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Some Observations on *Plasmodium falciparum* Gametocytaemia in Natural Infections in an Endemic Area of Koraput District, Orissa

S.S.S. MOHAPATRA*, P. GOVARDHINI*, P. JAMBULINGAM* and S.P. PANI*

Peripheral *P. falciparum* gametocytaemia with respect to its minimum average time taken for appearance in peripheral blood, its peak density, duration of persistence in peripheral circulation, conversion rate and sex ratio was studied in 22 persons with natural infections in a village of Koraput district, Orissa. The minimum average time taken for the appearance of gametocyte was 9.3 days in children (below 15 years) and 8.9 days in adults (15 years and above). The peak count was low, the maximum density being 31/μl. The duration of gametocytaemia in peripheral circulation was also short, the longest being 32 days. The gametocyte conversion rate on an average was 0.74% in children and 2.04% in adults. The mean sex ratio of micro:macro gametocytes was 1:2.6 in children and 1:6.2 in adults.

INTRODUCTION

Koraput district in Orissa state is highly endemic for *P. falciparum* malaria where a higher proportion of asymptomatic parasite carriers exist. While high peripheral gametocytaemia is the usual occurrence in endemic areas[1-3], the paucity of peripheral blood *P. falciparum* gametocytaemia is an interesting feature observed from the early part of the century in stable and endemic malarious areas. The paucity of gametocytaemia has been confirmed again recently in Koraput[4]. Asymptomatic, low-density gametocyte carriers were implicated as the main human reservoir of *P. falciparum* infection under conditions of moder-ate endemicity[5]. Also, a longer period of infectivity of gametocytes is seen to occur at low density in the absence of clinical symptoms in non-immune subjects[6]. Thus low peripheral gametocytaemia and asexual parasitaemia without fever have remained as interesting phenomena in endemic areas.

Hence an observation made on the course of events of peripheral gametocytaemia on partially immune subjects is reported here which may also be useful in ascertaining (by further experiments) the infectivity of gametocytes of such carriers to the vectors of the area.

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MATERIALS AND METHODS

K. Maliguda, a foot-hill village in Rabanaguda PHC, with a population of 158 (predominantly tribals) was selected for the study. The village recorded an annual malaria incidence of 26.1 per thousand population in 1989 and *P. falciparum* was the predominant species (75% of all positives – Data from PHC). The asymptomatic parasite load was moderate (35.4%; unlike in some other villages in the locality where it was very high).

A total of 22 *P. falciparum* asexual parasite carriers who were asymptomatic at the time of detection and had no history of fever for at least 30 days prior to detection was followed for peripheral blood gametocytaemia. Thick and thin blood smears were collected on alternate days for 30 days and weekly thereafter until the disappearance of gametocytes.

Two cases which did not show gametocytes were followed up for 155 and 134 days. Those patients who developed clinical manifestations were treated with a suppressive dose of chloroquine (600 mg adult dose). The schizonticidal therapy was necessary to prevent clinical illness and this was expected to have a negligible effect, if any, on the existing gametocytaemia. The thick blood smear slides were dehaemoglobinized and stained with 7% Giemsa at pH 7.2, for 55 min. Parasite count was made by examining 200 thick smear fields and the count was expressed per microlitre in accordance with the method described elsewhere[7]. Species confirmation was made by thin smear examination. At the end of the study all the patients were treated radically with both schizonticidal and gametocytocidal drugs.

RESULTS AND DISCUSSION

Of the 22 cases followed up, 11 were children (below 15 years) and 11 were adults (15 years or above). Since the immunological status of the latter group is expected to be different from that of the former as was suggested earlier[4], this classification was made.

Time lag between appearance of asexual forms and that of gametocytes in peripheral blood

The minimum average duration between the observation of asexual forms and gametocytes was found to be 9.3 days (range, 3-21 days) in children and 8.9 days (range, 4-28 days) in adults. There was no significant difference in the average duration between the two groups ('T' independent test; $t = 0.11$; $p = 0.92$).

Duration of persistence of gametocytaemia

The average duration of persistence of gametocytaemia was 14.4 days (range, 1-32 days) in children and 11.9 days (range, 1-22 days) in adults. The lower duration in adults might be due to the fact that older individuals eliminate the gametocytes faster from peripheral circulation than children. However, it is not known whether the gametocytes attain senescence faster in the older age group and eventually get eliminated, or get destroyed faster due to some other mechanism. The duration of gametocyte persistence in the observed subjects was much shorter as compared to several earlier reports ranging from 8 to 72 weeks [6,8-10]

The shorter duration of gametocytaemia might have been due to the administration of the suppressive dose of chloroquine, which would have influence on the asexual parasitaemia and further production of gametocytes.

Gametocyte conversion rate (GCR)

This is calculated as the percentage of gametocytes produced from initial asexual parasite count in each individual. In children, GCR ranged between 0.01% and 5.77% and in adults, between 0.89% and 6.32%. The average GCR was 0.74% in children and 2.04% in adults (difference between the two groups by proportion 't' test; $t = 11.11$; $p = 0.05$). The comparatively higher GCR

in adults may again be due to their higher immunity level. Thompson[11] had also recorded that crescent production was higher in older adults (above 20 years) than in children and young adults (below 20 years).

Peak gametocyte count

The peak gametocyte count was found to be 314 per microlitre of blood in children and 20 per microlitre in adults. These values are low, compared to those of earlier observations[10,12]. The lower mean counts in adults may be due to the rapid elimination of gametocytes, resulting in the arrest of density build-up in spite of a higher GCR.

Gametocyte sex ratio

Generally the ratio of micro to macro gametocytes is 1:3 and this, according to Garnham[10], may vary during the course of infection. In our study the sex ratio was also 1:3, when the total sample population (n = 22) was considered. However, the micro:macro gametocyte ratio was 1:2.6 in children and 1:6.2 in adults. The precise cause of this difference in the two groups is not known and it is difficult to explain. Further investigations are required to understand the dynamics of macro and micro gametocytaemia.

It was observed that 4 out of 22 persons with initial asexual forms followed (18%), remained asymptomatic throughout the period. Thus it appears that a majority of the subjects, i.e., 81.8% (18 out of 22) had only partial immunity as they became symptomatic at some time or other during the observation period. The remaining 4 cases might have been in their terminal stage of infection.

Out of the total 22 cases, two did not show any gametocytaemia during the entire follow-up period of 155 days and 134 days respectively. One of these cases (3-year-old male) became negative on 20th day of asexual parasitaemia and was positive again for 84 days from 71st day to 155th day, harbouring asexual parasite only. It is difficult to know whether this was due to reinfection or recrudescence. The other case (6-year-old female) continued asexual parasitaemia for 134 days without showing gametocytes at any time. The blood smears of these two cases were subjected to a thorough examination (for 500 thick smear fields) and were found negative for gametocyte consistently (other sensitive techniques such as collecting venous blood and subjecting to gradient/chemical concentration were not feasible in the field).

We are not certain whether the peripheral blood smear technique was not sensitive enough to detect scanty gametocyte or whether these two cases actually did not produce any gametocyte. Though under experimental conditions it has been shown that some strains of *P. falciparum* lose their ability to produce gametocytes *in vitro*[13], the actual reason for the absence of gametocyte is still not clear. Jeffery[14] had recorded a gametocyte less *P. falciparum* line which did not produce gametocyte (appeared after 6th passage in human neuro-syphilitic patients). He concluded that this loss of capacity to produce sexual forms is not due to any intrinsic change in the parasite line but is associated with passage through a specific individual. Further studies are required to confirm this phenomenon.

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Effect of Tissue Culture Media on Multiplication of *Plasmodium falciparum* in vitro

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To date RPMI-1640 has been the best medium for cultivation of *Plasmodium falciparum in vitro*. In addition to this medium, several alternative media, essentially the ones used in animal and plant tissue culture, were employed for the cultivation of *P. falciparum*. Only the media rich in glucose content, viz. Nitsch medium and White's medium S-3, supported the parasite multiplication.

INTRODUCTION

Several workers have used a variety of tissue culture media for the cultivation of *Plasmodium falciparum in vitro*. Divo and Jensen[1] used Ham's F-12 medium, Medium-199 with Earle's salts and Medium-199 with Hank's salts for this purpose. These authors reported that Ham's F-12 medium was superior to RPMI-1640 (Table 1) when used with minimally dialysed pooled human serum. However, it was not superior to RPMI-1640 when used with normal non-dialysed human serum. Pradhan[2] carried out experiments in which she maintained cultures of *P. vivax* in Korthof's medium, a peptone-rich medium employed for the culture of Leptospira. She observed that Korthof's medium consisting essentially of peptones and salts when fortified with human serum supported parasite growth comparable to that by RPMI-1640. Today, a large number of media are being employed for plant and animal tissue culture. We have evaluated the suitability of various plant and animal tissue culture media for the cultivation of human plasmodia. The results of this study are reported in this paper.

MATERIALS AND METHODS

The studies were carried out with erythrocytic stages of *Plasmodium falciparum*. The following media were used:

1. Gerbera Multiplication Medium*
2. Nitsch Medium*
3. White's Medium S-3*
4. Nutrient Mixture F-10 (HAM)*
5. Dulbecco's Modified Eagle Medium*
6. Minimum Essential Medium (Eagle) with L-Glutamine*

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7. Minimum Essential Medium (Eagle) w/o L-Glutamine*
8. Medium-199 with Hank's salts, L-Glutamine w/o $NaHCO_3$
9. Medium-199 with Earle's salts, L-Glutamine (Ca-, Mg-free) w/o $NaHCO_3$*

Preparation of various media

Gerbera Multiplication Medium: In 50 ml double-distilled water (DDW) 2.5 g of Gerbera multiplication medium was dissolved. To this was added 297 mg of Hepes buffer. The medium was sterilized through 0.22 μm millipore filters (Table 2).

Table 1. Composition of RPMI-1640

Component	Amount mg/l
Inorganic salts	
$Ca(NO_3)_2.4H_2O$	100.00
KCl	400.00
$MgSO_4$	48.84
NaCl	6000.00
$NaHCO_3$	2000.00
Na_2HPO_4	800.00
Others	
Glucose	2000.00
Glutathione (reduced)	1.00
Phenol Red	5.00
Amino acids	
L-Arginine (free base)	200.00
L-Aspargine	50.00
L-Aspartic acid	20.00
L-Cystine	65.00 (2 HCl)
L-Glutamic acid	20.00
L-Glutamine	300.00
Glycine	10.00
L-Histidine (free base)	15.00
L-Hydroxyproline	20.00
L-Isoleucine (allo-free)	50.00
L-Leucine (methionine-free)	50.00
L-Lysine-HCl	40.00
L-Methionine	15.00

contd...

* Himedia Laboratories Pvt. Ltd., Bombay, India.

Table 1. Composition of RPMI-1640 *(contd.)*

Component	Amount mg/l
L-Phenylalanine	15.00
L-Proline (hydroxy-L-proline-free)	20.00
L-Serine	30.00
L-Threonine (allo-free)	20.00
L-Tryptophane	5.0
L-Tyrosine	28.94 (sodium salt)
L-Valine	20.00
Vitamins	
Biotin	0.20
D-Calcium pantothenate	0.25
Choline chloride	3.00
Folic acid	1.00
Isoinositol	35.00
Nicotinamide	1.00
p-Aminobenzoic acid	1.00
Pyridoxine-HCl	1.00
Riboflavin	0.20
Thiamine-HCl	1.00
Vitamin B_{12}	0.005

Table 2. Gerbera multiplication medium w/ sucrose, w/o kinetin and IAA

Component	Amount (mg/l)
$CaCl_2.2H_2O$	440.0
$CaCl_2.6H_2O$	0.025
$CuSO_4.5H_2O$	0.025
$FeSO_4.7H_2O$	27.8
H_3BO_3	6.2
KH_2PO_4	170.0
KI	0.83
KNO_3	1900.0
$MgSO_4.7H_2O$	370.00
$MnSO_4.4H_2O$	22.3
$Na_2MoO_4.2H_2O$	0.25
Na_2-EDTA	37.3
NH_4NO_3	1650.0
$ZnSO_4$	8.6
NaH_2PO_4	85.00
Thiamine HCl	30.0
Nicotinic acid	10.0
Pyridoxine HCl	1.0
i-Inositol	100.0
Adenine sulphate	80.0
L-Tyrosine	100.0
Sucrose	45000.0

Nitsch Medium: 1.10 g of Nitsch medium was dissolved in 50 ml of DDW. 297 mg of Hepes buffer was also added. The medium was filtered through 0.22 μm millipore filter. 2.1 ml of 5% $NaHCO_3$ and 10% human serum were added. Five per cent cell suspension was prepared with the Nitsch medium (Table 3).

White's Medium: In 50 ml DDW 1.65 g of powdered White's medium was dissolved. 297 mg of Hepes buffer was also added and the solution was filtered through 0.22 μm millipore filter (Table 4).

Nutrient Mixture F-10: In 50 ml DDW 490 ml of powdered nutrient mixture was dissolved. 297 mg of Hepes buffer was added and the solution was sterilized through 0.22 μm filter. Five per cent $NaHCO_3$ (2.1 ml) and 10% human serum were added to the medium.

Dulbecco's Modified Eagle Medium: 499 mg of the medium was suspended in 50 ml DDW by adding the powder slowly to the water while stirring. 297 mg of Hepes buffer was added and the solution was mixed thoroughly with the help of a glass rod.

Table 3. PT0012-Nitsch medium w/ sucrose, vitamins w/o agar, $CaCl_2$

Ingredient	mg/l
KNO_3	950
NH_4NO_3	720
KH_2PO_4	68
$MgSO_4.7H_2O$	185
$FeSO_4.7H_2O$	27.8
Na_2-EDTA	37.3
$MnSO_4.4H_2O$	25
H_3BO_3	10
$ZnSO_4.4H_2O$	10
$CuSO_4.5H_2O$	0.025
$Na_2MoO_4.2H_2O$	0.25
Biotin	0.05
Glycine	2
i-Inositol	100
Nicotinic acid	5
Pyridoxine HCl	0.5
Thiamine HCl	0.5
Folic acid	5
Sucrose	

Table 4. White's medium S-3 for plant culture (modified)

Composition	Amount (mg/l)
$Ca(NO_3)_2$	200.0
Na_2SO_4	200.0
KNO_3	80.0
KCl	65.0
NaH_2PO_4	16.50
$MgSO_4$	360.00
$MnSO_4$	4.50
$ZnSO_4$	1.50
H_2BO_3	1.50
KI	0.750
$Fe_2(SO_4)_3$	2.50
Sucrose	20000.0
Thiamine	0.10
Pyridoxine	0.10
Nicotinic acid	0.50
i-Inositol	100.00
Choline	10.0
Riboflavin	0.10
Ascorbic acid	0.10
D-Ca-Pantothenate	0.10
Biotin	0.01
Hypoxanthine	2.50
Aspartic acid	6.0
L-Arginine HCl	7.80
L-Cystine	1.50
L-Glutamic acid	14.00
Glycine	13.00
L-Histidine	2.60
DL-Isoleucine	10.40
L-Leucine	15.60
L-Lysine	15.60
DL-Methionine	13.00
L-Phenylalanine	2.50
L-Proline	5.0
L-Threonine	6.50
L-Tryptophane	4.0
L-Tryptosine	40.0
DL-Valine	13.0
L-Glutamine	50.0
L-Asparagine	20.0
Chlorophenol	4.0

The solution was filtered through 0.22 μm millipore filter and dispensed in sterile bottles (Table 5).

Minimum Essential Medium (MEM) (Eagle) w/o L-Glutamine: 515 mg of powdered MEM was

dissolved in 50 ml DDW by adding the medium slowly to the water. 297 mg of Hepes buffer powder was also added and mixed thoroughly with the help of a glass rod. The medium was sterilized by autoclaving at 121°C (15 lb) for 15 min. The medium was dispensed in sterile screw-capped bottles (Table 6).

Minimum Essential Medium (MEM) (Eagle) with L-Glutamine: 480 mg (MEM) was suspended in 50 ml DDW by adding the powder slowly to the water while stirring with the help of a glass rod. Stirring was continued until the medium was completely dissolved in the water. 297 mg of Hepes buffer powder was added to it and the solution was subjected to filtration by using a millipore filter (0.22 μm pore size) (Table 7).

Medium-199 with Hank's salts, L-Glutamine w/o $NaHCO_3$: 1.09 g of Medium-199 was dissolved in 100 ml DDW. To this was added 594 mg of Hepes buffer. The medium was filtered through 0.22 μm millipore filter (Table 8).

