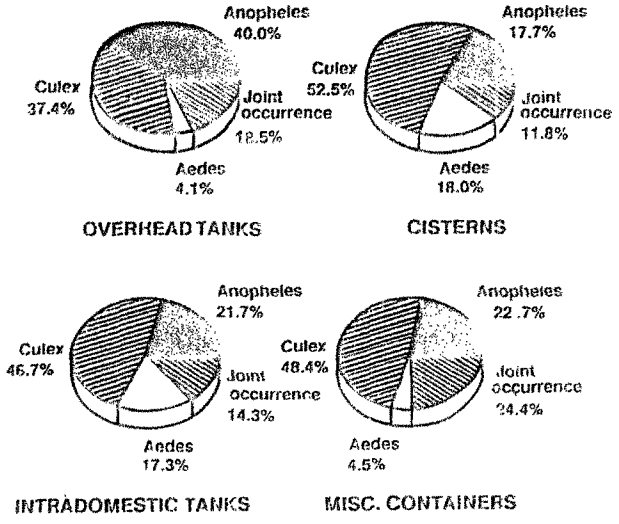

Fig. 1: Composition of mosquitoes in intradomestic breeding sources.

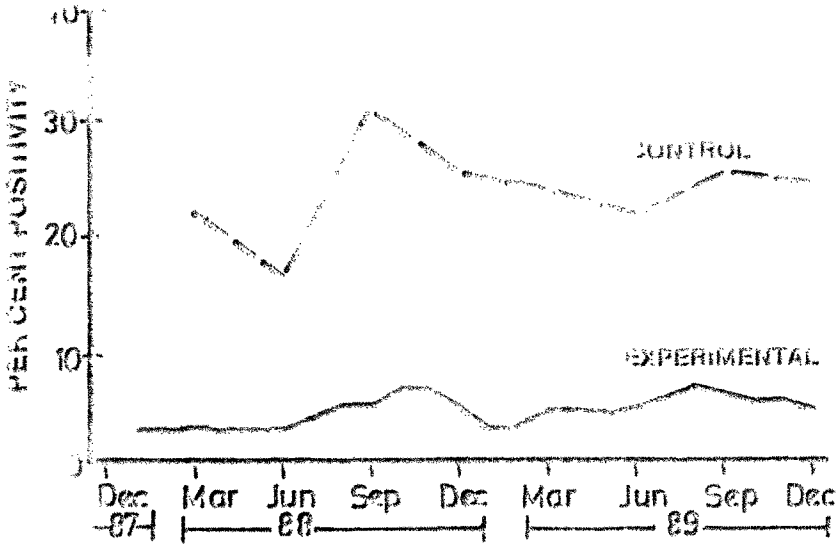

Fig. 2: Month-wise intradomestic positivity.

fishes, the intradomestic positivity in the experimental area was kept under control (< 5%) whereas in control area the positivity rate was above 20% throughout the period.

In urban area the intradomestic breeding sources play a major role in disease transmission[6]. Among the larvicides used under Urban Malaria Schemes, only Temephos (an organophosphorus compound) is being used to control mosquito breeding in intradomestic water collections. For the treatment of intradomestic containers, the ready-to-use Temephos (0.0125%) is poured into containers at a weekly dose of 20 ml/m^2. Although the mammalian toxicity of Temephos is very low, it has a prolonged residual effect[7]. At times it may not be possible to measure the water surface of the breeding container and to calculate the exact dose of Temephos. Beside, most of the larvicides are not only costly but toxic. However, the usage

Table 3. Intradomestic positivity in experimental and control areas

Type	1988						1989					
	Experimental			Control			Experimental			Control		
	Checked	+ve	%	Checked	+ve	%	Checked	+ve	%	Checked	+ve	%
OHT	88160	2918	3.3	3570	906	25.4	24423	1662	6.8	1585	422	26.6
Cisterns	557588	22463	4.0	3370	1005	29.8	198484	9640	4.8	1146	362	31.6
IDT	1198866	26167	2.2	42521	9963	23.4	299032	10443	3.5	21780	4992	22.9
Misc. Cont.	166072	16403	9.9	4500	1604	35.6	18281	2880	15.7	2515	744	29.6
Total	2010686	67951	3.4	53961	13478	25.0	540220	24625	4.5	27026	6520	24.1

OHT — Overhead tank; IDT — Intradomestic tank; Misc. Cont. — Miscellaneous container.

Table 4. Elimination of mosquito breeding and introduction of fishes in intradomestic containers

Type	1988		1989	
	Breeding eliminated	*Fish* introduced	*Breeding* eliminated	*Fish* introduced
OHT	910	5663	306	2592
Cisterns	13665	13841	5611	8298
IDT	16691	16960	6318	8483
Misc. Cont.	14331	947	2750	66
Total	45597	37411	14985	19439

OHT—Overhead tank; IDT—Intradomestic tank; Misc. Cont.—Miscellaneous container.

of fish in intradomestic containers is environmentally safe.

An. stephensi was found predominantly breeding in almost all types of breeding containers, which indicates the potential and preference of *An. stephensi* to breed in intradomestic water collections. Vishwanathan[8] observed that *An. stephensi* was a likely subsidiary vector in urban areas of Gujarat state, and the same species was responsible for malaria incidence in Ahmedabad[9] and Broach town[6]. Yadav *et al.*[10] also found the maximum percentage of *An. stephensi* (69.46%) in intradomestic containers, followed by *An. subpictus* (27.84%), *An. culicifacies* (2.58%), *An. annularis* (0.11%) and *An. tessellatus* (0.01%) in Kheda distt., Gujarat. Therefore, the role of intradomestic breeding and its control becomes more important particularly in urban or semi-urban areas, to interrupt disease transmission and mosquito nuisance. The control of *Ae. aegypti* in household containers in Central-Southern part of China, leading to dengue outbreak, by Chinese cat fish (*Clarias fuscus*) has been successfully demonstrated[11].

The method to control intradomestic breeding through the introduction of larvivorous fishes along with source reduction is environmentally safe and socially acceptable. More emphasis on such methods is likely to produce results under the National Malaria Control Programme both in rural and urban areas.