Medium-199 with Earle's salts, L-Glutamine (Ca-, Mg-free) w/o $NaHCO_3$: 0.93 g of powdered Medium-199 was dissolved in 100 ml DDW. 594 g of Hepes buffer was added in the medium. This solution was filtered through 0.22 μm millipore filter for sterilization. Before use, 4.2 ml of 5% $NaHCO_3$ was added by a sterile disposable syringe in the medium.

In all the above media, gentamycine sulphate (80 μg ml^{-1}) and 5-fluorocytosine (10 μg ml^{-1})

Table 5. Dulbecco's modified Eagle medium w/L-glutamine w/o $NaHCO_3$ and antibiotics

Component	Amount (mg/l)
L-Arginine HCl	84.0
L-Cystine. 2HCl	62.57
L-Glutamine	584.0
Glycine	30.0
L-Histidine HCl. H_2O	42.0
L-Isoleucine	105.0
L-Leucine	105.0
L-Lysine HCl	146.0
L-Methionine	30.0
L-Phenylalanine	66.0
L-Serine	42.0
L-Threonine	95.0
L-Tryptophane	16.0
L-Tyrosine (sodium salt)	104.2
L-Valine	94.0
D-Ca-Pantothenate	4.0
Choline chloride	4.0
Folic acid	4.0
i-Inositol	7.2
Nicotinamide	4.0
Pyridoxal HCl	4.0
Riboflavin	0.4
Thiamine HCl	4.0
$CaCl_2$ (anhyd.)	200.00
$Fe(NO_3)_3.9H_2O$	0.1
KCl	400.0
$MgSO_4$ (anhyd.)	97.72
NaCl	6400.0
$NaH_2PO_4.H_2O$	124.0
Glucose	1000.0
Phenol Red	15.0
Sodium pyruvate	110.0

Table 6. Minimum essential medium (MEM) (Eagle) with Earle's salts, NEAA, L-glutamine w/o $NaHCO_3$

Composition	Amount (mg/l)
Amino acids	
L-Arginine HCl	126.4
L-Cystine	24.0
L-Glutamine	292.0
L-Histidine HCl.H_2O	41.9
L-Isoleucine	52.5
L-Leucine	52.4
L-Lysine HCl	73.1
L-Methionine	14.9
L-Phenylalanine	33.0
L-Threonine	47.6
L-Tryptophane	10.2
L-Tyrosine	36.2
L-Valine	46.8
Vitamins	
D-Ca-Pantothenate	1.0
Choline chloride	1.0
Folic acid	1.0
i-Inositol	2.0
Nicotinamide	1.0
Pyridoxal HCl	1.0
Riboflavin	0.1
Thiamine HCl	1.0

Table 7. Minimum essential medium (MEM) (Eagle) (Autoclavable) with Earle's salts, NEAA, Phenol red w/o L-glutamine, $NaHCO_3$ and antibiotics

Composition	Amount (mg/l)
$CaCl_2$ (anhyd.)	200
KCl	400
NaCl	6400
D-Glucose	4500
L-Arginine HCl	84
L-Histidine HCl	42
L-Leucine	105
L-Methionine	30
L-Serine	42
L-Tryptophane	16
L-Valine	94
Choline chloride	4
i-Inositol	7.2
Pyridoxal HCl	4
Thiamine HCl	4
$Fe(NO_3)_3.9H_2O$	0.1
$MgSO_4$ (anhyd.)	97.67
$NaH_2PO_4.H_2O$	125
Phenol Red	15
L-Cystine 2HCl	62.57
Glycine	30
L-Isoleucine	105
L-Lysine HCl	146
L-Phenylalanine	66
L-Threonine	95
L-Tyrosine (sodium salt)	103.79
D-Ca-Pantothenate	4
Folic acid	4.0
Nicotinamide	4.0
Riboflavin	0.4

Table 8. Composition of Medium-199

Components	Amount (mg/l)
Amino acids	
L-Alganine	25.0
L-Arginine HCl	70.0
L-Aspartic acid	30.0
L-Cystine HCl	0.1
L-Cystine	20.0
L-Glutamic acid	67.0
L-Glutamine	100.0
L-Glycine	50.0
L-Histidine H_2O	22.0
L-Hydroxyproline	10.0
	contd...

Table 8. Composition of Medium-199 *(contd.)*

Components	Amount (mg/l)
L-Isoleucine	20.0
L-Leucine	60.0
L-Lysine HCl	70.0
L-Methionine	15.0
L-Phenylalanine	25.0
L-Proline	40.0
L-Serine	25.0
L-Threonine	30.0
L-Tryptophane	10.0
L-Tyrosine	40.0
L-Valine	25.0
Vitamins	
p-Aminobenzoic acid	0.050
Ascorbic acid	0.050
D-Biotin	0.010
Calciferol	0.100
D-Calcium pentothenate	0.010
Cholesterol	0.200
Choline chloride	0.500
Folic acid	0.010
i-Inositol	0.050
Menadione	0.010
Nicotinamide	0.025
Nicotinic acid	0.025
Pyidoxal-HCl	0.025
Pyridoxine-HCl	0.025
Riboflavin	0.010
Thiamine-HCl	0.010
DL-α-Tocopherol phosphate (Na_2)	0.010
Tween-80	5.00
Vitamin-A	0.100
Other components	
Adenine $HCl.2H_2O$	12.10
Adenosine-5-monophosphoric acid, dehydrate (AMP) (muscle adenylic acid)	0.20
Adenosine-5-Triphosphate disodium, tetrahydrate (ATP)	1.00
Deoxyribose	0.50
Dextrose	1000.00
L-Glutathione	0.05
Guanine HCl. H_2O	0.33
Hypoxanthine	0.30
Phenol Red	20.00
Ribose	0.50
Sodium acetate, $3H_2O$	83.00
Thymine	0.30
Uracil	0.30
Xanthine	0.30
$Fe(NO_3)_3.9H_2O$	0.70

were added to prevent bacterial and fungal contamination. Fresh medium was added every 24 h and the old medium pipetted off. Smears were made after 48 h and 96 h. The smears were stained with Giemsa stain. The experiments were terminated at 96 h.

Erythrocyte suspension

Fresh erythrocytes of A^+ group were used for the experimental work for diluting the parasitaemia. The blood was obtained from healthy donors and 5% suspension was prepared in RPMI-1640 complete medium. The erythrocytes stored in CPD were used within 3-4 weeks of collection.

Serum

Fresh 10% human serum AB^+ was used in every medium –pH 7.2.

For all cultures medium was fortified with glucose 2 mg ml^{-1}, bactopeptone 4.5 mg ml^{-1}, in addition to HEPES buffer 25 mmol and 29 mmol $NaHCO_3$.

Culture of parasites

Parasitized erythrocytes were obtained by collecting 6 ml aliquots of blood in 1 ml citrate-phosphate-dextrose (CPD) by venipuncture from clinically diagnosed cases of *P. falciparum* malaria from Malaria Unit, Vashi, New Bombay, District Thane (National Malaria Eradication Programme Unit). The blood samples were collected and brought to our laboratory at Haffkine Institute. The samples were examined by preparing thick and thin smears, and staining the smears with 10% Giemsa stain. The levels of per cent parasitaemia of the samples were recorded. The blood samples of *P. falciparum* were processed as above. 5% cell suspension was prepared in complete RPMI-1640 medium for controls and 5% cell suspension was prepared with any of the above media used for the particular experiment. Cultures were set up by dispensing 0.2 to 0.5 ml parasitized cell suspension in each sterile disposable plastic culture dish (35 × 10 mm). To this was added 1 ml of complete medium. The dishes were placed in a sterile desiccator (size 160 × 255 mm) containing a white candle. The candle was lit and the lid was sealed leaving the stopcock open until the candle was extinguished. The stopcock was closed and the desiccator was placed in 37°C incubator. As a result the requisite atmosphere of CO_2 could be maintained inside the desiccator[3].

Changing the culture medium

The culture medium was changed every 24 h. The old medium was removed from the cultures by gently tipping the petridish and aspirating off the medium with a sterile pasteur pipette. One and a half millilitres of fresh medium was added back to each culture dish; cells were resuspended by gently swirling and plates were replaced in the desiccator. Thick and thin smears were prepared after 48 h and 96 h. Smears were stained with Giemsa and the parasite count was determined.

Parasite multiplication rate

This was calculated by the following formula:

$$\frac{\text{Final parasitaemia}}{\text{Initial parasitaemia}} \times 100$$

Subculture

The experiments with different culture media were carried out for three 96 h cycles. Subcultures were undertaken every 96 h. The parasitaemia was brought down to 0.1-0.2 per cent at the time of each subculture by adding fresh erythrocyte suspension (5%) and growth was studied up to 96 h which was followed by further subculture. In this manner, the growth was studied for 3 cycles and the parasite multiplication rate was recorded for every 48 h and 96 h.

Maintenance of *P. falciparum* isolates with Nitsch medium and White's medium S-3

As experiments with various media showed that

Nitsch medium and White's medium S-3 can support *P. falciparum* cultures, these media were employed for maintenance of *P. falciparum* cultures continuously for four weeks.

RESULTS

Growth of P. falciparum with different media

Gerbera Multiplication Medium: This medium supported *P. falciparum* multiplication *in vitro*, the growth was high at 48 h level when compared to that with RPMI-1640 complete medium. However, the growth was not satisfactory at 96 h level in comparison to that with RPMI-1640 (Table 9).

Nitsch Medium: Nitsch medium with 10% human serum supported *P. falciparum* growth *in vitro*. The parasite multiplication rate was comparable to that observed with RPMI-1640 (Table 9).

White's Medium S-3: White's medium consists of sucrose in a very high concentration (20 mg/l). Hence the medium produced good parasite multiplication. Parasite multiplication rate was higher than in RPMI-1640 medium (Table 9). The smears from the cultures showed 4% schizonts (Table 10).

Dulbecco's Modified Eagle Medium: The growth of the malarial parasite with this medium was comparatively low. The parasite multiplication rate observed was 200 for *P. falciparum*. When smears from cultures with this medium were examined, they were found to contain 2.50% schizonts in the case of *P. falciparum* (Tables 11 and 12).

Minimum Essential Medium (Eagle) with L-Glutamine: Minimum essential medium with L-glutamine supported *P. falciparum* multiplication *in vitro*. The growth, however, was lower than that observed with RPMI-1640 medium. Similarly, the smears also showed a lower percentage of schizonts in *P. falciparum* in comparison to that with RPMI-1640 (Tables 11 and 12).

Minimum Essential Medium (Eagle) w/o L-Glutamine: The minimum essential medium w/o L-glutamine also supported *P. falciparum* growth *in vivo*. The presence or absence of L-glutamine in the medium did not affect parasite multiplication. The parasite growth was lower in comparison with that in RPMI-1640. The per cent parasite multiplication rate in the case of the above media is recorded in Table 11. Table 12 gives details of various stages of malarial parasites observed in the smears of cultures.

Medium-199: Two formulations of Medium-199 were employed for the cultivation of *P. falciparum in vitro*. These were:

(i) Medium-199 with Hank's salts, L-Glutamine w/o $NaHCO_3$.

(ii) Medium-199 with Earle's salts, L-Glutamine (Ca-, Mg-free) w/o $NaHCO_3$.

In both the formulations of Media-199, *in vitro P. falciparum* multiplication was slightly lower than with RPMI-1640 (Table 13). Both the media, however, supported parasite multiplication as could be seen from the percentage of schizonts present in the smears (Table 14).

None of the media used was superior to RPMI-1640. White's medium, Medium-199, Nitsch medium, and MEM (Eagle) supported *P. falciparum* growth. However, this was somewhat lower in comparison to that with RPMI-1640. Cultivation of *P. falciparum* for a period of four weeks with Nitsch medium and White's medium showed that these media supported parasite multiplication comparable to that with RPMI-1640 (Table 9).

DISCUSSION

For the initial work on the continuous cultivation of *P. falciparum*, Trager and Jensen[4] used RPMI-1640 medium developed for the cultivation of human leucocytes. This medium supplemented with HEPES still remains the medium of choice.

Table 9. Effect of tissue culture media on multiplication of *Plasmodium falciparum in vitro*

Species	Media	% Parasitaemia ± SD		Multiplication rate	
		48 h	96 h	48 h	96 h
P. falciparum	Gerbera Multiplication Medium + Serum	1.10 ± 0.14*	0.10 ± 0.00*	733.33	66.66
	Nitsch Medium + 10% Serum	1.15 ± 0.21*	0.20 ± 0.00	766.66	133.33
	White's Medium S-3 + 10% Serum	0.75 ± 0.07**	0.60 ± 0.00*	500	400
	RP-S (M) (control)	0.20 ± 0.0	0.30 ± 0.01	133.33	200

n = 2; Initial % parasitaemia – P.f. – 0.15%; Experimental values are compared with the control values; * Indicates significant difference $p < 0.05$; ** Indicates significant difference $p < 0.01$; Student's unpaired 't' test.

Table 10. Effect of tissue culture media on multiplication of *Plasmodium falciparum in vitro*

Media	Per cent stages parasitaemia ± SD					
	48 h			96 h		
	Rings	Trophozoites	Schizonts	Rings	Trophozoites	Schizonts
Gerbera Multiplication Medium + 10% S	68.00± 0.00	22.00± 0.00	10.00± 0.00	64.00± 2.8	33.00± 1.41	3.00± 1.41
Nitsch Medium + 10% S	70.00± 0.00	24.00± 0.00	6.00± 0.00	68.00± 2.83	27.00± 1.41	5.00± 1.41
White's Medium S-3 + 10% S	69.00± 1.41	21.00± 1.41	10.00± 0.00	74.00± 0.00	22.00± 2.83	4.00± 2.83
RP-S (M) (control)	80.00± 0.00	18.00± 0.00	2.00± 0.00	69.00± 7.07	25.00± 4.24	6.00± 2.83

n = 2.

Table 11. Effect of Dulbecco's medium and MEM medium on the growth of *Plasmodium falciparum in vitro*

Species	Media	Per cent parasitaemia ± SD		Multiplication rate	
		48 h	96 h	48 h	96 h
P. falciparum	Dulbecco's Modified Medium + 10% serum	0.07 ± 0.00**	0.20 ± 0.00*	70	200
	MEM (Eagle) + 10% serum	0.07 ± 0.00**	0.15 ± 0.07**	70	150
	MEM (Eagle) + 10% serum	0.17 ± 0.00*	0.29 ± 0.12*	170	290
	RP-S (M) (control)	0.29 ± 0.02	0.38 ± 0.03	290	380

n = 2; Initial % parasitaemia – Pf – 0.10%; Medium – RP-S (M); Experimental values are compared with the control values; * indicates significant difference $p < 0.05$; ** indicates significant difference $p < 0.01$; Student's unpaired 't' test.

Table 12. Effect of tissue culture media on the growth of *P. falciparum in vitro*

Media	Per cent stages parasitaemia ± SD					
	Rings	Trophozoites	Schizonts	Rings	Trophozoites	Schizonts
	48 h			96 h		
Dulbecco's Modified Eagle Medium + 10% S	72.50± 3.54	27.00± 2.83	0.50± 0.70	66.00± 1.41	31.50± 2.12	2.50± 0.70
NEM (Eagle) with L-glutamine + 10% S	67.50± 3.54	32.50± 3.54	0.00 –	66.50± 3.54	31.50± 3.54	2.00± 0.00
NEM (Eagle) without L-glutamine + 10% S	72.50± 6.37	27.50± 6.37	0.00 –	74.00± 5.66	24.00± 5.66	2.00± 0.00
RP-S (M) (control)	67.00± 4.24	29.00± 1.41	4.00± 2.83	67.00± 8.49	27.50± 3.54	5.50± 4.95

n = 2.

Table 13. Effect of Medium-199 on the growth of *Plasmodium falciparum in vitro*

Medium	Per cent parasitaemia ± SD		Multiplication rate	
	48 h	96 h	48 h	96 h
Medium-199 with Hank's salts, L-glutamine without $NaHCO_3$	3.18 ± 0.21	0.24 ± 0.05	1590	120
Medium-199 with Earle's salts, L-glutamine without $NaHCO_3$	1.45 ± 0.31	0.10 ± 0.00	725	50
RP-S (M) (control)	0.79 ± 0.16	0.44 ± 0.05	395	220

n = 2; Initial % parasitaemia – 0.20%; Student's unpaired 't' test.

Table 14. Effect of Medium-199 on the growth of *Plasmodium falciparum in vitro*

Media	Per cent stages parasitaemia ± SD					
	48 h			96 h		
	Rings	Trophozoites	Schizonts	Rings	Trophozoites	Schizonts
Medium-199 with Hank's salts, L-glutamine without $NaHCO_3$	68.50± 6.36	27.00± 4.24	4.50± 2.12	66.50± 9.19	31.00± 8.49	2.50± 0.71
Medium-199 with Earle's salts, L-glutamine (Ca-, Mg-free) without $NaHCO_3$	67.00 ± 2.83	31.50± 2.12	1.50± 0.71	74.50± 7.78	25.00± 7.07	0.50± 0.71
RP-S (M) (control)	74.50± 2.12	24.50± 0.71	1.00± 1.41	76.00± 1.41	23.50± 0.71	0.50± 0.71

n = 2.

Medium-199 was used by Haynes *et al.*[5] and Chen *et al.*[6] in a greatly supplemented form. Divo and Jensen[1] compared RPMI-1640, Ham's nutrient mixture F-12, and medium-199 containing Eagle's or Hank's salts solution, and found that RPMI-1640 was superior to both formulations of 199 and equivalent to Ham's F-12 for culturing isolates of *P. falciparum*.

In the present study, several plant and animal tissue culture media were employed for the cultivation of isolates of *P. falciparum*. These included Gerbera multiplication medium, Nitsch medium, White's medium, Dulbecco's modified Eagle's medium, minimum essential medium (MEM) with L-glutamine, MEM w/o L-glutamine, medium-199 with Hank's salts, L-glutamine w/o $NaHCO_3$, and medium-199 with Earle's salts, L-glutamine (Ca-, Mg-free) w/o $NaHCO_3$. All the media supported *P. falciparum* multiplication *in vitro*. The growth was, however, lower in the case of Gerbera multiplication medium, Dulbecco's modified Eagle medium, minimum essential medium and medium-199 than in RPMI-1640 complete medium. In the case of Nitsch medium and White's medium S-3, the growth was comparable to that obtained with RPMI-1640. It is concluded that Nitsch medium and White's medium may be suitably employed as a substitute to RPMI-1640 complete medium. These observations are in agreement with those of earlier workers regarding the favourable influence of glucose on parasite growth as all the three media are rich in glucose [7-10].

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Microscopic Diagnosis of Malaria in Kheda District of Gujarat

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A study conducted in 1990 revealed that 2% (range 0.6-4.8) of negative blood smears were mislabelled as positive, and 6.7% of positive blood smears were mislabelled as negative. A result of such mislabelling would be inadequate treatment of a large number of patients. Hence the need to look into the training aspect and system of supervision of laboratory technicians. The present system of cross-checking of blood smears at different levels also needs to be reviewed. A study which could address itself to these needs is indicated.

INTRODUCTION

During the last decade (1981-90) in India, on an average, over 67 million blood smears were collected to detect about 2 million malaria cases annually. Ray and Sharma[1] emphasized case detection and treatment to reduce the parasite reservoir in the community, along with drug distribution to fever cases through drug distribution centres (DDCs). Clyde and Beljaev[2] found that the quality of microscopic diagnosis suffered a setback due to lack of supervision and support. An in-depth evaluation report of NMEP[3] mentions that laboratory technicians were overloaded with work because of which the quality of blood smear examination suffered a setback. In Gujarat, new PHCs are being established covering a population of about 30,000. In this situation malaria microscopy is expected to be of high quality. This paper is a case-study which highlights the status of microscopic diagnosis and suggests ways and means of improving this method.

Methodology

In the malaria control programme in Gujarat, all blood smears are examined by laboratory technicians at their respective malaria clinics/Primary Health Centres (PHCs). Ten per cent of the blood smears so examined are randomly cross-checked at different levels, i.e. 2.5% each at district, region, state and central level. However, due to constraints in manpower in cross-checking laboratories, all positive slides are not cross-checked and at times it is difficult to examine even the slides collected for the purpose. In the present study, carried out between January and December 1990,

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up to 50 already examined blood smears from each of the 9 PHCs of Nadiad taluka in Kheda district were randomly collected every month along with their results. The smears were re-examined at Malaria Research Centre's laboratory. One hundred fields of thick smear only were examined before declaring it negative for malaria parasites as practised by laboratory technicians of PHCs under study. Information regarding qualification and training of laboratory technicians and condition of microscopes, particularly the lenses, was also collected.

RESULTS AND DISCUSSION

The results of cross-examination of blood smears of 9 PHCs of Nadiad taluka in Kheda district are given in Table 1. The average Discrepancy Rate (DR) in the blood smears already declared negative by the laboratory technicians was found to be 2.0% (range 0.6-4.8%).

In 1990, in rural Nadiad taluka, 63,747 blood smears were examined to detect 2140 malaria cases in a population of 361,537, giving an API of

Table 1. Quarterly results of cross-examination of blood smears of 9 PHCs of Nadiad taluka

A: Negative slides

PHC	Jan–Mar	Apr–Jun	Jul–Sep	Oct–Dec	Total	DR(%)
1. Akhdol	96 (2)	127 (4)	125	140 (1)	488 (7)	1.4
2. Alina	93 (3)	94 (4)	50	97 (9)	334 (16)	4.8
3. Alindra	100	137	138 (2)	140 (7)	515 (9)	1.7
4. Chaklasi	100 (1)	147 (4)	96 (2)	144 (2)	487 (9)	1.8
5. Mahudha	127	142 (1)	87	138 (1)	494 (3)	0.6
6. Mohorel	127	145 (1)	149 (2)	95 (4)	516 (7)	1.4
7. Narsanda	135 (2)	148 (6)	148 (4)	146 (4)	577 (16)	2.8
8. Palana	98 (1)	147 (4)	47 (1)	147 (4)	439 (10)	2.3
9. Pij	117	139 (4)	93 (2)	138 (6)	487 (12)	2.5
Total	993 (9)	1226 (28)	933 (14)	1185 (38)	4337 (89)	2.0

Figures in parentheses are the numbers found positive.

B: Positive slides

PHC	Jan–Mar	Apr–Jun	Jul–Sep	Oct–Dec	Total	DR(%)
1. Akhdol	2	4	9	10	25	0.0
2. Alina	1	6	0	3	10	0.0
3. Alindra	11 (3)	13	12	10	46 (3)	6.5
4. Chaklasi	0	3	4 (1)	6	13 (1)	7.7
5. Mahudha	11	9	11	12 (1)	43 (1)	2.3
6. Mohorel	10	5	1	5 (1)	21 (1)	4.7
7. Narsanda	6 (4)	2 (1)	2	4 (1)	14 (6)	42.8
8. Palana	0	3	2 (2)	3	8 (2)	25.0
9. Pij	0	11	7	12	30	0.0
Total	41 (7)	56 (1)	48 (3)	65 (3)	210 (14)	6.7

Figures in parentheses are the numbers found negative; DR – Discrepancy rate.

5.9. According to the present finding (DR = 2.0%), 1262 malaria cases might have been missed in 9 PHCs (API = 3.5). Thus the corrected API should be 50% higher (9.0) against the reported figure of 5.9 if we subtract 6.7% (143 cases) from total positive cases, as 6.7% is the discrepancy rate among smears declared positive (Table 1). Also to be noted is that the discrepancy rate is highest in the months of October (4.4%) and November (4.2%), which is also the peak season for *P. falciparum* infection. DR was low in the months of February, March, August, September and December (< 1%). The high DR during October and November may probably be attributed to high *P. falciparum* prevalence, identification of which has probably been missed particularly in cases not having gametocytes.

The results of routine cross-examination of blood smears of the same period from Kheda district to which Nadiad taluka belongs are given in Table 2. The results of cross-examination of blood smears at district, region and state headquarters and the regional office of the Government of India showed no discrepancy at any level as regards the blood smears declared positive by the laboratory technicians at malaria clinic/PHC level. Also, among the declared negative smears, no discrepancy was observed at district level. In 1990 at regional and state headquarters, discrepancy rates of 0.5 and 2.1% respectively were observed whereas the figure was 0.01% at central level. Our results (DR = 2.0%) are close to the findings at state level in negative smears (DR = 2.1%). Differences observed at different levels in the results of the routine cross-checking of blood smears are difficult to explain. This is indicated while looking at the results (Table 2) of cross-examination of blood smears at different levels during the last three years (1988-90).

However, we tried to find out the reasons for the varied performance of technicians posted in 9 different PHCs of Nadiad taluka. Of the nine laboratory technicians (one each in a PHC), eight were graduates and one was tenth standard pass (Table 3). All were trained for malaria microscopy either at the district laboratory or at the laboratory of Regional Office for Health and Family Welfare, Ahmedabad. Six out of nine had 1-4 years' experience whereas three had 17-29 years' experience. The condition of microscopes was unsatisfactory (objectives being hazy) in 4 PHCs. The condition of smears and their staining was reported to be satisfactory by all the PHCs. From the information gathered in Table 3, no correlation could be established between the performance and the parameters listed in the table. However, discrepancy due to defective microscopes cannot be ruled out. Perhaps a bigger sample size may help establish such a correlation. Sharma and Dwivedi[4] mentioned carelessness on the part of microscopists to be the reason for discrepancy.

Discussions with several officers working in the programme revealed that the results of the routine cross-checking of blood smears are not taken seriously in the absence of corrective measures. Consequently, laboratory technicians work as they are used to – a conclusion borne out by the results of our study. It is important that the outcome of the re-examination be continuously analysed and necessary action taken. With decentralisation of laboratories at PHC level, a higher level of supervision is required to improve efficiency[3].

We conclude that the components of training programmes, the system of supervision of laboratory technicians and the maintenance of microscopes in PHCs, all need to be geared up. Also necessary is the improvement of the system of cross-checking of the blood smears at different levels. The field laboratories should send all the examined slides and the supervising laboratory should cross-check 10% of slides, selected randomly as recommended by Pampana[5]. About 95% of the positive slides, missed by laboratory technicians in peripheral laboratories, could be found positive by re-examining only about 23 fields of thick smear[4]. Therefore, a study which could address

Table 2. Results of routine cross-examination of blood smears from Kheda district at different levels (1988-90)

Level	Year	Negative blood smears			Positive blood smears		
		BSE	Discre-pancy	Discre-pancy rate (%)	BSE	Discre-pancy	Discre-pancy rate (%)
A. State level							
1. District headquarters	1988	8378	0	0.00	0		
	1989	4577	0	0.00	0		
	1990	7471	0	0.00	57	0	0.00
2. Regional headquarters	1988	10636	77	0.72	0		
	1989	6173	39	0.63	0		
	1990	9950	49	0.49	125	0	0.00
3. State headquarters	1988	14336	116	0.81	0		
	1989	6878	58	0.84	0		
	1990	11600	250	2.15	250	0	0.00
B. Central level							
1. Regional office	1988	10588	2	0.02	0		
of Government	1989	8037	0	0.00	0		
of India	1990	9533	1	0.01	81	0	0.00
Average		108157	592	0.55	513	0	0.00

BSE – Blood slides examined.
Source : District Malaria Officer, Kheda.

Table 3. Qualification and experience of laboratory technicians at PHCs of Nadiad taluka and other particulars

PHC	Population	Quali-fication	Trained at	Age	Experience (years)	Condition of		DR (%)	
						Micro-scope*	Smears	+ve	–ve
1. Akhdol	29728	BSc.	DL	25	1	D	S	Nil	1.4
2. Alina	25782	BSc., MLT	DL	23	2	D	S	Nil	4.8
3. Alindra	32024	BSc.	RO	52	29	S	S	6.5	1.7
4. Chaklasi	54343	BSc., MLT	DL	25	2	S	S	7.7	1.8
5. Mahudha	56351	SSC	RO	55	17	D	S	2.3	0.6
6. Mohorel	38128	BSc., MLT	DL	30	4	S	S	4.8	1.4
7. Narsanda	30664	BA	RO	47	28	S	S	42.8	2.8
8. Palana	28700	BSc.	DL	24	3	D	S	25.0	2.3
9. Pij	32287	BSc.	DL	24	2	S	S	Nil	2.4

* Objective/eyepiece; RO – Regional Office for Health and Family Welfare, Ahmedabad; DL – District Laboratory of NMEP, Nadiad; S – Satisfactory; D – Defective; DR – Discrepancy rate ; MLT – Medical laboratory technology.

itself to these problems in detail is called for. Such a study will help locate lacunae in the system for redressal. There is also an urgent need to develop not only a better diagnostic technique for detection of malaria parasite but for adopting criteria for collection of blood smears with more emphasis on actual fever cases suggestive of malaria.

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Problem of Antimalarial Drug Resistance in *Plasmodium falciparum* in Mizoram

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Studies conducted in Mizoram during 1981 to 1990 have shown areas with increasing RIII level of chloroquine resistance. These foci need urgent liquidation. Sulfalene-pyrimethamine drug combination was found suitable for treatment of *P. falciparum* cases in these areas with only 5.3 per cent cases showing RI level of *Plasmodium* response. Quinine combined with sulfalene-pyrimethamine showed 100 per cent success. Amodiaquine however was similar in response to chloroquine though the mean parasite clearance time with amodiaquine was slightly better.

INTRODUCTION

The state of Mizoram is situated between 22° 19′ and 24° 19′ N latitude and 92° 16′ E longitude. It has border with Myanmar on its east and south, Bangladesh and Tripura on west, and Assam and Manipur on north. Topographically the union territory is on steep hills and deep gorges.

Malaria is a persistent problem in Mizoram. In spite of the intensified surveillance and three rounds of regular spray with HCH since 1978, malaria incidence has been very high. Table 1 shows malaria incidence in the state during 1979 and 1988 as reported by the State Health Services.

Chloroquine resistance was first detected in Myanmar in 1969 and in northeast India in 1973 from Assam[1]. It is presumed that the resistance in India has come from Thailand via Myanmar. Pattanayak *et al.* [2] reported that chloroquine 600 mg base and pyrimethamine 50 mg base together failed in Aizawl district (Mizoram) to clear asexual parasites in about 30% of the cases on Day 6.

Monitoring of chloroquine resistance in Mizoram was started in 1981 and was continued up to 1990. The observations in these studies conducted in Sairang and Kolashib PHCs in Aizawl district and Hnanthial PHC of Lungleih district are reported in this paper.

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Table 1. Incidence of malaria in Mizoram in 1979 and 1988

Year	No. of blood smears examined	Positive cases	*Pf* cases	SPR	SFR	*Pf* ratio
1979	1,65,066	19,345	12,075	11.7	7.31	62.4
1988	2,05,514	20,030	8,880	9.75	4.32	44.33

SPR – Slide positivity rate; SFR – Slide falciparum rate; *Pf* – *Plasmodium falciparum*.

Table 2. Results of chloroquine sensitivity studies in *P. falciparum* cases in Mizoram

District	PHC	Year	No. tested	Sensitivity					Remarks
				S	S/RI	RI	RII	RIII	
Aizawl	Sairang	1981	47	19	–	19	8	1	28 days' test
		1988	23	10	–	5	1	7	-do-
Aizawl	Kolashib	1987	19	10	–	8	1	–	-do-
		1990	9	8	–	1	–	–	-do-
Lungleih	Hnanthial	1983	23	5	11	5	1	1	-do-
		1989	24	–	14	1	1	8	7 days' test
	Grand total		145	52	25	39	12	17	

S – Sensitive; RI – Resistant at level I; RII – Resistant at level II; RIII – Resistant at level III.

Table 3. Results of sensitivity studies with (a) Amodiaquine, (b) Sulfalene with Pyrimethamine, and (c) Sulfalene and Pyrimethamine with Quinine

Drug/ Study district	No. of cases tested	Sensitivity					Remarks
		S	S/RI	RI	RII	RIII	
Amodiaquine							
Lungleih	31	–	23 (74.19)	1 (3.23)	–	7 (22.58)	7 days' test
Sulfalene with Pyrimethamine							
Aizawl	14	13	–	1	–	–	28 days' test
Lungleih	24	23	–	1	–	–	-do-
	38	36 (94.74)	–	2 (5.26)	–	–	
Sulfalene and Pyrimethamine with Quinine							
Aizawl	14	14	–	–	–	–	28 days' test
Lungleih	23	23	–	–	–	–	-do-
	37	37 (100)	–	–	–	–	

Figures in parentheses indicate percentage.