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Reversal of Chloroquine Resistance with Verapamil in *P. berghei in vivo*

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The effects of verapamil on the parasite susceptibility to chloroquine were examined in mice infected with chloroquine-sensitive and chloroquine-resistant lines of *Plasmodium berghei*. Verapamil in a dose of 10-50 mg/kg daily s.c. for 4 days did not affect the growth of both sensitive and resistant parasites. When verapamil in the same dose range was combined with 1.5 mg/kg chloroquine diphosphate, the chloroquine-sensitive parasites became more susceptible to chloroquine. Similarly, verapamil severely suppressed the growth of chloroquine-resistant parasites in combination with 3 mg/kg (base) of chloroquine, but the reversal of resistance was not complete. Thus, still higher doses of verapamil, which are not tolerated by the host, are required for the complete reversal of resistance.

This observation was confirmed by the finding that verapamil at 50 mg/kg increased the acute toxicity of chloroquine in mice. Further toxicological evaluation of the reversing agents is called for before clinical trials are contemplated.

INTRODUCTION

An escalating problem in the treatment of malaria over the last 25 years has been the emergence in many parts of the world, including India, of malarial parasites that are resistant to multiple, differently structured antimalarial drugs[1]. Certain calcium channel blockers, phenothiazines, calmodulin inhibitors, tricyclic anti-depressants and antihistaminic drugs known collectively as reversing agents – reverse the resistance to chloroquine in *P. falciparum in vitro*[2-4].

Reversal is due to the inhibition of an efflux of chloroquine from the resistant parasites resulting in increased accumulation of chloroquine inside the parasite[3]. This process probably involves blockade of P-glycoprotein (Pgp) encoded by multiple drug resistant (mdr) genes akin to that of mdr genes of mammalian tumour cells[5-7]. Unfortunately, Pgp expression is not confined to drug-resistant cells; it is present in normal human tissues as well[8-9]. If normal tissues use Pgp to rid themselves of harmful substances, there might be abnormalities in secretory or excretory functions

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of these tissues owing to the use of such reversing drugs. Such accumulation of toxins/drugs inside the cytosol of normal cells may lead to toxicity.

In this study the effects of verapamil, a calcium channel blocker, were studied *in vivo* in mice infected with chloroquine-sensitive and -resistant lines of *P. berghei*. Acute and subacute toxicity tests were performed to find the implication for toxicity.

MATERIALS AND METHODS

The experiments were carried out in randomly selected Balb(c) mice of either sex weighing 20-25 g. Food and water were given *ad libitum* to all the animals.

In the first set of experiments the mice were divided into 5 groups, each consisting of 5 mice. Each animal was injected with 5×10^6 *P. berghei*

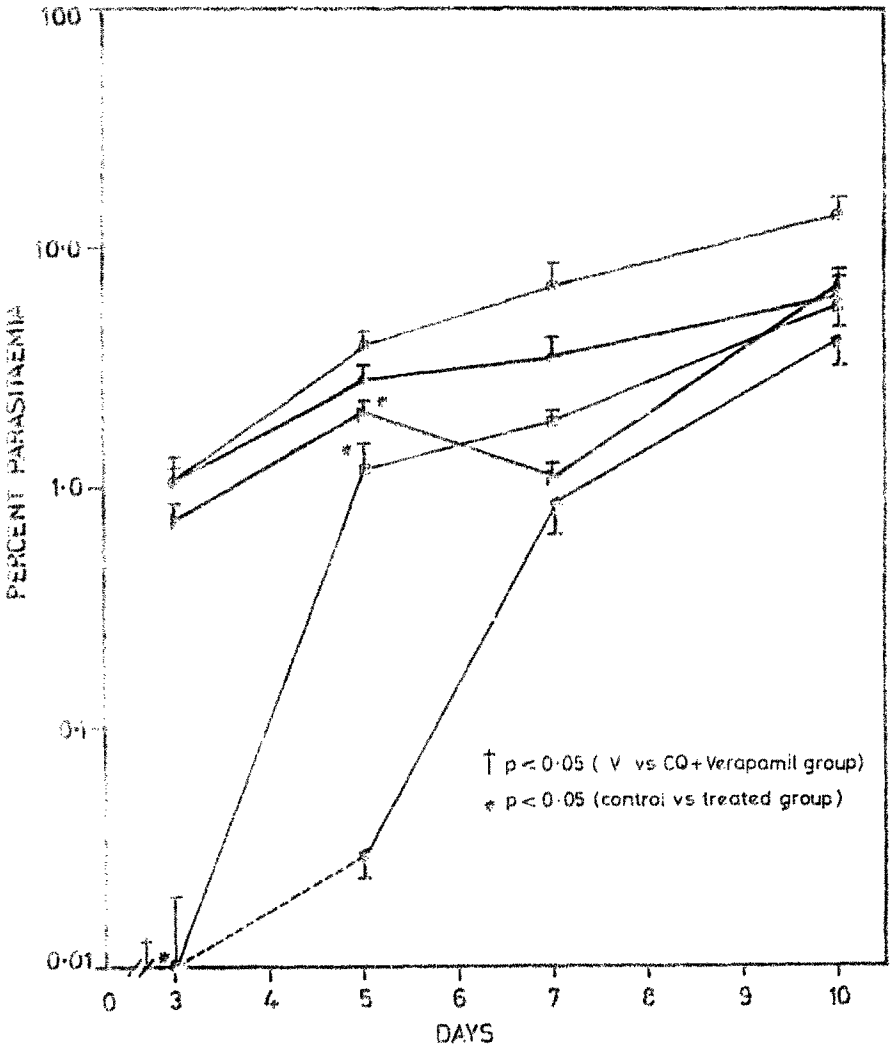

Fig. 1: Suppression of the growth of the chloroquine-sensitive strain of *P. berghei* in mice that received none (o), verapamil 30 mg/kg (●) or verapamil 30 mg/kg with chloroquine at 1 (◀) 2 (□) or 3 (■) mg/kg s.c. daily for 4 days.

(sensitive strain) infected RBCs in a volume of 0.1 ml. Group I served as control (no antimalarial drug treatment) and was injected with 0.9% saline in a dose of 0.1 ml/10g body weight. Drug sensitivity tests were carried out by the 4-day test as described by Peters[10]. All the drugs were given subcutaneously (s.c.) in a volume of 0.1 ml/10 g body weight from Day 0 (day of infection) to Day 3 consecutively. Group II received chloroquine diphosphate in a dose of 1.5 mg/kg, while groups III, IV and V received verapamil hydrochloride in doses of 10, 30 and 50 mg/kg respectively in addition to 1.5 mg/kg of chloroquine diphosphate.

The same procedure was followed for the second experiment but in this case, group I served as control, group II received verapamil hydrochloride in a dose of 30 mg/kg s.c., while groups III, IV and V received chloroquine diphosphate in doses of 1.2 and 3 mg/kg respectively in addition to the above dose of verapamil.

In the third experiment the mice were infected with chloroquine-resistant strain of *P. berghei* and the drug treatment schedule was: group I control, group II received chloroquine (base) at 3.0 mg/kg, and groups III, IV and V received in addition to chloroquine, verapamil hydrochloride in doses of 10, 30 and 50 mg/kg respectively.

Parasitaemias were determined in each animal in all the experiments on Days 3, 5, 7 and 10 by counting the number of parasitized RBCs per 10,000 RBC in tail blood smears stained with JSB.

Data were analysed by student 't-test', a p-value of less than 0.05 being considered significant.

Acute toxicity (LD50) tests were done using chloroquine alone and in combination with verapamil.

For subacute toxicity tests mice were administered chloroquine (base) alone at 6 mg/kg or chloroquine (base) at 6 mg/kg with verapamil hydrochloride (50 mg/kg) daily s.c., for 2 weeks and then killed by decapitation. Histopathological examination of liver, kidney, spleen, muscles, adrenals and eyes was carried out after fixing the tissues with normal saline and routine processing of paraffin sections.

RESULTS

Verapamil by itself at doses of 10-50 mg/kg did not affect the growth of chloroquine-sensitive lines of *P. berghei* ($p > 0.05$) (Fig. 1). The growth of chloroquine-sensitive *P. berghei* was slightly inhibited with 1.5 mg/kg chloroquine diphosphate on Days 3,5 and 10 ($p < 0.05$) as compared to control mice (Fig. 2). The parasite growth, however, was totally suppressed with 3.0 mg/kg of chloroquine diphosphate from Days 3 to 10 (data not shown). Addition of verapamil hydrochloride in doses of 10-50 mg/kg potentiated the effect of chloroquine (Fig. 2). No parasites appeared on Day 3 in mice given 1.5 mg/kg of chloroquine in combination with verapamil hydrochloride at 30 mg/kg ($p < 0.05$). It thus follows that verapamil increased the susceptibility to chloroquine in chloroquine-sensitive parasites.

In contrast to chloroquine-sensitive parasites, the resistant parasites were not affected by chloroquine (base) at 3 mg/kg ($p > 0.05$) (Fig. 3). Addition of verapamil hydrochloride in doses of 10-50 mg/kg showed marked effects. Verapamil hydrochloride in a dose of 50 mg/kg in combination with the same dose of chloroquine totally suppressed growth on Days 3 and 5. The inhibitory effect of verapamil continued to be statistically evident on Days 7 and 10 ($p < 0.01$) as compared to the group which received only chloroquine. Thus verapamil reversed the resistance to chloroquine in the chloroquine-resistant strains but even with the highest dose of 50 mg/kg, as used in this study, the parasite growth occurred subsequent to Day 5 (doses higher than 50 mg/kg produced some mortality thereby making the interpretation of the results difficult).

LD50 of chloroquine when given i.p. in male mice was 60 mg/kg (base). Addition of verapamil