Studies of treatment of *P. falciparum* cases with alternative drugs like (i) amodiaquine, (ii) sulfalene with pyrimethamine, and (iii) sulfalene and pyrimethamine with quinine were also done in these areas. These findings are also reported in this paper.

MATERIALS AND METHODS

In vivo studies were carried out in accordance with the procedures laid down by WHO[3]. Drug schedules and dosages used were as given below:

(i)	Chloroquine	:	25 mg per kg body weight in 3 days
(ii)	Amodiaquine	:	25 mg per kg body weight in 3 days
(iii)	Sulfalene + Pyrimethamine	:	100 mg + 50 mg (single dose)
(iv)	Sulfalene + Pyrimethamine and Quinine	:	100 mg + 50 mg (1st day) and 100 mg daily × 3 days.

Children received proportionately lower dosages. All the cases were followed up to Day 28 after starting treatment. The test at Hnanthial PHC was carried out for only 7 days' follow-up due to administrative reasons. Slides collected were cross-checked in the reference laboratory at the Directorate of NMEP, Delhi.

RESULTS

Chloroquine : Classification of cases into sensitive and resistant at RI, RII and RIII levels has been done according to WHO guidelines[3]. The results of the studies are given in Table 2. Out of a total of 145 cases tested, 52 (35.86%) were sensitive and 68 (46.90%) were resistant. Twenty-nine (42.65%) of the resistant cases were of RII and RIII levels. Variation in sensitive and resistant cases in all the three places between the first and second study is statistically insignificant, χ^2 value being 0.063 in Sairang, 3.482 in Kolashib and 0.647 in Hnanthial PHC.

The variation in RI and RII cases against RIII cases in Sairang PHC during 1981 and 1988 is highly significant statistically ($\chi^2 = 14.257$, $p < 0.001$). Similarly in Hnanthial PHC the variation is highly significant ($\chi^2 = 7.158$; $p < 0.01$). No RIII level of resistance was detected in Kolashib PHC during 1987-1990.

Mean parasite clearance times (MPCT) after drug administration in sensitive and RI level resistant cases were 2.66 days in Sairang, 2.92 days in Kolashib and 2.47 days in Hnanthial PHC.

The results of studies with amodiaquine, sulfalene with pyrimethamine, and sulfalene and pyrimethamine with quinine are given in Table 3.

Amodiaquine: Eight (25.81%) of the 31 cases treated with amodiaquine showed resistance at RI and RIII levels. Studies on treatment with chloroquine at the same time showed 41.67% resistant cases in this area. However, statistically no significant difference in the two groups of drugs was found ($\chi^2 = 1.534$, $p < 0.05$). MPCT with amodiaquine was 1.92 days.

Sulfalene with Pyrimethamine: Only two (5.26%) cases were resistant at RI level. All the cases showed initial clearance of parasitaemia by Day 3, MPCT being 1.89 days.

Sulfalene and Pyrimethamine with Quinine: All the 37 cases (100%) tested were sensitive to this drug combination, MPCT being 1.86 days.

DISCUSSION

Mizoram is strategically placed between Bangladesh and Myanmar (Fig. 1) where chloroquine resistance is very acute[4,5]. The present study has indicated that chloroquine resistance is pre-valent in Mizoram. A significant increase in RIII level of resistance was found in both Sairang PHCof Aizawl district and Hnanthial PHC of Lungleih district. However, no RIII level of resistance was detected in Kolashib PHC under Aizawl district. A change in the drug policy in the Sairang and

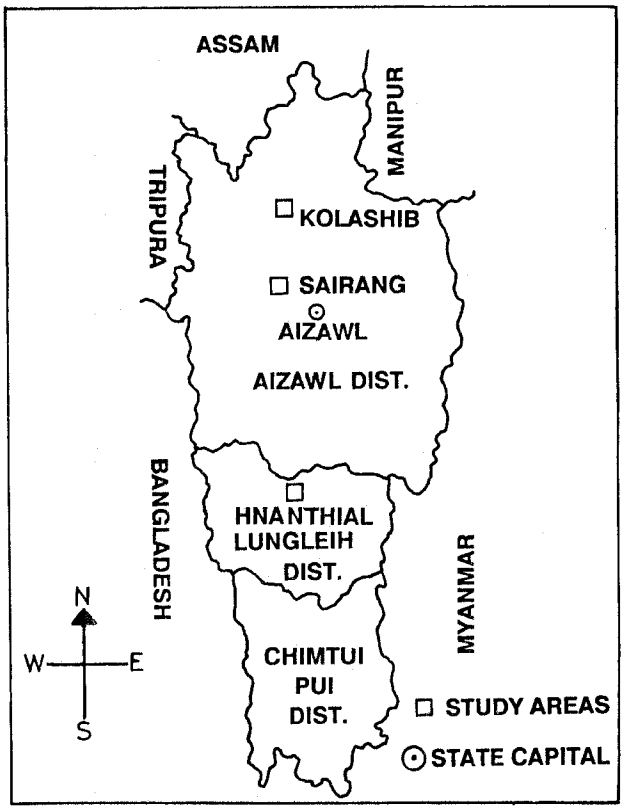

Fig. 1 : Map of Mizoram showing the study areas.

Hnanthial areas is therefore necessary. The technical advisory committee of NMEP agreed to changing the drug policy in the Sairang and Hnanthial PHC of Lungleih district, and the new policy is being implemented from 1991.

Alternative drug treatment is very essential to wipe out the RIII level of resistance, in addition to effective insecticide spray operation. Das and Baruah[6] incriminated *An. minimus* and *An. balabacensis balabacensis* as vectors of malaria in Mizoram. Both these vectors are highly susceptible to DDT. Amodiaquine is similar to chloroquine in efficacy. But the mean parasite clearance time with amodiaquine (1.92 days) is slightly better.

In our study, the results of treatment with sulfalene and pyrimethamine combination were encouraging with only 5.26% cases showing RI level of response. MPCT is 1.89 days, which is lower than with amodiaquine and chloroquine. Das *et al.*[7] reported from Manja areas of Karbi-Anglong district of Assam that this drug combination against chloroquine-resistant *P. falciparum* showed 5.5 per cent initial failure rate on Day 7 but 50 per cent of the cases showed parasitaemia by Day 28. Barkakaty *et al.*[8] reported 13 per cent failure rate with this drug combination in different states of northeast India.

When quinine is given in combination with sulfalene and pyrimethamine, the result is extremely

satisfactory with 100 per cent success rate. Quinine alone may not be responsible for 100% cure rate of this drug combination. First, the dose of quinine was very low (1000 mg daily for 3 days). Secondly, in another study carried out in Sairang PHC in 1988 in which quinine (900 mg × 5 days) plus pyrimethamine (50 mg × 3 days) was used, the results showed only 58.3% sensitive and 33% and 8.34% showed RI and RII level resistance respectively (unpublished).

Though Mizoram has the problem of RIII level chloroquine resistant focus in certain locations, it has also the alternative drugs to treat the cases and an effective insecticide (DDT) to control the vectors. Efforts are needed to liquidate the focus of RIII level resistance immediately.

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Management of Admitted Malaria Cases in Four Major Hospitals of Delhi : A Case Study

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A hospital-based retrospective case study of admitted patients was undertaken in four major hospitals of Delhi during 1991, with a view to assessing (i) recording and reporting system of malaria cases, (ii) diagnostic criteria being followed, (iii) management of complicated and severe malaria cases, and (iv) availability of life-saving antimalarials.

The study showed that none of the hospitals either followed the international coding system for recording or adopted the National Malaria Eradication Programme guidelines for diagnostic criteria of malaria, i.e. by blood smear examination. Diagnosis of malaria in three out of four hospitals was not preceded by blood examination in all cases. Only 55% of the 283 clinically suspected malaria cases were screened for malaria parasite with overall positivity of 20.14 per cent and of 38.25 per cent in examined cases. Age and sex break-up indicated that males suffered more and 65 per cent of the patients belonged to 16-40 years' age groups as compared to 38.4 per cent population falling in this age group according to 1981 census. Out of 263 recovered study cases, 13 per cent came from adjoining states while this percentage went up to 35 per cent (7 out of 20 cases) in the case of malaria deaths. Over 80 per cent of the clinically suspected cases presented with signs and symptoms of fever or fever with rigour, chills or vomiting. In 38 per cent of the cases there was a definite time lag in reporting of the cases to hospitals but most of the cases (91 per cent) were administered antimalarials within 24 h of admission. Out of 283 clinically labelled malaria cases, seven were neither screened for malaria parasite, nor given any antimalaria treatment. In addition, about 13 per cent cases were screened after starting the antimalarials. Clinicians were neither found to be fully aware of the first line of treatment for *Plasmodium vivax* and *P. falciparum* cases being advocated by the Directorate of NMEP nor were the hospitals stocked with the life-saving drug 'injectable quinine'.

INTRODUCTION

Malaria had been one of the major causes of morbidity and mortality in the country about three and a half decades ago when an organized national level health programme was initiated to eradicate this menace. Initially this disease was localized to a large extent in rural areas so the major emphasis of this programme was on rural areas, but after achieving initial success by mid-sixties there was complacency on the part of medical authorities as well as the community because it was no longer their felt need. After resurgence

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of malaria during 1970s the epidemiological scenario of this disease went topsy/turvy as the urban areas were showing a definite increase while in rural areas the problem was more or less under control.

Under the National Malaria Eradication Programme, urban areas do not have any active surveillance machinery, unlike in rural areas, to detect malaria cases and provide treatment. Basically hospitals, dispensaries and private practitioners cater to this need. In view of the changed situation, the Directorate of NMEP thought it appropriate to create awareness about this problem and apprise the physicians working in the hospitals about the absolute need of laboratory confirmation of clinically suspected malaria cases and correct methodology and the latest knowledge and practices for efficient management of complicated and severe malaria cases, by conducting a number of workshops in the country from 1988 to 1990. The basis of the present study is the observation made in workshops that clinicians do not generally pay adequate attention to differential diagnosis of the common local health problems vis-a-vis malaria, diagnosis being made mostly clinically though the laboratory facilities are available and case management of malaria is not based on the national drug policy formulated by the Directorate of NMEP.

This case study was conducted during 1991 to assess (i) the recording and reporting system of malaria cases, (ii) diagnostic criteria adopted, (iii) attention followed for management of complicated and severe malaria cases, and (iv) availability of life-saving antimalarials in the hospitals.

The overall objective was to have first-hand experience on all the above-mentioned aspects with an intention to highlight the need for bringing up suitable changes in the undergradute teaching curriculum of medical colleges and to introduce refresher training courses-cum-workshops for physicians working in hospitals/medical colleges and to laboratory technicians on a large-scale.

MATERIALS AND METHODS

This study was conducted in four different major hospitals of Delhi city. These hospitals were selected randomly but the only criterion of selection was that they should be government hospitals run by different authorities. The hospitals have not been named because of obvious reasons but mentioned as A, B, C and D. The categories are as follows:

A – A hospital under the administrative control of the Central Government catering to general patients as well as Central Government Health Scheme beneficiaries.

B – A hospital under the administrative control of the Central Government but attached to a medical college and catering exclusively to female patients.

C – A hospital under the administrative control of a local health authority not directly responsible for implementation of the programme and catering to general population.

D – A hospital under the administrative control of a local health authority responsible for the implementation of the programme in the city.

Case sheets of all the patients admitted in these hospitals with clinical diagnosis of malaria from January to December 1990 were studied for detailed analysis.

RESULTS AND DISCUSSION

A total of 283 case sheets of patients admitted during 1 January to 31 December 1990 and classified under the diagnosis of malaria by the hospital authorities were retrieved from these four hospitals. Of these, 20 case sheets were of the patients whose deaths were recorded as due to cerebral malaria. The detailed findings are as follows:

Recording and reporting system of malaria cases and deaths

As per the guidelines of the International Classification of Diseases[1], the reporting of malaria is classified under codes 084.0–084.9. All the codes are designated for either specific to infection by a particular species of parasite or a particular type of presentation of malaria except code 084.6 which has been assigned for unspecified malaria, which in other words may mean clinical malaria.

According to the guidelines of NMEP[2], blood smears of all patients who are reporting with history of fever, should compulsorily be screened for malaria parasite and only parasitologically positive cases should be recorded and reported as suffering from malaria.

In hospital A, out of 122 clinical malaria cases, blood slides of only 75 patients were examined and 22 were positive for malaria. In addition, this hospital reported 13 deaths due to malaria, but owing to some clerical mistake 2 deaths were wrongly classified under malaria as they died of TB, meningitis and rabies, going by the diagnosis entered in the case records.

In hospital B, out of 61 cases of clinical malaria, blood smears were examined in 34 cases, only 11 being positive cases.

In hospital C, out of the 83 case sheets clubbed under malaria, 14 cases were wrongly classified as malaria under code 084.6, but they were suffering from other diseases. Out of remaining 69 cases, only 15 were screened for malarial parasite with two positives.

In hospital D, only patients having peripheral blood smear positive for malarial parasite were recorded and reported as malaria cases.

These observations show that the hospitals considered for retrospective case study are neither following the international coding system nor the guidelines of the Directorate of NMEP, as three hospitals are reporting all clinically diagnosed malaria cases as malaria while they are taking blood smears from only a few of them, of whom a small percentage are positive for malaria.

As per the reporting system of malaria cases by these hospitals to the local health authority responsible for running the programme in the city, the prevalent practice is that monthly information about clinical as well as parasitologically confirmed malaria cases and deaths due to malaria are reported in a prescribed pro forma. All these hospitals are regularly sending this information but in response to this input the local health authorities are expected to investigate each and every death due to malaria reported to them. But this is not being done as is evident from the fact that the four hospitals reported 20 deaths due to malaria during 1990 but the epidemiological information supplied by the local health authority responsible for running the National Malaria Eradication Programme in the city to the Directorate of NMEP gave 'nil' report on this account and no death investigation form – a prescribed norm under NMEP – was submitted.

Diagnostic criteria being followed

In clinically diagnosed malaria patients who survived, it was observed that during 1990 only 140 out of a total 263 patients admitted in these hospitals with provisional diagnosis of malaria were subjected to blood examination for malaria parasite (Table 1).

The positivity rate for malaria parasite as a whole was 20.5 per cent of all admitted patients, and clinically diagnosed as malaria, in spite of the fact that it was 100 per cent in hospital D, which is recording malaria parasite positive cases only. Blood smears for malaria parasite were examined in other fever patients also in this hospital but the negative cases were not labelled as malaria, so positivity was 100 per cent.

Table 1. Hospital-wise distribution of clinical malaria cases (who recovered) showing positivity for malaria parasite

Hospital Code	No. of patients admitted with provisional diagnosis of malaria	No. of patients whose blood was examined for MP*	Results of BS examined			Positivity rate in all admitted patients
			P. vivax	*P. falciparum*	Total	
A	122	75	13	9	22	18.0
B	59	34	7	4	11	17.3
C	63	12	–	2	2	3.4
D	19	19	5	14	19	100.0
Total	263	140	25	29	54	20.5

*Malaria parasite.

In death cases which were clinically labelled as due to malaria, it was observed that out of 20 such deaths recorded by the four hospitals during 1990, blood smear was examined for malaria parasite in only nine cases (45 per cent). In addition, it was noticed that only three death cases (15 per cent) were positive for malaria parasite and two of them were positive for *P. falciparum* (10 per cent), which is known to cause death, the third case being positive for *P. vivax*, which in itself cannot cause mortality. So it is evident that these hospitals are putting more emphasis on their clinical judgement to diagnose the cases than on the more scientific method of parasitology.

Age-wise and sex-wise distribution

There was preponderance of males both in clinical malaria cases who recovered and in death cases labelled as due to malaria clinically in A, C and D hospitals (about 65 per cent cases) but this fact has not come up in the distribution of total cases (Table 2) of all the four hospitals as hospital B caters only to female patients and thus has diluted this distribution.

Age-wise distribution of these cases brought out the fact that a majority of the patients (about 68 per cent) in both the cases, i.e. those who recovered and those who expired, were in the age group of 16-40 years as compared to 38.4% population falling in risk age group according to 1981 census[3].

This distribution in specific age and sex group may

Table 2. Age-wise and sex-wise distribution of clinical malaria cases and cerebral malaria deaths

Age group (years)	Clinical malaria cases			Clinical malaria deaths		
	Male	Female	Total	Male	Female	Total
< 1	3	1	4	0	0	0
1–4	9	7	16	1	1	2
5–15	22	24	46	1	1	2
16–40	87	92	179	7	6	13
> 40	11	7	18	2	1	3
Total	132	131	263	11	9	20

be due to the fact that (i) it is not a population-based study, (ii) immunity in older age group which was rather characteristic of the erstwhile hyperendemic area is absent at present[4,5], (iii) a majority of the male population of this age group sleep outdoors and are more exposed to mosquito bites and consequently to malaria, and (iv) about 15 per cent of the malaria cases treated in these hospitals belong to the adjoining states and a majority of them are males coming to the city for occupation.

Epidemiological classification

All the admitted cases were classified into two categories – indigenous and imported – based upon the residential address of the patient entered in the case sheets. This approach was adopted as it was not possible to follow the accepted norms of epidemiological classification in the present study. It was observed that about 13 per cent of the clinical malaria cases who recovered were imported (from outside Delhi). This proportion was higher (35 per cent) in the case of deaths due to malaria clinically, which may be due to the fact that a large number of patients from outside Delhi with serious symptoms are either referred by the attending physicians/hospitals (about 10 per cent in the study) or they themselves rush to hospitals in Delhi seeking expertise and better facilities. It may not be out of context to mention here that one death case was referred to hospital A by a hospital situated in Haryana, which is considered to be much better equipped as it even undertakes coronary bypass surgery. As a result, the patient lost crucial time, for it is a well-known fact that in cerebral malaria, a majority of severe *P. falciparum* cases, death takes place in the first 48 hours[6].

It was also noticed that the case fatality rate in imported cases (21.2 per cent) was much higher in comparison to indigenous cases (5.6 per cent), thus reflecting that imported cases which were generally from neighbouring states of Delhi came at a late stage and in more critical conditions.

Geographical distribution of malaria cases admitted

It was observed, after plotting the admitted cases on the map of Delhi according to their residential addresses, that catchment areas of hospitals C and D were limited to their surrounding areas only. But the origins of patients admitted to hospitals A and B were widely dispersed, which may be due to the fact that hospital A not only caters to general category of patients but to beneficiaries of Central Government Health Scheme also who are living in all parts of the city, and hospital B is a specialist hospital for only females who come from all over the city.

Clinical spectrum of malaria cases admitted in these hospitals

It was observed that 263 patients who were admitted to these hospitals with clinical diagnosis of malaria and recovered from the disease presented with a wide range of symptom complexes (Table 3).

The table shows that a majority of the patients presented themselves with either fever alone (34.2%) or fever with chills and rigour (34.6%), which is according to the accepted pattern of malaria presentation[5]. Only about 52 per cent of all the cases were subjected to blood smear examination for malaria parasite. Slide positivity rate was found to be in the following order: Symptoms of fever with altered sensorium (66.7%), fever with loose motion (62.5%), and fever alone (52%).

The clinical spectrum of declared malaria deaths was much more severe. A majority of them presented with history of fever (95%) but associated with altered sensorium (40%), unconsciousness (20%), convulsion (15%) and jaundice (15%). Out of these 20 death cases, blood smear was advised only in 9 cases, but the results are available on the case sheet only of 7 cases, and out of these 4 were negative. Two cases were positive

Table 3. Clinical spectrum of surviving malaria cases admitted in 4 hospitals of Delhi during 1990

Presenting symptoms	No. of patients (clinically suspected malaria)	Blood smears examined	Positive for malaria parasite	Positivity (%)
Fever	90 (34.2)	50	26	52
Fever with chills and rigours	91 (34.6)	47	12	25.5
Fever + vomiting	31 (11.8)	16	6	37.5
Fever + chills/rigours and vomiting	6 (2.3)	4	1	25
Fever + convulsions	5 (1.9)	3	0	0
Fever + convulsions + unconsciousness	9 (3.4)	3	0	0
Fever + altered sensorium	14 (5.3)	6	4	66.7
Fever + loose motions	13 (4.9)	8	5	62.5
Fever + loss in weight and appetite	4 (1.5)	3	0	0
Total	263 (100.0)	140	54	38.6

Figures in parentheses indicate percentage.

for *P. falciparum*, and one was positive for *P. vivax*, which in itself cannot cause mortality, and others were negative. The patient who was positive for *P. vivax* was a young female presenting with 3 days' history of fever with chills and rigour along with jaundice. This patient might have been suffering either from infective hepatitis (IH) with concomitant infection of *P. vivax* or from mixed infection with *P. falciparum* (as the blood slide was not examined properly).

Time lag between onset of symptom and admission

Among the recovered cases, only 39% of the patients reported to the hospital within 4 days of onset of symptoms (Table 4), which indicates that a majority of the patients might have taken treatment from medical practitioners or other health functionaries before reporting to the hospital. It was also observed that among the recovered cases

Table 4. Time lag between onset of symptom and date of admission and their positivity for malaria parasite in clinical malaria cases who recovered

Time lag (days)	No. of cases	No. of blood smears examined	Positive	Positivity (%)
0–1	29 (11.0)	7	6	85.7
2–4	71 (27.0)	35	8	22.9
5–9	61 (22.2)	40	19	47.5
10–15	36 (13.7)	25	9	36.0
> 15	66 (25.1)	33	12	36.4
Total	263	140	54	38.6

Figures in parentheses indicate percentage.

whose peripheral blood smear examination was conducted, only 37% were found positive. It can also be concluded from Table 4 that positivity for malaria parasite decreased with increasing time lag between the onset of symptoms and admission to hospitals, perhaps due to their taking antimalarials in inadequate doses from various agencies before admission. This aspect can be explained by the fact that slide positivity rate (SPR) was observed to be highest in the group of patients who reported to the hospital within 24 h of the start of illness.

Time lag between admission and treatment with antimalarials and microscopic examination of blood

Analysis of information about these aspects (Table 5) brought out the following facts:

(i) Out of 283 clinical malaria cases admitted in these hospitals, seven patients were never given antimalaria treatment and they were not screened for malaria parasite but still they were labelled as suffering from malaria.

Table 5. Distribution of all patients admitted with clinical malaria according to time lag between admission and BS examination and time lag between admission and antimalaria treatment

Time lag	No. of cases between admission and BS examination	No. of cases between admission and anti-malaria treatment
< 24 h	115	249
24-47 h	21	12
48-71 h	7	5
72-95 h	1	5
96 h and above	5	4
Not known	5	1
Not carried out	129	7
Total	283	283

(ii) Although a majority (about 91%) of those malaria cases who were given antimalarial drugs were administered the treatment within 24 h of admission, only 78% of the patients who were screened for malaria parasite were examined within the first 24 h. This indicates that some cases were screened for parasite after starting the antimalarials, which is not proper as both these aspects should be initiated simultaneously and reviewed later.

Type of treatment

Although about 98 per cent of all clinical malaria cases were administered antimalaria treatment, many of them were not screened for malaria parasite and clinicians were not sure of their diagnosis, and so this medication was given along with a large number of antibiotics.

The anti-malarial drug of choice is chloroquine, unless resistance is suspected, but in the study about 15% of clinically diagnosed malaria cases who had recovered were administered presumptively alternative drugs, i.e. Pyrimethamine + long acting sulpha combination single or along with chloroquine. It may be stated that the combination drug is not the drug of choice in *P. vivax* as many reports from Southeast Asia and South America stress that the response of *P. vivax* malaria to the combination of sulphadoxine with pyrimethamine has been much less satisfactory than in *P. falciparum* infection. Thus chloroquine followed by primaquine is still the best treatment for vivax malaria[7]. Further, chloroquine and sulphapyrimethamine drugs by themselves do no provide radical cure for *P. vivax* or *P. falciparum* as these drugs do not act on the liver stage of *P. vivax* and gametocytes of *P. falciparum*. In the absence of primaquine, the treatment would be incomplete and result in relapses in *P. vivax* malaria and *P. falciparum* cases will continue to act as reservoir of infection. Therefore, all cases are to be administered primaquine but in the study none of the hospitals, except hospital D prescribing this drug. Even in hospital D, primaquine was

given to only 10% of parasitologically positive malaria cases.

In clinically diagnosed malaria cases who succumbed to the infection and were admitted in these hospitals with very serious symptoms such as cerebral malaria, although the drug of choice should have been I.V. quinine, only one out of 20 such cases was treated with this drug and 16 were treated with injectable chloroquine. This may be due to unawareness of the physician about this aspect or nonavailability of this drug as in two death cases I.V. quinine was prescribed but injectable chloroquine was administered due to nonavailability of the former.

All these aspects about the management of malaria cases have been mentioned in the drug policy for malaria, formulated by the Directorate of National Malaria Eradication Programme, Delhi, and have been circulated to all the state malaria programme officers. As it was observed that a majority of the hospitals are not following the drug policy, there is a need for the state health authorities to bring this fact to their notice.

In addition, three death cases were not given antimalarials but the cause of death was mentioned as cerebral malaria. Out of these three cases, two were even not screened for malaria parasite. One patient who was given I.V. quinine, was screened for malaria parasite on the day of admission but was found negative so this death may not be due to malaria. A majority of these patients were administered many types of antibiotics along with chloroquine and supportive management. Although corticosteroids are not indicated in the management of cerebral malaria[8], one death case was administered steroids in addition to antimalarials. Although parenteral administration of chloroquine in children is not recommended by some workers due to its potential toxicity[8] there were four death cases belonging to the age group of 0-15 years who were administered intramuscular chloroquine.

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Biochemical Alterations in *Plasmodium vivax*-Infected Malarial Patients before and after Radical Treatment

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Biochemical alterations in 152 malaria patients infected with *Plasmodium vivax* were studied and the effect of parasitaemia on these changes was assessed. The degree of parasitaemia correlated positively with plasma uric acid, total and unconjugated bilirubin. A decrease in the levels of serum total protein, albumin, serum total, free and ester cholesterol was observed in vivax malaria. A follow-up study done on a section of the above patients after administration of chloroquine and primaquine for radical treatment of malaria showed that most of the alterations observed were brought back to normal. However, blood haemoglobin level was not restored to normal even after ten days of commencement of treatment.

INTRODUCTION

Malaria is one of the major parasitic diseases affecting humans today, contributing greatly to the high mortality rates in the developing world[1]. Sinden[2] has cautioned against fundamental generalizations against the genus *Plasmodium* because of species-related differences in ultrastructural, pathological and molecular organizations. Although the human disease caused by infection with *Plasmodium falciparum* is well documented, research on *Plasmodium vivax* has attracted scant attention because of its low fatality and fewer complications, the difficulty of culturing its blood stages and paucity of clinical cases in developed countries[3,4]. *Plasmodium vivax*, though not an intrinsically life-threatening disease, is important because of the morbidity and debility it produces as a result of relapses and frequent infections. Primaquine is the only drug in current clinical use for the radical cure of *P. vivax* infection as it is highly active against the latent exoerythrocytic stages. Very little study has been done on the course of the disease after drug therapy in humans.

We therefore investigated the alterations in blood haemoglobin, packed cell volume, blood glucose, plasma protein, serum uric acid, serum total, free and ester cholesterol and serum total, conjugated and unconjugated bilirubin levels in *P. vivax*-infected malaria patients before and after commencement of radical treatment.

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Table 1. Levels of blood haemoglobin, PCV, glucose, plasma protein, serum uric acid, bilirubin and cholesterol in malarial patients at different levels of parasitaemia and healthy and negative control subjects

Particulars	Healthy control	Negative control	Malaria	Malaria : Parasite density/mm^3 blood				
				< 250	251-450	451-650	> 650	F-ratio
Haemoglobin (g/dl)	14.8 ± 1.6 (183)	14.9 ± 1.3 (24)	13.7 ± 1.7‡a (102)	13.9 ± 1.6 (38)	13.5 ± 1.7‡a (34)	13.1 ± 2.1†a (15)	12.6 ± 1.5‡a (15)	2.4
PCV (%)	46.0 ± 3.3 (37)	44.3 ± 2.7 (21)	42.9 ± 5.0‡a (62)	43.0 ± 5.2 (26)	41.8 ± 5.3 (14)	40.9 ± 5.7 (4)	40.9 ± 2.4 (7)	0.5
Glucose (mg/dl)	95.3 ± 20.4 (192)	91.8 ± 14.3 (22)	100.4 ± 17.2 (61)	101.4 ± 19.1 (29)	99.5 ± 12.4 (13)	90.3 ± 14.8 (8)	106.1 ± 16.8 (11)	1.4
Uric acid (mg/dl)	5.3 ± 2.0 (160)	4.2 ± 2.1 (26)	5.0 ± 1.9 (76)	4.7 ± 1.7 (38)	4.8 ± 1.9 (22)	5.6 ± 1.1 (9)	6.7 ± 2.8*b (7)	2.9
Total protein (g/dl)	7.6 ± 0.7 (101)	7.8 ± 0.4 (21)	7.4 ± 1.0 (45)	7.5 ± 0.6 (22)	7.4 ± 0.6 (12)	7.5 ± 0.9 (5)	6.8 ± 2.1 (6)	0.9
Albumin (g/dl)	3.9 ± 0.6 (101)	3.7 ± 0.4*a (21)	3.5 ± 0.6‡a (45)	3.4 ± 0.7‡a (22)	3.7 ± 0.6 (12)	3.2 ± 0.6†a (5)	3.6 ± 0.7 (6)	0.8
Total bilirubin (mg/dl)	0.42 ± 0.21 (143)	0.42 ± 0.17 (20)	1.12 ± 0.56‡a (66)	0.73 ± 0.43‡a (31)	1.27 ± 0.38‡a (21)	1.48 ± 0.40‡a (6)	1.89 ± 0.36‡a (8)	22.3‡
Conjugated bilirubin (mg/dl)	0.05 ± 0.13 (143)	0.06 ± 0.11 (20)	0.38 ± 0.31‡a (66)	0.17 ± 0.25‡a (31)	0.50 ± 0.23‡a,b (21)	0.55 ± 0.20‡a†b (16)	0.74 ± 0.29‡a,b*c (8)	18.5‡
Unconjugated bilirubin (mg/dl)	0.37 ± 0.12 (143)	0.36 ± 0.11 (20)	0.75 ± 0.33‡a (66)	0.56 ± 0.24‡a (31)	0.78 ± 0.30‡a (21)	0.93 ± 0.26‡a†b (6)	1.14 ± 0.29‡a,b†c (8)	11.8‡
Cholesterol (mg/dl)	200.2 ± 32.2 (174)	173.6 ± 26.6‡a (28)	150.9 ± 40.1‡a (98)	161.3 ± 37.7‡a (47)	142.0 ± 40.6‡a (30)	144.8 ± 39.7‡a (9)	137.3 ± 42.9‡a (12)	2.1

Values are expressed as mean ± SD; Figures in parentheses indicate number of samples; Values are statistically significant, when $^{*}p < 0.05$; $^{\dagger}p < 0.01$; $^{\ddagger}p < 0.001$; a – significantly different compared to healthy control; b – significantly different compared to parasite density < 250/mm^3 blood; c – significantly different compared to parasite density 251 – 450/mm^3 blood.

MATERIALS AND METHODS

The subjects for this study included 152 adult, male malaria patients who had the trophozoite and gametocyte stages of *P. vivax* in the peripheral blood smear. These patients had not suffered an attack of malaria six months prior to the time of blood sampling. They reported to the free out-patient clinic of the Central Malaria Laboratory, Elephant Gate, Madras (55th Division), run by the Corporation of Madras. A section of the above patients (n = 44) who consented to repeated blood sampling formed the group after therapy. Radical treatment involved oral administration of 600 mg chloroquine and 15 mg primaquine/day for 2 days followed by 15 mg primaquine/day for 3 days in the presence of a basic health worker. Blood was drawn from these patients on every alternate day beginning with the day of commencement of treatment till the fifth day. A final sampling was done on the tenth day. Both thick and thin film smears were made in the out-patient clinic and stained according to the procedure given by Jaswant Singh and Bhattacharji[5]. Parasites per 100 leucocytes were counted in blood films and values per cubic millimetre of blood were estimated from the total leucocytic count[6]. Age-matched controls were 214 apparently healthy, adult male volunteers, employed by the Corporation of Madras. In addition, blood was withdrawn from 44 patients who complained of similar or malaria-like symptoms, but whose peripheral blood smear did not reveal any malarial parasites and were classified as negative controls. As only 4-5 ml of blood was withdrawn from patients and control subjects, all the parameters could not be studied for every sample and hence the uneven sample size observed.