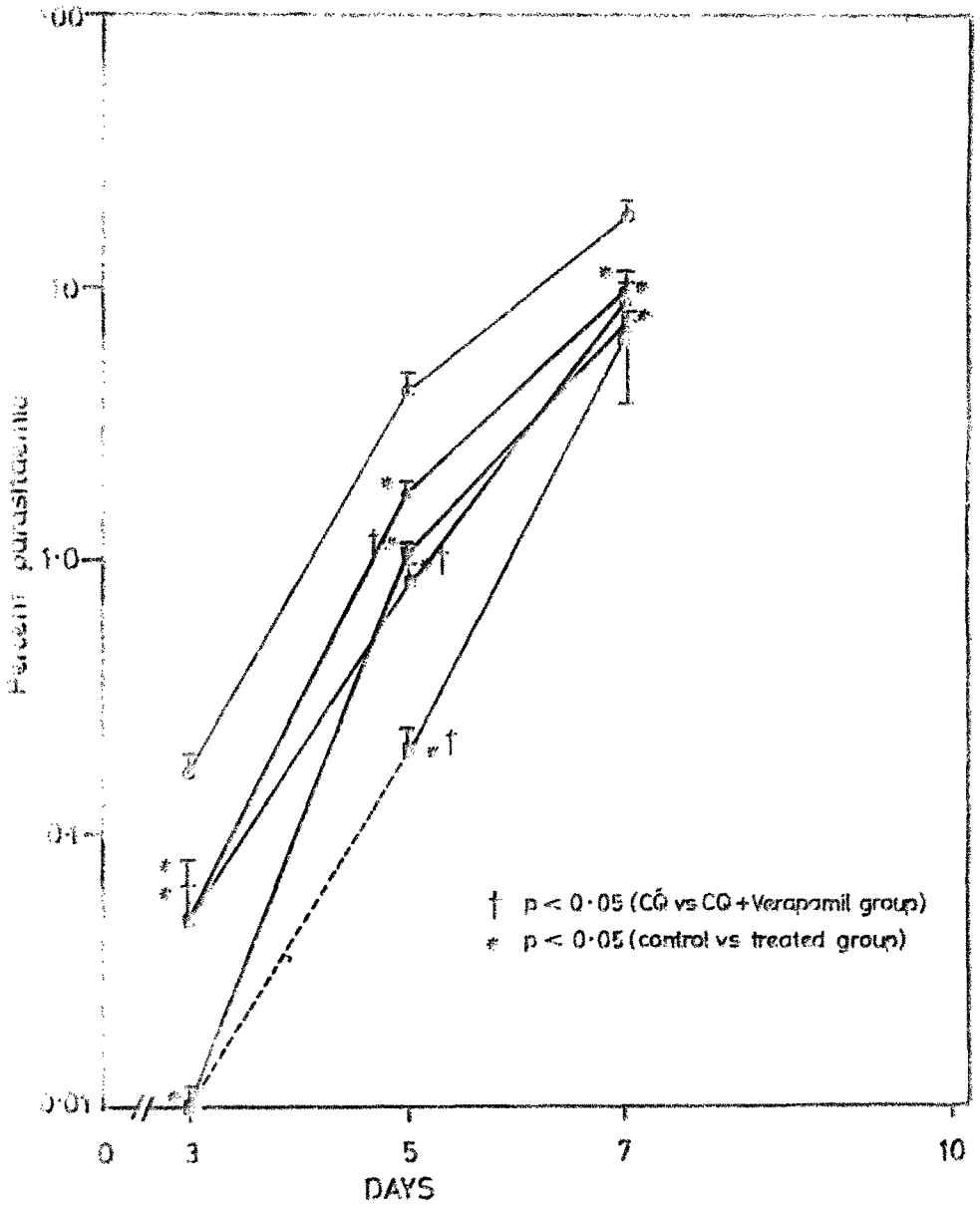

Fig. 2: Effect of verapamil on the chloroquine-sensitive strain of *P. berghei* in mice that received none (o), 1.5 mg/kg of chloroquine (●) or chloroquine 1.5 mg/kg with verapamil at 10 (◀), 30 (●) and 50 (□) mg/kg s.c. daily for 4 days.

hydrochloride in a dose of 50 mg/kg s.c. decreased the LD50 of chloroquine (base) to 40 mg/kg while verapamil alone in a dose 50 mg/kg did not produce any mortality. Histopathological analysis of spleen, liver, lung, eye and muscle did not reveal any significant changes in combination-treated groups except for occasional focal areas of necrosis in liver of 2 out of 5 animals. Such changes were observed in 1 out of 3 animals which were treated with only chloroquine.

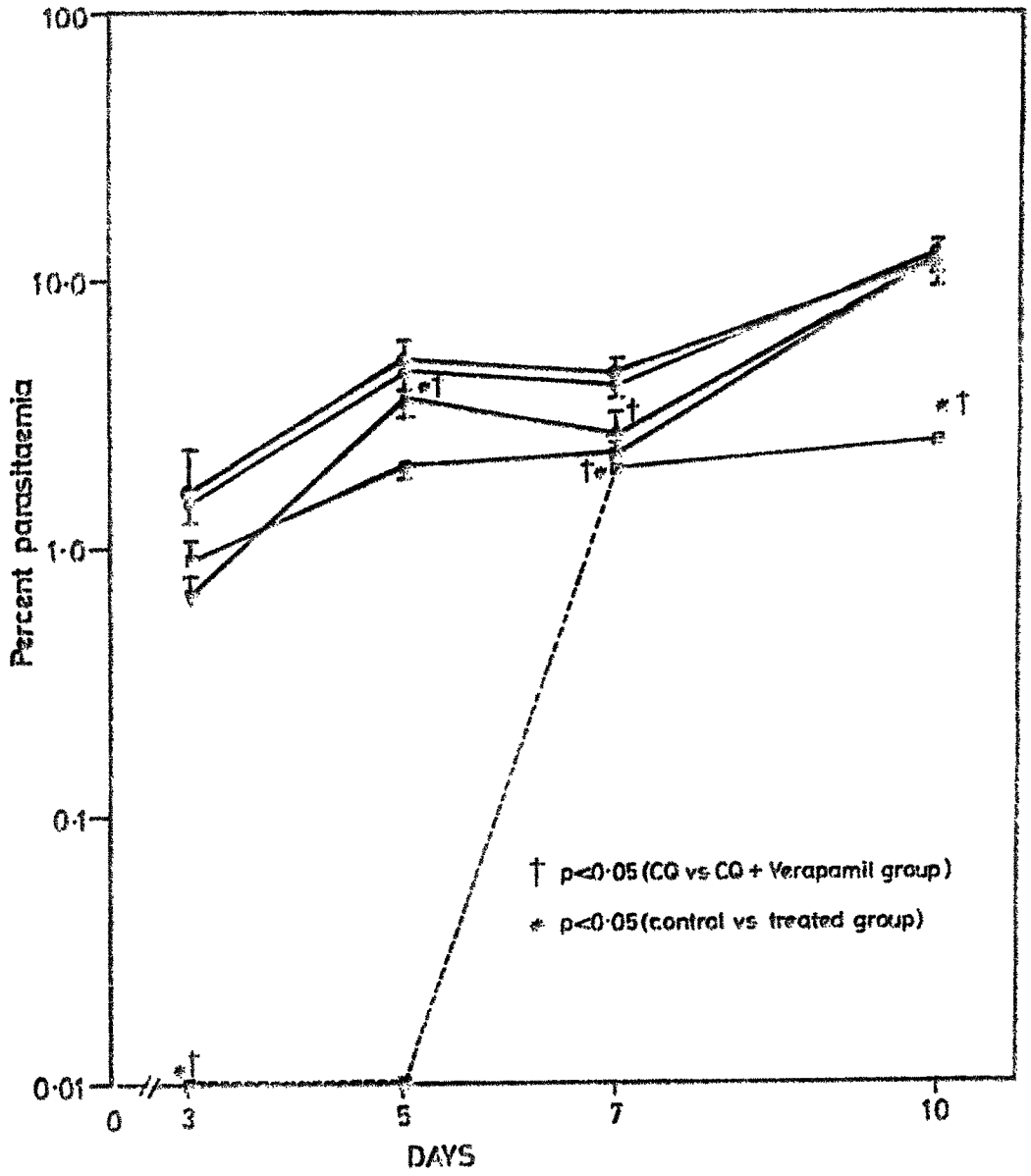

Fig. 3: Effect of verapamil on the chloroquine-resistant strain of *P. berghei* in mice that received none (o), 3.0 mg/kg (base) of chloroquine (●) or chloroquine 3.0 mg/kg (base) with verapamil at 10 (◢), 30 (▽) or 50 (□) mg/kg s.c. daily for 4 days.

DISCUSSION

Chloroquine-resistant *Plasmodium* is reported to accumulate less chloroquine than the susceptible parasites, the reason being increased efflux of the drug[11-13]. Calcium channel blockers, tricyclic antidepressants, etc., interfere with the efflux and reverse the resistance[3,13]. The reversal has been noticed only in chloroquine-resistant *P. falciparum* strains[2,13]. However, our study indicates that

verapamil increases susceptibility of chloroquine-sensitive strains of *P. berghei* also to chloroquine. Similar results were reported by Peters *et al.*[4] with cyproheptadine against chloroquine-sensitive lines of *P. falciparum* and *P. berghei* and by Tanabe *et al.*[14] with calcium antagonists against *P. chabaudi*.

Though the exact mechanism is not clear, both sensitive and resistant lines may have genes equivalent to mdr genes, which may be expressed to a lesser extent in sensitive strains.

As far as the clinical use of such combinations is concerned, their iatrogenic toxicity should be considered. Watt *et al.*[15] have reported deleterious effects in Hep-G2 liver cells when supraphysiological concentrations of chloroquine and verapamil that were nontoxic individually were combined *in vitro*. The explanation given was that increased chloroquine accumulation in normal tissues produced cell death.

This study shows that although verapamil at 50 mg/kg enhances the acute toxicity of chloroquine, it does not induce any histopathological changes in the tissues. However, for complete reversal of chloroquine-resistance still higher doses of verapamil will be required for clinical use, which is not recommended in the light of the above findings.

Toxicity studies with other more potent reversing agents which act by a similar mechanism and have less systemic side effects are called for before clinical trials are contemplated.

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SHORT NOTES

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Anopheline Breeding in Ponds of Central Gujarat with Reference to Water Hyacinth Infestation

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Water hyacinth (*Eichhornia crassipes*), a free-floating aquatic plant found in warmer parts of the world, also occurs throughout India in a variety of water bodies. The weed disturbs flora and fauna and supports the breeding of various vectors of diseases[1]. It also provides shelter to adults and prevents the predation of mosquito larvae by their natural enemies. The association of anopheline breeding with aquatic plants has been studied by several workers[2-9]. Many ponds of the Kheda distt. of Gujarat were found heavily infested with water hyacinth. In view of the ever changing agroclimatic conditions and mosquito behaviour, this study was undertaken to investigate the composition of anophelines in water hyacinth-infested and weed-free ponds.

Eight ponds in seven villages of Kheda distt. were selected, four densely infested with new as well as old water hyacinth plants and four free from any major aquatic vegetation. The total area of the ponds infested with water hyacinth was 22.40 ha with an average density of 71 plants/m^2, whereas ponds free from aquatic vegetation had an area of 53.40 ha. In the latter category, some infestation with vegetation like *Hydrilla*, *Ipomea*, *Azolla*, *Nymphea* and *Lemna* occurred during winter season. Fortnightly larval collections from March 1989 to February 1990 were made from the periphery of all the eight ponds using a dipper (9.5 cm dia.; 300 ml capacity) and larval densities were recorded. Immatures were brought to the laboratory, reared up to adults and identified using the key of Christophers[10]. The mean diversity per individual ($\bar{d}$) was calculated for each category of pond for different seasons to evaluate the apportionment of individuals among species by applying the uncertainty function of Shannon and Weaver[11]. To quantify the associations of different species occurring together, C_g Index of association of Hurlbert[12] was used. The statistical significance was assessed with the corrected χ^2 formula as indicated by Pielou[13] for approximating a discrete distribution. When any cell value was equal to or less than five, Fisher's exact test was applied.

Twelve anopheline species were observed breeding in water hyacinth-infested ponds, whereas only nine species were encountered from weed-free ponds (Table 1). *An. jamesii*, *An. stephensi* and *An. tessellatus* were absent in the latter. Five anopheline species, viz. *An. subpictus*, *An. aconitus*,

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Table 1. Composition (per cent) of anophelines in water hyacinth-infested and weed-free ponds

Species	Water hyacinth-infested ponds				Weed-free ponds				ANOVA
	Summer	Monsoon	Winter	Total	Summer	Monsoon	Winter	Total	
1. *An. aconitus*	15.03	31.25	13.25	20.54	0.60	3.48	2.05	2.13	†
2. *An. annularis*	6.53	11.97	27.10	15.26	37.8	18.40	66.43	44.59	*
3. *An. barbirostris*	1.30	23.95	7.83	11.93	1.82	4.47	19.86	10.65	ns
4. *An. culicifacies*	6.53	4.16	1.20	3.91	2.43	4.47		1.97	ns
5. *An. fluviatilis*	2.61			0.78			1.02	0.45	ns
6. *An. jamesii*	1.30			0.39					ns
7. *An. nigerrimus*	11.76	7.29	19.87	12.72	28.04	9.95	8.21	13.69	ns
8. *An. pallidus*			0.60	0.19			0.68	0.30	ns
9. *An. stephensi*	0.65			0.19					ns
10. *An. subpictus*	37.90	18.22	29.51	27.78	29.26	59.20	0.34	25.57	ns
11. *An. tessellatus*	15.03	2.60		5.47					ns
12. *An. varuna*	1.30	0.52	0.60	0.78			1.36	0.60	ns
Total adults which emerged	153	192	166	511	164	201	292	657	
Total samples collected	32	28	28	88	28	28	29	85	

Single factor analysis of variance for significance; † ($p < 0.05$); * ($0.05 > p < 0.25$); ns — ($p > 0.25$); na — Not applied due to rare occurrences.

An. annularis, *An. nigerrimus* and *An. barbirostris*, contributed 88 per cent of the total emergence in water hyacinth-infested ponds; except for *An. tessellatus* (5.47%) and *An. culicifacies* (3.91%) each of the other five species accounted for less than one per cent. *An. annularis* (44.59%) was the most dominant species in weed-free ponds followed by *An. subpictus* (25.57%), *An. niger-*

Table 2. Number of species which emerged, total larval density (TLD) per dip and the diversity ($\bar{d}$) during different seasons

Type of pond	Attribute	Summer (Mar - Jun)	Monsoon (Jul - Oct)	Winter (Nov - Feb)	Mean
A. Water hyacinth-infested	No. of spp.	11	8	8	9[a]
	TLD	11.31	5.98	7.98	8.42[a]
	$\bar{d}$	1.84	1.62	1.62	1.69[a]
B. Weed-free	No. of spp.	6	6	8	6.66[a]
	TLD	12.87	6.77	9.69	9.78[a]
	$\bar{d}$	1.28	1.25	1.04	1.19[b]

Mean values followed by the same letter are not significantly different from each other using least significant range test ($p < 0.05$).

rimus (13.69 %) and *An. barbirostris* (10.65%), and except for the former the differences in the composition of the latter three species in water hyacinth-infested and weed-free ponds were insignificant ($p > 0.25$).