The biochemical analyses carried out included blood glucose[7], total cholesterol[8], free and ester cholesterol[9], uric acid[10], total protein and albumin[11], and total, conjugated and unconjugated bilirubin[12]. Haemoglobin in blood was estimated by the cyanmethemoglobin method[13] and packed cell volume (PCV) by the Wintrobe macromethod[14].

Student's t-test was used to determine statistically significant differences between healthy control and malarial population and one-way analysis of variance was done to compare the sample means at different levels of parasitaemia and the F-ratio was computed. Pearson's correlation coefficient 'r' was arrived at to asses the degree of linear association among variables, taken two at a time. These analyses were carried out on a computer using a statistical software package.

RESULTS

Alterations before therapy

Malarial patients showed a significant decrease ($p < 0.001$) in blood haemoglobin level with a concomitant decrease in packed cell volume (Table 1). Haemoglobin values ranged from 9.0 to 16.4 g/dl in vivax malaria. From Fig. 1A, it is observed that 23% of patients had haemoglobin values below 12.0 g/dl and 2 patients had haemoglobin values below 10.0 g/dl. Malaria patients had a median haemoglobin value of 13.8 g/dl compared to 14.7 g/dl of healthy control. There was a pro-gressive decrease in blood haemoglobin levels with increasing parasite density and significant decreases were observed with higher parasit-aemia compared to healthy controls. However, the F-ratio indicated that the alterations observed at different parasitaemia levels were not significant. A similar decrease was also observed in packed cell volume with increasing parasitaemia.

Blood glucose level was not significantly altered in vivax malaria. Serum uric acid (Table 1; Fig. 2B), which did not show any significant change in malarial patients, showed a progressive increase with increasing parasitaemia and correlated positively ($r = 0.33$, $p < 0.05$, $n = 76$). Analysis of variance done at different parasitaemia levels

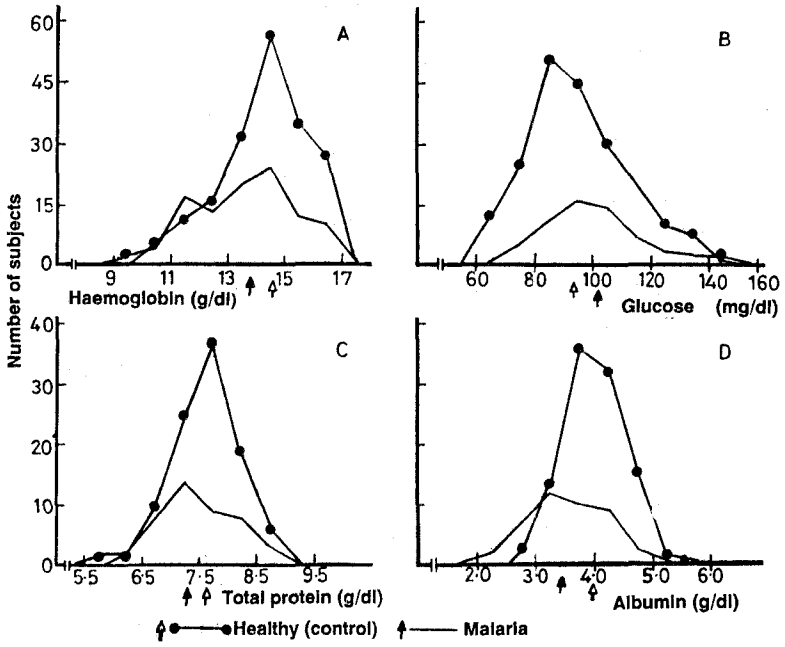

Fig. 1: Frequency distribution of blood haemoglobin, glucose, plasma total protein and albumin in healthy control and malaria subjects. Median values are indicated by arrows.

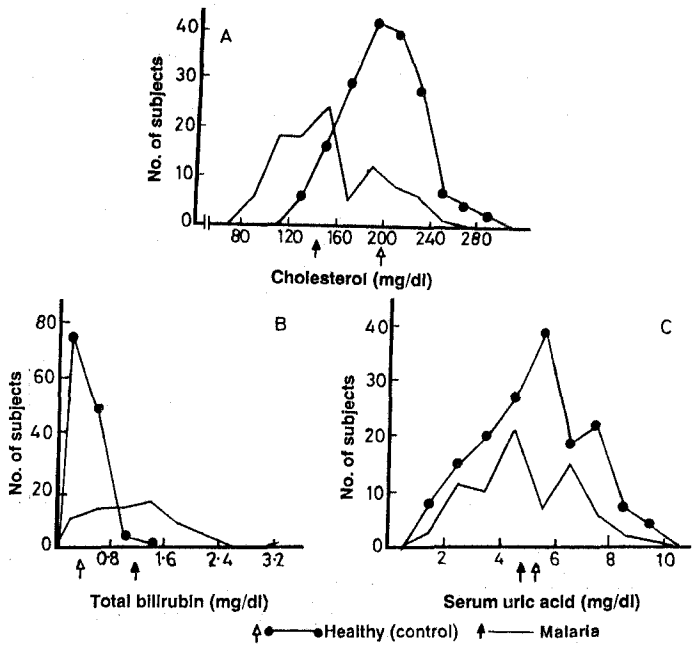

Fig. 2. Frequency distribution of serum cholesterol, total bilirubin and uric acid in healthy control and malaria subjects. Median values are indicated by arrows.

showed that the F-ratio for uric acid was significant at 5 per cent level and uric acid content at high parasitaemia ($> 650/mm^3$) was significantly different from low parasitaemia ($< 250/mm^3$). Though a marginal, insignificant decrease was observed in total proteins, albumin levels were significantly decreased ($p < 0.001$) in malaria compared to those of healthy controls (Table 1). Fig. 1C and D illustrate the frequency distribution of total protein and albumin levels in healthy controls and malarial patients. The healthy control population showed a total protein peak between 7.5 and 8.0 g/dl, while the malarial population showed a peak between 7.0 and 7.5 g/dl. Nearly 68% of the healthy control population had albumin levels between 3.5 and 4.5 g/dl, while only 42% of the malarial population fell within that range.

Serum cholesterol level was significantly decreased ($p < 0.001$) in malarial patients (Table 1). Negative control population also displayed a significant decrease ($p < 0.001$) compared to healthy controls. However, the decrease of serum cholesterol was more pronounced in malaria than that in negative control. The frequency distribution for serum cholesterol showed two peak frequencies in the malarial population (Fig. 2A). The major frequency peak was between 140 and 160 mg/dl, while the minor peak corresponded with that of the normal healthy population. More than 65% of the malarial population had cholesterol levels below 160 mg/dl and nearly 25% below 120 mg/dl. Six cases even had serum cholesterol below 100 mg/dl. Serum cholesterol levels progressively decreased with increasing parasitaemia, but the mean cholesterol levels at different parasite densities were not significantly different from each other. Free and ester cholesterol levels were significantly decreased ($p < 0.001$) in malaria (Fig. 3). However, the ratio of ester to free cholesterol remained unaltered.

Total bilirubin levels were significantly elevated ($p < 0.001$) in vivax malaria (Table 1). This increased level of bilirubin was found to be associated with significant increases ($p < 0.001$) in both conjugated and unconjugated bilirubin. Total bilirubin ranged from 0.20 to 2.75 mg/dl in malarial patients (Fig. 2B). Conjugated bilirubin levels were elevated over 6-fold compared to those of healthy controls. Total bilirubin levels showed a progressive increase with increasing parasite density. There was a significant increase ($p < 0.001$) in all the groups compared to healthy control. A positive correlation was observed for total ($r = 0.35$, $p < 0.005$, $n = 66$) and unconjugated bilirubin ($r = 0.61$, $p < 0.05$, $n = 66$). The F-ratio was also statistically significant at 0.1 per cent level which indicated that the mean bilirubin levels at different parasite densities were significantly different from each other.

Alterations after therapy

Following radical treatment, blood haemoglobin values were decreased even further on Day 3 and Day 5. Blood samples obtained on Day 10 revealed that haemoglobin levels were still signi-

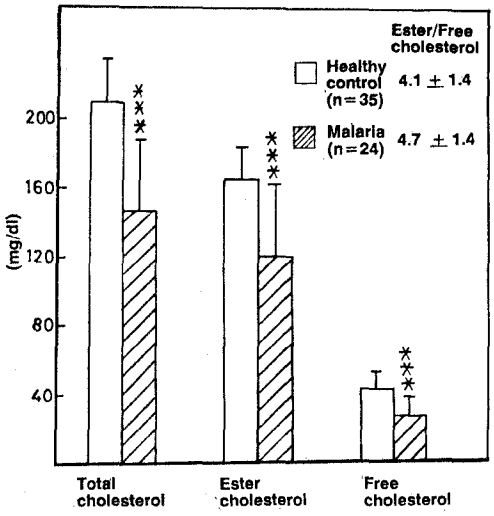

Fig. 3: Levels of serum total, free and ester cholesterol in healthy controls and malaria subjects.

Figures in parentheses indicate the number of samples. Values are statistically significant compared to those of healthy control, when ***$p < 0.001$.

Table 2. Concentrations of blood haemoglobin, packed cell volume, glucose, plasma protein and serum uric acid following drug treatment in *P. vivax*-infected malaria patients

Particulars	Healthy control	Malaria : After therapy			
		Day 1	Day 3	Day 5	Day 10
Haemoglobin (g/dl)	14.4 ± 1.6 (183)	13.4 ± 1.9 (21)	12.4 ± 2.6 (14)	12.1 ± 1.6 (7)	13.2 ± 1.2* (11)
PCV (%)	46.0 ± 3.3 (37)	39.3 ± 3.2 (6)	40.4 ± 5.0 (6)	40.3 ± 5.1 (6)	44.7 ± 2.0 (6)
Glucose (mg/dl)	95.3 ± 20.4 (192)	99.1 ± 27.9 (19)	84.9 ± 8.2 (15)	92.2 ± 23.4 (7)	81.5 ± 10.2* (11)
Total protein (g/dl)	7.6 ± 0.7 (101)	7.6 ± 0.8 (16)	8.3 ± 0.3 (10)	8.0 ± 0.4 (8)	7.5 ± 0.3 (8)
Albumin (g/dl)	4.0 ± 0.6 (101)	3.5 ± 0.7 (16)	3.8 ± 0.4 (10)	3.5 ± 0.5 (8)	3.6 ± 0.2 (8)
Uric acid (mg/dl)	5.3 ± 2.0 (160)	6.2 ± 2.1 (20)	4.1 ± 0.8 (15)	3.7 ± 1.4 (12)	4.2 ± 1.2 (11)

Values are expressed as mean ± SD; Figures in parentheses indicate number of samples; Day 10 values are statistically significant compared to healthy control, when *$p < 0.05$.

ficantly decreased ($p < 0.05$) compared to those of healthy controls. However, packed cell volume content approached normal levels by the tenth day (Table 2).

Total protein and albumin levels showed a varied pattern during the course of treatment and were restored to normal by the tenth day. Serum uric acid level showed a progressive decline on Day 3 and Day 5 and by Day 10, it was lower than in healthy controls. However, this lowered value was not statistically significant (Table 2).

Total cholesterol, ester and free cholesterol reached near normal levels by Day 10. The ester/free cholesterol ratio, which showed a fluctuation during therapy, was also restored by the tenth day (Table 3). Total bilirubin, conjugated and unconjugated bilirubin, which were significantly elevated on Day 1, showed a progressive decrease through Days 3 and 5 and reached near normal levels by the tenth day (Table 3).

DISCUSSION

Malaria has been the scourge of tropical populations and Madras city has been known to be endemic to malaria. *P. vivax* has largely been the infective species responsible for malarial morbidity. In our study, the 5-day radical treatment was followed as it has been shown to give good results and follow-up studies for one year showed only 1.3% relapse after a primaquine course of 5 days' duration[15].

Haemolytic anaemia is one of the hallmarks of malaria and is attributed to premature destruction of red cells. However, unlike *P. falciparum* infection, *P. vivax* is limited in its multiplication potential as they invade only reticulocytes[16]. In our study, mean haemoglobin levels at high parasitaemia did not fall below 12.0 g/dl and the degree of anaemia and PCV did not correlate significantly with parasite density. Earlier studies from our laboratory had shown no alteration of mean corpuscular volume, mean cellular haemoglobin and mean corpuscular haemoglobin concentrations in vivax malaria[17]. Only a few reports are available about the course of anaemia after an attack of malaria. We have observed that haemoglobin level continues to fall for a variable period following treatment. This trend may be due to the fact that

Table 3. Distribution of serum cholesterol and bilirubin following drug treatment in *P. vivax*-infected malaria patients

Particulars	Healthy control	Malaria : After therapy			
		Day 1	Day 3	Day 5	Day 10
Total cholesterol (mg/dl)	200.2 ± 32.2 (174)	145.5 ± 32.2 (18)	152.4 ± 32.1 (14)	184.0 ± 31.3 (8)	193.7 ± 23.9 (11)
Ester cholesterol (mg/dl)	164.7 ± 19.2 (35)	121.7 ± 30.9 (18)	126.0 ± 29.4 (14)	156.1 ± 26.1 (8)	155.8 ± 19.6 (11)
Free cholesterol (mg/dl)	43.3 ± 11.9 (35)	23.7 ± 5.8 (18)	21.8 ± 5.8 (14)	29.7 ± 7.4 (8)	37.9 ± 6.2 (11)
Ester/Free cholesterol (mg/dl)	4.1 ± 1.4 (35)	5.3 ± 1.3 (18)	6.0 ± 2.0 (14)	5.9 ± 1.4 (8)	4.2 ± 0.5 (11)
Total bilirubin (mg/dl)	0.42 ± 0.21 (143)	1.51 ± 0.53 (15)	1.43 ± 0.71 (10)	0.75 ± 0.38 (8)	0.50 ± 0.32 (10)
Conjugated bilirubin (mg/dl)	0.05 ± 0.13 (143)	0.58 ± 0.38 (15)	0.51 ± 0.35 (10)	0.21 ± 0.22 (8)	0.07 ± 0.13 (10)
Unconjugated bilirubin (mg/dl)	0.37 ± 0.12 (143)	0.93 ± 0.21 (15)	0.92 ± 0.39 (10)	0.56 ± 0.32 (8)	0.43 ± 0.21 (10)

Values are expressed as mean ± SD; Figures in parentheses indicate number of samples; Day 10 values are statistically significant compared to healthy control.

erythropoietin production is not being impaired during malaria and erythrocyte destruction exceeds the rate of erythropoiesis[18]. Primaquine has been known to be a haemolytic drug and the degree of haemolysis is related to drug dosage[19]. Clark and Hunt[20] have shown that reactive oxygen intermediates cause haemolysis and parasite death in *P. vinckei*-infected mice. We have already reported increased lipid peroxidation in erythrocytes of *P. vivax*-infected malaria patients following radical treatment[21].

As only 'random' blood samples could be obtained from malarial patients, blood glucose levels seem to be unaltered significantly compared to those of healthy and negative controls. Though there are no reports of hypoglycaemia in vivax malaria, it is increasingly recognized as a manifestation of falciparum malaria[22]. The hypoalbuminaemia observed in vivax malaria with decrease in total proteins may be attributed to decrease in albumin synthesis by the liver. However, no correlation has been observed between total protein and albumin with parasitaemia and a similar finding has been reported by Seshadri *et al*[23]. Uric acid has been demonstrated to be a powerful scavenger of singlet oxygen, peroxyl and hydroxyl radicals[24]. The increased uric acid concentration at high parasitaemia is suggestive of its role as an antioxidant *in vivo*. Stocker *et al.*[25] have observed a similar increase in the plasma concentration of uric acid in *P. vinckei*-infected mice.

The subnormal levels of serum total, free and ester cholesterol observed during malarial infection reaching normal levels after treatment suggests an active involvement of lipid metabolism. The decrease observed in malaria is more pronounced than in negative control. Serum cholesterol is reported to decrease during fever, pneumonia and other acute infections[26-28]. Reduction in total lipids and cholesterol of plasma has been observed in mice infected with *P. vinckei*[29]. Rapid exchange of cholesterol between serum and erythrocytes has been reported[30]. The decreased serum cholesterol observed in vivax malaria may be associated with the impairment of cholesterol synthesis in the liver and increased uptake by the

infected erythrocytes. In severe falciparum malaria, jaundice is quite common resulting mainly from haemolysis[31]. In our study, a parasitaemia-related increase of total bilirubin content was observed. A similar observation on bilirubinaemia mainly of the unconjugated type has been reported in malarial mice[32]. The elevated levels of both unconjugated and conjugated bilirubin observed in this study may indicate a sign of hepatic dysfunction. In *P. vivax* infection, parasitaemia rarely exceeds 2 per cent unlike the high parasitaemia that is observed in human falciparum malaria and in cells under culture. Despite the low parasitaemia levels in vivax malaria, the marked alteration in the host metabolism, especially the role of serum cholesterol, in the diseased state, points to the need for further investigation.