Marked differences were observed in the seasonality of breeding in both types of ponds. In water hyacinth-infested ponds, *An. subpictus* was predominant during summer and winter seasons, whereas, during monsoon, *An. aconitus* was dominant. In weed-free ponds, *An. subpictus* bred profusely during monsoon; however, during summer and winter it was replaced by *An. annularis*. *An. aconitus* showed predilection for ponds with water hyacinth plant ($p < 0.05$) and bred pro-

Table 3. Associations among anophelines collected from ponds of Kheda district, Gujarat
(Upper triangular matrix = Hurlbert's index (* $p < 0.05$), lower triangular matrix = number of joint occurrences)

a. Water hyacinth-infested ponds

Species	*A.ac.*	*A.an.*	*A.b.*	*A.c.*	*A.f.*	*A.j.*	*A.n.*	*A.p.*	*A.st.*	*A.su.*	*A.t.*	*A.v.*
An. aconitus (A.ac.)		-0.224	0.368	0.276	0.0		-0.306*		0.0	0.0	0.0	1.0
An. annularis (A.an.)	4		0.0	-0.570			-0.762*	0.0	0.0	-0.226	0.0	
An. barbirostris (A.b.)	13	4		0.156			-0.288		0.0	-0.176	0.0	
An. culicifacies (A.c.)	9	1	6		0.0	0.0	0.271			0.0		0.0
An. fluviatilis (A.f.)	1			1			1.0*				0.0	
An. jamesii (A.j.)					1		0.0				0.0	
An. nigerrimus (A.n.)	8	1	5	8	3	1				-0.377	0.0	0.0
An. pallidus (A.p.)		1										
An. stephensi (A. st.)	1	1	1							0.0	0.0	
An. subpictus (A.s.u.)	14	4	7	6			7		1		0.0	0.0
An. tessellatus (A.t.)	1	1	1		1	1	1		1	1		
An. varuna (A.v.)	3			1			2			1		
Total positive	33	12	20	15	3	1	26	1	1	32	3	3
Total negative	41	62	54	59	71	73	48	73	73	42	71	71

b. Weed-free ponds

Species	*A.ac.*	*A.an.*	*A.b.*	*A.c.*	*A.n.*	*A.p.*	*A.su.*
An. aconitus (A.ac.)		0.0	0.189	0.0	0.0		0.0
An. annularis (A.an.)	5		-0.272	0.0	-0.091	0.0	-0.196
An. barbirostris (A.b.)	4	7			0.316		-0.196
An. culicifacies (A.c.)	2	5			0.0		0.407
An. nigerrimus (A.n.)	4	14	10	3			-0.171
An. pallidus (A.p.)		2					
An. subpictus (A.su.)	4	10	5	5	8		
Total positive	10	39	17	8	27	2	25
Total negative	58	29	51	60	41	66	43

fusely throughout the year, a finding which is in agreement with previous observations[4]. *An. annularis* was more abundant during winter in both types of ponds, but showed preference for weed-free ponds ($0.05 > p < 0.25$). Considerable changes were also observed in the composition of *An. barbirostris*, *An. nigerrimus* and *An. subpictus* during different seasons. *An. culicifacies* showed preference to water hyacinth and was most abundant during summer.

Maximum number of species (11) were observed breeding in water hyacinth-infested ponds during summer, whereas in weed-free ponds most species preferred to breed during winter. This may perhaps be due to the fact that the former offers favourable microclimatic conditions and simultaneously other breeding sources become scarce; hence larval densities were also high during summer in both the categories of ponds (Table 2). Diversity ($\bar{d}$) was more in water hyacinth-infested ponds, which is characteristic of fauna where an equitable number of specimens among species is present. Maximum diversity was observed during summer.

Thirty-seven pairings from water hyacinth-infested ponds revealed six positive and eight negative associations (Table 3a). Maximum positive (+1) association was observed between *An. aconitus* and *An. varuna*, and *An. nigerrimus* and *An. fluviatilis* ($p < 0.05$). *An. nigerrimus* was negatively associated with *An. annularis* and *An. aconitus* ($p < 0.05$).

Fifteen pairings from weed-free ponds revealed three positive and five negative associations (Table 3b). *An. subpictus* was negatively associated with *An. barbirostris*, *An. annularis* and *An. nigerrimus* in both categories of ponds. Also, *An. annularis* and *An. nigerrimus* produced negative C_8 values and *An. aconitus* and *An. barbirostris* were positively associated in both — water hyacinth-infested and weed-free ponds. A most striking difference in associations of different species in the two types of ponds was between *An. barbirostris* and *An. nigerrimus*, which indicated repulsion for each other in water hyacinth-infested ponds and attraction in weed-free ponds, respectively.

Removal of water hyacinth from ponds in many ways is beneficial as it improves the quality of water. Also, mosquito breeding can be controlled using larvivorous fishes along with food fish culture[14]. However, further studies are required to ascertain the breeding potential of vectors of diseases like Japanese encephalitis and filaria.