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Gambusia affinis : Dispersal due to Floods and its Failure to Colonize New Water Bodies in Shahjahanpur District (U.P.)

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In villages of District Shahjahanpur, 122 decentralized *Gambusia* multiplication ponds were established to cover the need of the entire district. Profuse breeding of *Gambusia* was observed in these ponds. The fishes are being successfully used in mosquito control all over the district. In July 1990 there was a widespread flood due to which 70 *Gambusia* multiplication ponds were affected and the fish was washed away in large numbers, leaving only a scanty population in the flood-affected ponds. We utilized this opportunity to study the natural dispersal and colonization of *Gambusia* in different aquatic habitats. The study revealed that *Gambusia* was either not found in most habitats or was present in very small numbers, and on its own *Gambusia* was unable to eliminate the local fauna to become a dominant species. Predatory fishes and birds played a major role in eliminating *Gambusia*. *Gambusia* is therefore unlikely to pose any ecological hazard in vector-control programmes.

INTRODUCTION

Gambusia affinis has been used extensively for mosquito control throughout the world[1]. The species was imported in India by B.A. Rao in 1928 and since then it is being used in the urban malaria scheme (UMS) to control mosquito breeding. Sitaraman[2] showed that introduction of *Gambusia* in wells in Hyderabad city controlled *An. stephensi* breeding and curtailed malaria transmission. *Gambusia* was introduced in Shahjahanpur villages in 1988 for the first time to control malaria. The initial stock of about 10,000 was brought from MRC's field station at Haldwani (Nainital district). The fishes were released in a village pond after removing the weeds and predatory fishes. *Gambusia* multiplied enormously in a few weeks in this pond. Thereafter, *Gambusia* from this pond were introduced on a large scale into various other ponds of the district during 1989-1990. A total of 122 multiplication ponds being established throughout the district. The growth and survival of *Gambusia* was very good in village ponds where weeds and predatory fishes had been removed. The species is being produced by the millions in these ponds and whenever required these are collected and released in different types of mosquito-breeding habitats such as

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ponds, wells, marshes, riverine areas, borrow pits, stored waters, rice fields and drains.

Because of the voracious feeding and high multiplication rate of *Gambusia*, questions have been raised about the possibility of ecological harm to the local fauna and flora[3,4]. During July 1990 there was a heavy flood in Shahjahanpur district and as a result *Gambusia* were washed away along with flood waters and got dispersed to far-off habitats. The flood therefore provided an opportunity to study *Gambusia* dispersal and associated hazards. An extensive survey of different aquatic habitats in the district was made to assess the dispersal and colonization of new habitats by *Gambusia*. The results of this study are reported in this paper.

MATERIALS AND METHODS

Study area

In Shahjahanpur there are seven rivers, viz. Garrah, Khannaut, Ram Ganga, Katna, Gomti, Behgul and Ganga in addition to Sharda canal, and a number of its tributaries form a network of several minor canals in the area. Table 1 shows PHC-wise total number of wells, ponds and the number utilised for *Gambusia* production in the entire district.

Survey of different types of aquatic habitats

Surveys were carried out prior to the rainy season, from April to June 1990, to find out the natural occurrence of *Gambusia* in different water bodies in Shahjahanpur. After the onset of monsoon the water level of all major rivers started rising and some of them like Garrah crossed the danger level (148 - 148.9 m) during 12-14 July 1990 which resulted in heavy floods. The flood adversely affected about 500 villages of Dadraul, Nigohi, Bhawal Khera, Kant, Tilhar, Powayan, Jalalabad, Banda and Khutar PHCs. The flood water also entered *Gambusia* multiplication ponds in rural

Table 1. Establishment of *Gambusia* multiplication ponds and wells in Shahjahanpur district

S. No.	PHC	Total no. of wells	No. of wells with *Gambusia*	Total no. of ponds	No. of ponds used for multiplication
1.	Nigohi	1678	4	479	19
2.	Sindhauli	1316	6	402	22
3.	Powayan	1260	7	872	13
4.	Bhawal Khera	1968	7	843	14
5.	Khudaganj	1678	–	887	09
6.	Tilhar	2414	11	885	24
7.	Kant	2233	–	515	17
8.	Jalalabad	2929	–	779	10
9.	Mirjapur	1194	–	318	7
10.	Kalan	1266	–	382	9
11.	Banda	1414	–	825	9
12.	Khutar	984	–	802	15
13.	Jaitipur	2878	–	441	10
14.	Dadraul	2790	1073	505	104
	Total	26268	1108	8936	282*

*Out of 282 *Gambusia* multiplication ponds 122 are being maintained

areas, thus providing an easy escape for the fish to enter in new habitats. About 70 *Gambusia* hatcheries were adversely affected by the floods and about 10 million *Gambusia* were washed away.

An extensive survey of *Gambusia* in different types of aquatic habitats was carried out from August 1990 onwards covering about 80 km^2 area in Dadraul PHC. A total of 5 rivers (excluding Ganga), 10 canals, 2 drains, 51 low-lying areas, 88 ponds, 99 pools and 270 rice and sugarcane fields were thoroughly searched for the presence of *Gambusia*. Nets of 30 × 3 m, 20 × 2 m, and 10 × 2 m were used for fish collection taking into consideration the size of the water body and convenience in the netting operation. In the rice and sugarcane fields the density of *Gambusia* was monitored by using an iron drum of 1 m dia., open at both ends as netting was not possible in such aquatic habitats. The drum was placed at 4 different corners and one in the centre of the rice/sugarcane fields. The fishes trapped inside the drum were collected and the density (No./m^2) of *Gambusia* was calculated. In ponds, pools, ditches and low-lying areas, fishes were captured through repeated netting. Fishes caught during each netting were brought to the laboratory, counted and identified, and the mean density of *Gambusia* was calculated.

RESULTS AND DISCUSSION

The results of the survey are summarised in Table 2. There was a wide dispersal of *Gambusia* during floods and the density of fish varied considerably from habitat to habitat. A large number of other fish species were also found in these habitats. Table 3 gives the list of 44 fishes found in addition to *Gambusia*. Nineteen fish species were predatory and the rest were non-predatory. Of the former, 18 species were abundant in all the five rivers. The predatory fishes were also available in other aquatic habitats but in small numbers.

An analysis of the fish catches from different aquatic habitats showed that although there was a wide dispersal of *Gambusia* with floods, the establishment of the fish in new environments was very poor. In rivers, not a single *Gambusia* could be caught even after extensive netting, perhaps because of the presence of a wide variety of predatory fishes and their poor survival in running waters.

Out of 116 spots of six canals searched, only 6 spots showed the presence of *Gambusia* which were restricted to a minor canal where floods resulted in the intermixing of ponds and canal water. The population density of the fish in the minor canals varied between 0.3 and 0.6 fish per square metre during the post-flood period (September-November) and in later surveys (December-January) *Gambusia* was absent. Newly hatched *Gambusia* were rarely found, suggesting poor survival in the canals.

The establishment of *Gambusia* in ponds, pools and ditches through natural dispersal was also very poor. Only seven ponds out of 88 contained *Gambusia*. In this area most ponds and pools are seasonal and get filled up during the rainy season in which predatory fishes from adjacent areas find their way and consume *Gambusia*. These ponds and pools dry up gradually and the fishes are not only captured by local people but also consumed by birds.

Out of 51 low-lying areas searched, *Gambusia* was found in 32 places. The density of *Gambusia* varied between 1.8 and 3.5 fish per metre square but decreased quickly because of the predatory fishes, birds and drying of these habitats. *Gambusia* population in agricultural fields was found high owing to raised level of water in *Gambusia* ponds adjacent to fields because of heavy rains. The density of *Gambusia* was highest (5.6/m^2) during the flood period and declined to 0.3 in the post-flood period (Table 2). The number of fishes in rice fields gradually declined owing to decrease in water level with paddy growth. The dispersal of *Gambusia* in rice fields and low-lying

Table 2. Natural dispersal of *Gambusia* in Shahjahanpur district

No. of water bodies searched	Pre-flood period			Flood period	Post-flood period				
	Apr 90	May 90	June 90	July and Aug 90	Sep 90	Oct 90	Nov 90	Dec 90	Jan 91
1. Rivers	2	4	5	2	4	5	5	3	3
Spots surveyed	29	13	12	4	32	28	12	21	23
Gambusia present	0	0	0	0	0	0	0	0	0
Density*	0	0	0	0	0	0	0	0	0
2. Canals	1	0	0	0	2	3	3	7	10
Spots surveyed	3	0	0	0	32	35	14	15	17
Gambusia present	0	0	0	0	2	3	1	0	0
Density*	0	0	0	0	0.55	0.3	0.6	0	0
3. Drains	1	1	1	2	2	2	1	1	1
Spots checked	3	5	4	12	6	5	3	5	3
Gambusia present	1	1	0	2	2	1	0	0	0
Density*	0.01	0.03	0	2.6	1.2	0.68	0	0	0
4. Low-lying areas	4	2	3	12	13	7	0	3	7
Gambusia present	0	0	0	3	3	1	0	0	0
Density*	0	0	0	3.5	1.8	1.8	0	0	0
5. Ponds	13	16	6	13	9	12	3	7	9
Gambusia present	1	2	0	1	3	0	0	0	0
Density*	0.62	1.06	0	0.3	1.6	0	0	0	0
6. Pools/ditches	16	9	11	6	19	16	3	13	6
Gambusia present	0	0	1	1	6	6	0	1	0
Density*	0	0	0.6	2.8	0.35	1.05	0	0.7	0
7. Rice/Sugarcane fields	0	0	0	118	81	59	12	–	–
Gambusia present	0	0	0	76	66	12	–	–	–
Density*	0	0	0	5.6	4.2	0.2	–	–	–

*Density, No./m^2.

areas during the flood was certainly useful for mosquito control.

Out of 46 spots in two major drains of the area searched, only seven were found with *Gambusia*. The population density varied between 0.1 and 2.6 fish per sq m. In drains the density of fish was higher during the post-flood period than in the pre-flood period. The absence of *Gambusia* in drains from November onwards was mainly due to infestation of water hyacinth which completely choked the drains, allowing netting only at a few spots. Moreover, predatory fish *Channa punctatus,* found abundantly in the drains, might have consumed *Gambusia.*

The results point to the conclusion that *Gambusia* is unable to colonize new habitats specially the rivers on its own owing to the presence of a large number of predatory fishes and birds and also to

Table 3. Fishes found during search for *Gambusia* dispersal

S. No.	Category/Fish species	Rivers	Ponds/ Pools	Canals	Drains
Predatory fishes					
1.	*Wallago attu*	+	+	+	Nil
2.	*Mystus cavasius*	+	Nil	Nil	Nil
3.	*Mystus tengara*	+	+	Nil	Nil
4.	*Mystus vittatus*	+	+	Nil	Nil
5.	*Mystus oar*	+	Nil	+	Nil
6.	*Mystus seenghala*	+	+	Nil	Nil
7.	*Rita rits*	+	Nil	Nil	Nil
8.	*Bagarius bagarius*	+	Nil	+	Nil
9.	*Clupisoma garua*	+	Nil	Nil	Nil
10.	*Eutropichthys vacha*	+	Nil	Nil	Nil
11.	*Pseudeutropius* sp.	+	Nil	Nil	Nil
12.	*Heteropneustes fossilis*	+	+	Nil	+
13.	*Clarias batrachus*	+	+	Nil	+
14.	*Channa punctatus*	+	+	+	+
15.	*Channa striatus*	+	+	+	Nil
16.	*Channa marulius*	+	Nil	+	Nil
17.	*Channa gachua*	Nil	+	Nil	+
18.	*Notopterus notopterus*	+	+	+	+
19.	*Notopterus chitala*	+	Nil	Nil	Nil
Non-predatory fishes					
20.	*Labeo rohita*	+	+	+	Nil
21.	*Labeo calbasu*	+	+	Nil	Nil
22.	*Labeo gonius*	+	+	Nil	Nil
23.	*Labeo pata*	+	+	+	Nil
24.	*Catla catla*	+	+	Nil	Nil
25.	*Cirrhinus mrigala*	+	+	Nil	Nil
26.	*Cirrhinus reba*	+	+	+	Nil
27.	*Chanda nama*	+	+	+	+
28.	*Chanda ranga*	+	+	+	+
29.	*Colisa fasciata*	+	+	+	+
30.	*Chela bacaila*	+	+	+	+
31.	*Esomus danricus*	+	+	+	+
32.	*Nandus nandus*	Nil	+	Nil	+
33.	*Mastacembelus armatus*	+	+	+	+

contd...

Table 3. Fishes found during search for *Gambusia* dispersal *(contd.)*

S. No.	Category/Fish species	Rivers	Ponds/ Pools	Canals	Drains
34.	*Mastacembelus puncalus*	+	+	+	+
35.	*Puntius stigma*	+	+	+	+
36.	*Puntius sophore*	+	+	+	+
37.	*Puntius ticto*	+	+	+	+
38.	*Puntius sarana*	+	+	+	Nil
39.	*Danio rerio*	+	+	+	+
40.	*Anabas testudinus*	Nil	+	Nil	+
41.	*Rasbora daniconius*	+	+	+	+
42.	*Amblypharyngodon mola*	+	+	+	+
43.	*Xenentodon cancila*	+	+	+	Nil
44.	*Lepidocephalus guntea*	+	+	+	+

+ – Present.

fact that most water bodies are infested with weeds. *Gambusia* multiplication in any water habitat, therefore, requires protection from predatory fishes as well as from water weeds like water hyacinth. Under such circumstances, it is unlikely that *Gambusia* will pose any significant ecological hazards, thus can be used extensively in mosquito control programmes.

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SHORT NOTES

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Socio-cultural Factors Associated with Malaria Transmission: A Review

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When investigating malaria, social scientists try to relate its continuance to a variety of socio-cultural factors, ranging from those operating at the individual level to those at the community level. The principal socio-cultural factors that need to be examined in research on malaria are the socio-economic status of the people, illiteracy, system of beliefs, norms and values, and sleeping habits of people. There has been, however, little comprehensive research to understand how socio-cultural factors may contribute to creating preconditions for malaria transmission.

Socio-economic status of people and malaria

The fact that the incidence of malaria is more common among the low socio-economic groups in tropical countries is a good indication of the importance of socio-cultural factors in contributing to malaria transmission. Even though most studies on socio-economic conditions and malaria are confined to desk work, they shed light on the needs and new avenues for further research in this domain. Khan[1], Banguero[2], Wessen[3], Rebello and Verma[4], Mata[5], Brown[6], and Pinikahana[7] provide some insights into the socio-economic status of the people and malaria. According to Wessen[3] the vicious cycle of malaria is a matter of great significance specifically in the sense that low socio-economic status of the people not only contributes to creating the preconditions for malaria but also that the syndrome of poverty provides ways in which malaria may spread, hindering the control of the disease. Wessen further states that although it is not precisely known to what extent malnutrition reduces resistance to disease like malaria, we may suspect an important relationship.

Brown[6] suggests that the malaria eradication programmes in Surdinia and Sri Lanka were based on a mental model of the vicious cycle which characterises 'people who are sick because they are poor and they become poorer because they are sick'. Banguero[2] studied the association of socio-economic factors with malaria in Colombia in which 217 households (cases) were investigated by comparing a similar number of households as controls (in which no cases were reported in the same period). It was shown that the prevalence and incidence of malaria were associated with the low income of the family. Mata[5] points out that poor housing and deficient personal hygiene are due to

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poverty and low educational level. Mata also found that poor housing and outdoor activities after dark are of great significance as socio-cultural determinants of malaria transmission. By contrast, Banguero[2] found no relationship between the degree of completion of the house (roof, walls, windows, doors) and malaria incidence, nor did he find any association between the education level and the disease incidence.