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Malaria Morbidity Survey in Schoolchildren in Age Group 5-15 Years in an Urban Area

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Malaria morbidity surveys have a significant role to play in school health programmes. The present study is aimed at finding malaria prevalence among schoolchildren in two private schools of old Hyderabad city, as a previous Malaria Survey in 1989 in the surrounding areas resulted in the detection of a sizable number of positive cases including asymptomatic carriers[1]. It also aims at applying certain recent methods in epidemiology, namely tests of validity of measurements[2, 3] which had not been used hitherto in malariometry and consequently are not covered in the WHO monograph on Statistical Methods in Malaria Eradication[4]. A random sample of two schools out of a total of 15 privately managed schools in wards 17 and 18 of Hyderabad city was chosen for this study in March 1990. The two schools together had 311 children in the age group of 5-15 years on the rolls, out of which 20 (6.43%) could not be included in the medical check-up on account of their continued absence. Among the 291 screened, 140 were boys and 151 were girls. The history of pyrexia during the previous fortnight was elicited and blood smears were collected. A team from the National Institute of Nutrition, Hyderabad, consisting of one doctor, one biochemist and two laboratory technicians, simultaneously collected blood samples from these schoolchildren to assess the Hb concentration with filter paper technique, using modified cynmeth-haemoglobin method. The blood smears were stained with Leishman's. The history of pyrexia along with that of any treatment received thereof was elicited by paramedical staff of Department of Social and Preventive Medicine from the schoolchildren and their parents after home visits. The children under study did not receive any antimalarials during the period of enquiry regarding history of pyrexia. Out of 291 schoolchildren, 22 (6 boys and 16 girls) were found positive for malaria (20 *Pv* + 2 *Pf*). Out of 20 *Pv*, there were 11 in schizont stage and nine in trophozoite stage and the two positive for *Pf* showed gametocytes. A history of pyrexia in the fortnight preceding the inquiry was present in 72 schoolchildren, and this is shown in Table 1. The history of pyrexia here as a screening procedure for malaria would miss 36.4% (100-sensitivity) of true positive cases, resulting in a false negative rate of 2.7% which otherwise would continue to be a hidden reservoir of infection but for the mass blood survey of this study. There is a significant relationship between pyrexia and parasite positivity ($p < 0.001$) but the positive predictive value of pyrexia is

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Table 1. Relationship between history of pyrexia and lab. diagnosis of malaria in the study population

		Lab. diagnosis of malaria		Total
		+ve	–ve	
History of pyrexia	Present	14 (*a*)	58 (*b*)	72
	Absent	8 (*c*)	211 (*d*)	219
Total		22	269	291 (*n*)

Sensitivity = $[a/(a+c)] \times 100 = 63.6\%$;
False –ve rate = $(c/n) \times 100 = 2.7\%$

Specificity = $[d/(b+d)] \times 100 = 78.4\%$;
False +ve rate = $(b/n) \times 100 = 18.9\%$

Positive predictive value = $[a/(a+b)] \times 100 = 19.44$

Negative predictive value = $[d/(c+d) \times 100 = 96.34$

$Q = -0.728$; $\chi^2 = 17.039$; $p < 0.001$.

<20% (19.44). Both the positives for *Pf*, detected here, were five years old and had no history of pyrexia, which meant persistence of infection and sufficient scope for disease spread in the community and therefore needed a drastic approach to cut down transmission specially in urban areas.

Out of 291 study population, 101 (34.7%) were found anaemic as per the criteria laid down in the report of WHO Expert Group on Anaemia[5]. The relationship between malaria and anaemia is given in Table 2, which shows negative correlation between the two (Q = –0.851). This finding, which is by no means new, is in accord with the data of an earlier study carried out in the neighbourhood of DCMS, which ruled out malaria as a principal cause of this form of under-nutrition[6]. It is therefore inferred that malaria may be at best one of the factors in 'unmasking' the already existing borderline dietary deficiency states including anaemia[7].

Table 2. Relationship between presence of malaria and anaemia

		Anaemia		Total
		Present	Not present	
Lab. diagnosis of malaria	+ve	1	21	22
	–ve	100	169	269
Total		101	190	291

$Q = -0.85$; $\chi^2 = 8.1689$; $p < 0.01$.

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Enhancing the Efficacy of *Gambusia affinis* to Control Mosquito Breeding in Ponds

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The Bhabar and Terai areas of distt. Nainital are well known for high transmission of *vivax* and *falciparum* malaria. The slide positivity rate was as high as 90 in 1982, and in subsequent years it varied from 40 to 60[1-3]. *An. culicifacies* and *An. fluviatilis* are the two well-known vectors which have been incriminated in recent years[4].

In Bhabar, water is scarce because soil is porous and has a high content of sand, pebbles and stones. The area is in the foothills and the detritus material has been deposited here gradually. The porous soil does not retain water for long, and the sources of water are canals and subcanals, which run 2 to 4 times in a month. People use canal water for drinking, for their cattle and for other domestic purposes in addition to irrigation.

Water for domestic use is stored in cement tanks of a variety of shapes and sizes which may be underground or on the ground and covered or uncovered. Small ponds, called *pokhars*, are constructed for storing water for the cattle.

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These ponds have become impermeable owing to silting and get periodically refilled by the canal water.

These ponds are the breeding places for the important malaria vector *An. culicifacies* and other anophelines such as *An. maculatus*, *An. subpictus*, *An. nigerrimus*, *An. barbirostris* and *An. vagus*. *Culex quinquefasciatus* was also found breeding in ponds. Under the bioenvironmental malaria control strategy, efforts were made to tackle the mosquito breeding by the application of *Gambusia affinis*. In the beginning the shorelines were not clear and therefore the results were not satisfactory. For fishes to act effectively it is important that shorelines are kept as smooth as possible. Weekly maintenance of each *pokhar* took a great deal of time, and the main problem was that weeds used to grow abundantly within a week's time owing to the availability of water and manure in the form of decomposing material in the pond.

The solution to the problem was to deweed and cut the margins vertically followed by thick mud-plastering of the walls. The mud-plaster dries up soon and arrests the growth of grass and as a result, fishes can effectively reach the margins and devour mosquito larvae. There are 745 ponds in the experimental area and 125 ponds in the

control area. In the control area, margins of ponds were not repaired and fishes were not released. In the experimental ponds mud-plastering was done and larvivorous fishes were released.

For mud-plastering the shoreline is first cut vertically and the vertical margin extends into the water. Most of the weeds are removed by this process but deeply situated roots remain. As far as possible efforts were made to remove roots. The fresh soil was then soaked in water and the mud thus formed was plastered thickly with force on the inner sides of vertical wall and on the horizontal upper bank. The wall surface was then smoothened and maintained in that condition by the communities.

The positivity of ponds for mosquito breeding was recorded every week. During 1988 the mosquito breeding percentage in plastered ponds was 8.7, as compared to 69.2 in unplastered ponds. Similarly, during 1989 the figures were 3.3 and 55.2 respectively. The results of two year's observations are summarized in Table 1 and Fig. 1. Some ponds in the experimental area were also

Table 1. Impact of repairing of ponds on mosquito breeding using *Gambusia* fishes

Month		1988			1989		
		No. surveyed	No. (+)ve	(%)	No. surveyed	No. (+)ve	(%)
Jan	Expt.	5779	196	(3)	1983	37	(2)
	Cont.	313	229	(73)	364	102	(28)
Feb	Expt.	3011	406	(13)	1761	55	(3)
	Cont.	283	217	(77)	303	61	(20)
Mar	Expt.	2834	379	(13)	2004	88	(4)
	Cont.	291	245	(84)	379	97	(26)
Apr	Expt.	2970	293	(10)	1622	67	(4)
	Cont.	295	241	(82)	256	112	(44)
May	Expt.	2325	372	(16)	2233	78	(3)
	Cont.	307	270	(88)	518	348	(67)
Jun	Expt.	2590	410	(16)	2123	90	(4)
	Cont	317	297	(94)	487	455	(93)
Jul	Expt.	2606	189	(7)	2208	77	(3)
	Cont.	245	209	(85)	449	388	(86)
Aug	Expt.	1787	119	(7)	2494	66	(3)
	Cont.	403	243	(60)	180	143	(79)
Sep	Expt.	2115	105	(5)	2361	46	(10)
	Cont.	318	217	(68)	233	180	(77)
Oct	Expt.	2136	118	(6)	1832	70	(4)
	Cont.	302	151	(50)	185	106	(57)
Nov	Expt.	2020	163	(8)	1919	81	(4)
	Cont.	304	115	(38)	191	50	(26)
Dec	Expt.	2232	73	(3)	1847	54	(3)
	Cont.	295	106	(36)	328	97	(30)
Total	Expt.	32405	2823	(9)	24387	809	(3)
	Cont.	3673	2540	(69)	3873	2139	(55)

Expt. — Experimental (with *Gambusia* fishes); Cont. — Control (without fishes).

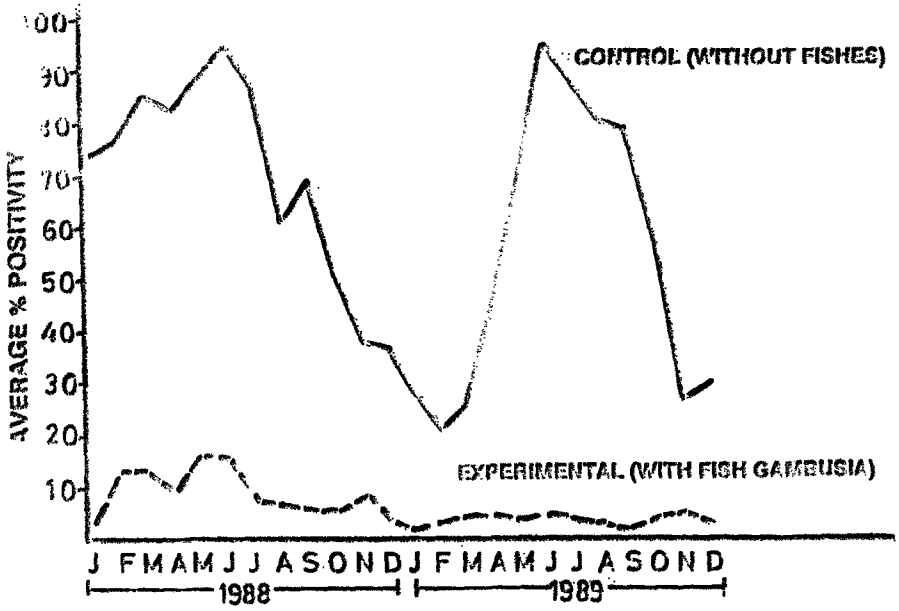

Fig. 1: Effect of shorelining on mosquito breeding in ponds having *Gambusia* fishes.

positive for mosquito larvae but only early instars were encountered, which were also consumed by fishes in a few days. In contrast, all immature stages were found in the ponds of control area. This clearly demonstrated that the thick growth of overhanging weeds contributes to mosquito

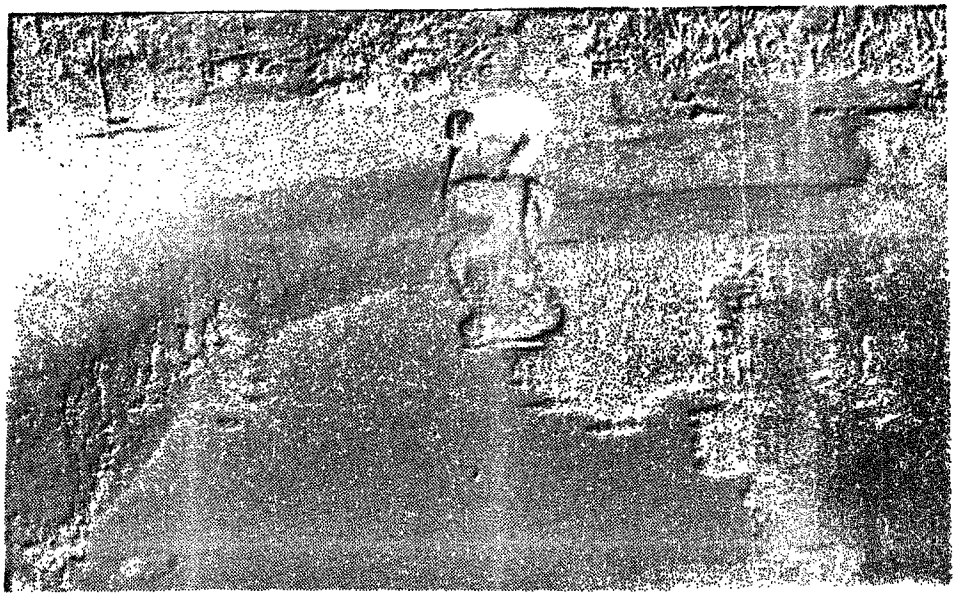

Fig. 2: Repairing of pond margins to allow *Gambusia* to act effectively.

breeding and the situation can be tackled by mud-plastering (Fig. 2). This method is currently used in the control of mosquito breeding in experimental villages of Bhabar.

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