Illness behaviour, socio-religious beliefs and malaria

The human behaviour with regard to the aetiology, treatment and prevention of malaria not only fosters the spread of the disease but also results in continuing of the disease within the community. People seek medical care depending upon the individual perceptions of illness. The concept of 'illness behaviour' under which individual perceptions of ill health are analysed has been reviewed by many workers[8-16]. In Mechanic's terms, 'Illness behaviour is the ways in which given symptoms may be differentially perceived, evaluated and acted (or not acted) upon by different kinds of persons'[10].

It appears that although most of the studies about beliefs and values of the people and the continuance of malaria are not conceptually focused on the 'illness behaviour' and malaria transmission, such studies seem to provide much wider perspectives which not only possess some practical significance but also some research interest.

As regards the beliefs of the people in relation to the causation of malaria, there are two typical examples, one from India[17] and another from Surinam[18]. The former study concerning 'tribal' populations in 18 villages in Orissa state identified the perceptions about causation, prevention and treatment of malaria. In general, the tribals believed that diseases are caused primarily by the spirits of the dead, anger of the local deities and black magic. People in these villages could not distinguish malaria from other types of fever, and regarded malaria as a mild and self-limiting disease. Malaria fever, they believed, is the result of climatic factors. Mosquito bites, like bed bug bites, were not viewed as harmful to health and as a result, treatments (even magico-religious treatments) were not taken for malaria.

Barnes and Jenkins[18] studied the perceptions of Bush Negroes in Surinam with regard to the cause of malaria. According to these authors, Bush Negroes do not think a mosquito could be a vector of malaria. Interestingly, the Negroes' world-view is reflected in the statement by a local man in this survey as:

"Malaria is a very big disease. Is not it? It makes a big man shake with fever. A mosquito is very small, so small you can hardly see it. How can such a little thing cause such a big disease?".

Ogunmekan's study[19] in Nigeria, in which 141 women took part, showed that 48% of the women thought that mosquitoes do not cause malaria and the causes given by them include germs, overwork, working in the sun, eating much too hot or cold food, eating bad food and drinking infected well water. Trent[20] found that in Ghana where he studied 202 children in 93 housing units more than half of the subjects did not know the cause of malaria. It was ascribed to mosquitoes by 33 of the respondents. Although most of the respondents knew that malaria was a disease or an illness, 22 said they had never heard the word malaria. Asked about the cause of malaria, only 4 respondents from 93 adults could describe accurately the role of mosquitoes in malaria transmission.

A survey carried out by the same author in which 227 adults and 161 children participated in Ghana concluded that 61% of children and 45% of adults did not know that malaria is caused by mosquitoes and that 25% of the adult subjects did not consider malaria a serious disease. Oghalu[21] found that of the 250 respondents drawn from two towns in

Nigeria, 75.8% were unable to identify the main breeding sites of mosquitoes. Asked about the cause of malaria in a separate research project carried out in that country, most of the respondents frequently mentioned that hard work, the will of Allah, lack of proper food and strong desert winds may result in malaria fever[22]. Pinikahana[7] found that insufficient knowledge of malaria among the people played a major part in high malaria transmission in Kudagam 1 in the Lunugamwehera irrigation project in Sri Lanka. The village folk thought that drinking filthy water with mosquito eggs, working in the sun, bathing in evenings, change in climate, personal contact and body weakness were responsible for malaria transmission.

The degree of perceived threat of malaria provides much insight into the understanding of the illness behaviour of the patient, which certainly leads either to increase or decrease in the risk of malaria within the community. If the perceived threat of malaria is mild then the tendency to seek medical treatments will also decline, which in turn leads to a large number of untreated patients who are capable of spreading malaria. This segment of 'disease iceberg' of malaria will neither intentionally contribute to destroy adult mosquitoes nor to reduce the mosquito-breeding sites. As regards the cultural perceptions of the threat of malaria, many studies demonstrate that most people in tropical countries where malaria is endemic regard malaria as a mild disease. Dhillon and Kar[17], Barnes and Jenkins[18], Dixit[23], Amelsoort[22], Trent[20], Rebello and Verma[4] and Arora and Salu[24] favour the above view. Rebello and Verma's study[4] in Haryana state in India shows that malaria is thought of as one of the least dangerous diseases. Trent[20] points out that one-fifth of the respondents in his survey in Ghana did not consider malaria a serious disease for children, and nothing was done by 79% of the respondents to protect themselves from mosquitoes. Jackson[25] studied the biocultural perceptions of malaria in Liberia and ranked the symptomatology of malaria for children and mothers by cultural severity.

If malaria is believed to be due to evil spirits, hard work or lack of food, the response will be inevitably oriented towards magico-religious rituals or indigenous herbals or no treatment. The lack of treatment for malaria may result in continuance of malaria patients within the community as potential sources of malaria infection.

The refusal of household spraying in many parts of the world has been recorded either as due to ignorance of mosquito control or to rigid folk social beliefs that vary by degrees. Dhillon and Kar's study[17] in Orissa identified some reasons for the refusal of household spraying. One of the reasons is that spraying produces a bad smell in rooms in which they live. In addition, spraying causes inconvenience and waste of time in shifting household goods. Since the people are not aware of its benefits, spraying is considered useless in these villages. In the survey of Surinam, Barnes and Jenkins[18] investigated the reasons for refusal of household spraying as: (i) fear of the loss of domestic animals (cats, dogs and chickens); (ii) fear that the insecticides would cause personal harm to the householders and their families; (iii) fear that the insecticides would destroy or weaken the protective power of the gods; (iv) jealousy between kinship groups for jobs with the malaria eradication programme; (v) enviable position of the employees of the Malaria Eradication Programme (MEP). Promiscuous behaviour of the MEP workmen with local women, leading to troubles in several households; (vi) use of unpleasant insecticides; (vii) dislike of modern medicine; and (viii) resistance to giving blood smears.

In Oghalu's study[21] in Nigeria it was found that although more than half (55.9%) of the respondents used insecticides, the rest of the respondents did not use them because of the bad smell, lack of money to buy it and fear that it could poison their food and domestic animals.

In Terai villages in Nepal, inhabitants mud-wash their houses every day, or on any '*pooja*' day in the family—a practice which is resorted to as soon as the spray teams left the house. In some houses, the housewives rubbed off the sprayed surface immediately after spray teams left the houses[23]. In the Terai villages, as seems to be the case in Orissa and Surinam, people fear that household spraying increases household rats, mice and bed bugs and hence many houses remain unsprayed because of the refusal of the people. Also, a study carried out in Baygada and Teypore areas in Orissa in India during 1973-74 showed that 48 to 60% of sprayed houses had been mud-plastered within 2 to 6 days[24].

Spray teams in Sri Lanka have faced difficulty in getting access to Muslim houses because of the practice of *purdah*, which forbids non-kin males from entering houses when only women are present. The Tamils, an ethnic minority who follow a strict caste ideology, pose the same problem to sprayers: 'high caste' Tamils deny entrance to sprayers if they are of a low caste, for fear of ritual contamination[26-28].

In Pinikahana's study[7] in the Lunugamwehera irrigation area in Sri Lanka, it was found that the effectiveness of household spraying with malathion is diminished by refusals or by allowing only partial spraying in some houses. Bad odour, fear of water and food being poisoned in homes, fear of killing domestic animals like pets, discolouration of walls, inconvenience caused by removing furniture and other belongings, the dirtiness of the house after spraying and the perceived ineffectiveness of spraying are the reasons for refusal of household spraying in this area.

Refusal to permit household spraying, for whatever cultural reasons, would inevitably increase the density of mosquitoes, which in turn leads to increase in the frequency of mosquito bites in the longevity of mosquitoes and in the spread of malaria disease.

Sleeping habits and malaria

Several studies have concluded without reservation that the sleeping habits of the people, for instance sleeping without mosquito nets or any other protective device, outdoor sleeping, sharing a bed among children etc., are associated with the spread of malaria[29-35].

Bruce-Chwatt[30] pointed out: "The use of bed nets as a protection from mosquito bites during the night has been practised from very early times. They still remain the most important of all measures of personal protection."

He suggests that bed nets, protective repellents, screening and site selection for living are salient protective devices which can be employed by dwellers so as to protect them from mosquito bites. Interestingly, a team of researchers carried out a survey in the Farafenni area of the Gambia to find, if any, the relationship between the use of bed nets and the morbidity of malaria. The survey carried out in 1982 involved 41 villages and hamlets with a total population of 12,313. The researchers found from clinical records parasitaemia in 40 out of 75 people (53%) who did not use bed nets, in 65 out of 177 people (37%) who used torn nets, and in 82 out of 262 people (31%) who used well-maintained nets. The researchers also concluded that bed nets protect from malaria Farafenni children (who were found with large spleens). The enlargement of the spleen was nearly 50% less in net users than in nonusers. MacCormack and Snow[35] found that in the Gambia where they studied three main ethnic groups (Mandinka, Fula, and Wolof), Mandinka children had a lower malaria prevalence than the other groups. The differences have been explained as due to the sleeping habits of the people. For instance, over 99% of the people in Mandinka used bed nets while in Fula and Wolof villages, only 1-6% of the people used them. In addition, while Mandinka children sleep in beds with mattresses, most Fula and Wolof children sleep on the floor and are vulnerable to bites from below.

Port and Boreham[32] conducted an experimental study in purpose-built huts in the Gambia to test the effectiveness of bed nets as mosquito deterrents. In this survey, several volunteers were asked to sleep in two purpose-built huts using their own nets. These tests were conducted under the scrutiny of the researchers, and tests were repeated on four or five nights. In re-examining the results, it was clear that a very high proportion of female *Anopheles* mosquitoes *(An. gambiae)* leaving a house where all the occupants had good nets, were unfed. It was therefore concluded that bed nets are a very effective means of reducing attacks by *An. gambiae.* Even a defective net provides some measure of protection and helps reduce the man-mosquito contact.

Apart from bed nets, net jackets and repellent-treated/impregnated nets have also been tested in many parts of the world and the results show that they were extremely effective against mosquito nuisance[36-39]. In Kenya most people insert wads of sisal fibres between the top of the walls and the roof as to prevent mosquitoes from getting into the house. In rural southern Sudan, a mixture of cow dung ash and cow's urine is applied in the evenings on the exposed body as a protection from mosquitoes[40].

All the investigations on bed nets and other protective devices have focused on their effectiveness in preventing malaria transmission. There has been little interest, however, on why some people do not use bed nets or other methods of personal protection, and why some people in spite of using bed nets and other protective measures get malaria. Furthermore, none of the researchers has aimed at comprehensively explaining the entire cycle of malaria transmission in which the socio-economic status of the people, beliefs, values and sleeping habits with or without protective measures and the escalation of the frequency of mosquito bites and eventually the spread of parasites from infected patients are interlinked.

So it seems that one possible line of inquiry might be to find whether some people who do not use bed nets or any other protective device are motivated by cultural beliefs and values or whether they are actually so poor that they cannot afford the cost of a mosquito net. Another line of inquiry might be to find out why people prefer outdoor sleeping to indoor sleeping and to determine the contribution made by outdoor sleeping *per se*. It may be worthwhile assessing what contribution sleeping without nets and outdoor sleeping make in relation to other contributing factors in malaria transmission in a given society.

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Involvement of Gastrointestinal Tract in *Plasmodium vivax* Malaria

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Although gastrointestinal symptoms, such as nausea, vomiting, dyspepsia, diarrhoea and abdominal pain, are common in *Plasmodium falciparum* malaria[1], in *P. vivax* malaria practically no literature exists on the involvement of the gastrointestinal tract. There are only two reports available wherein *P. vivax* severe diarrhoea took place[2].

To assess the function of the small intestine in clinical practice, D-xylose tolerance test is used[3]. Urinary D-xylose excretion test of 15 parasitologically positive patients (age, 20-35 years) suffering from *P. vivax* malaria has been conducted to evaluate the gastrointestinal involvement in *P. vivax* malaria. The method of Roe and Rice[4] has been followed. As morbidity due to diarrhoeal diseases is quite high in India, in these patients all other causes of acute gastrointestinal upset have been excluded by microscopic examination of stool and personal history. There was no evidence of ascites, bacterial overgrowth and intake of drugs like indomethacin and aspirin. The period of collection of complete urine was 5 h after ingestion of 25 g D-xylose. The test was repeated in the case of each patient i.e. first time on the day of detection of malaria parasite prior to administration of antimalarial drugs (Day 0) and again on Day 21 after treatment.

Urinary D-xylose excretion ranged between 1 and 3 g on Day 0, which on Day 21 ranged between 3.9 and 4.6 g. In 15 age- and sex-matched healthy control subjects, the value varied between 4.1 and 5.2 g. Serum urea and creatinine in *P. vivax* malaria patients and control subjects were within normal limits, showing normal kidney function in these persons.

The data were statistically analysed. On comparing the D-xylose excretion in 15 patients between the Day 0 and Day 21 of treatment, a significant level of difference at the 95% confidence was obtained. No significant difference between the values of D-xylose excretion in controls and Day 21 sample of patients was observed (Table 1).

Migasena and Maegraith[5] pointed out impaired D-xylose absorption in rhesus monkeys infected

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Table 1. Urinary D-xylose excretion in 15 parasitologically positive patients with *P. vivax* malaria on Day 0 and Day 21 after treatment with control

	D-xylose (g) in patient														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Day 0	1.2	1.5	2.7	2.8	3.2	1.0	2.0	1.0	2.5	3.1	2.7	1.5	2.5	3.0	3.1
Day 21	4.1	3.8	4.2	4.4	4.5	3.7	4.4	3.9	4.6	4.4	4.2	4.0	4.3	4.5	4.4
Control	5.2	4.8	4.0	4.2	4.5	4.2	5.1	5.2	4.1	4.1	4.2	5.1	4.1	4.2	4.5

with *P. knowlesi*, which phenomenon was substantiated in human beings. According to them, impaired D-xylose absorption could be due to ischaemic changes in the small intestinal mucosa resulting from vasoconstriction of the splanchnic bed as observed earlier by Skirrow *et al*[6].

About one-third of *P. falciparum* malaria cases are reported to suffer from gastrointestinal symptoms[7]. In the present series of 150 parasitologically positive *P. vivax* malaria cases, 46(30.6%) clinically showed gastrointestinal distress of mild to moderate degree, which included nausea, vomiting, dyspepsia and abdominal pain.

The D-xylose excretion test is a measure of effective intestinal absorptive surface area and mucosal permeability to D-xylose[3]. Therefore, altered value of D-xylose excretion in *P. vivax* malaria cases in our study points to impairment of normal functions of the small intestine. In the setting of a gut problem there seems to be evidence for a vascular aetiology for this phenomenon due to changes in perfusion of the mucosa. Another possibility is that gastric emptying is delayed. However, the change in D-xylose absorption in *P. vivax* malaria is minor and transitory.

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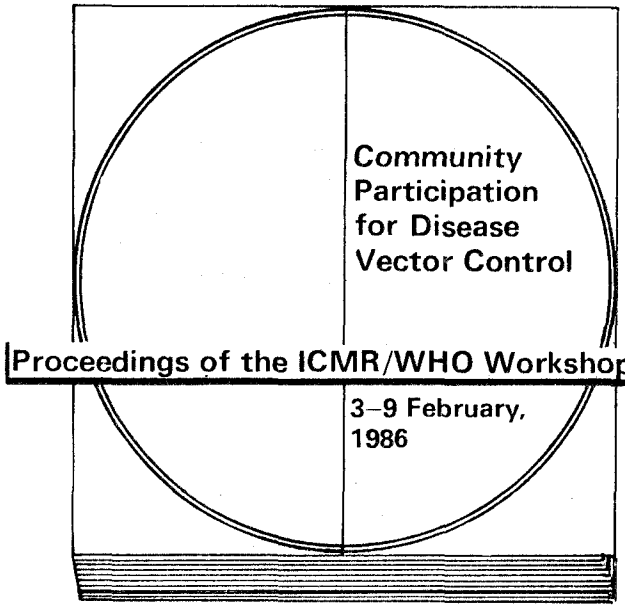

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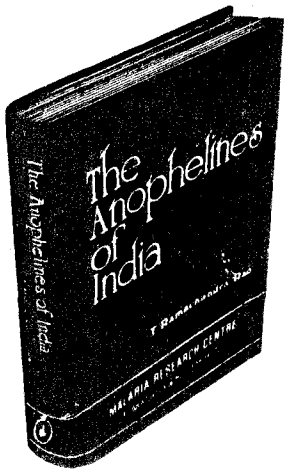

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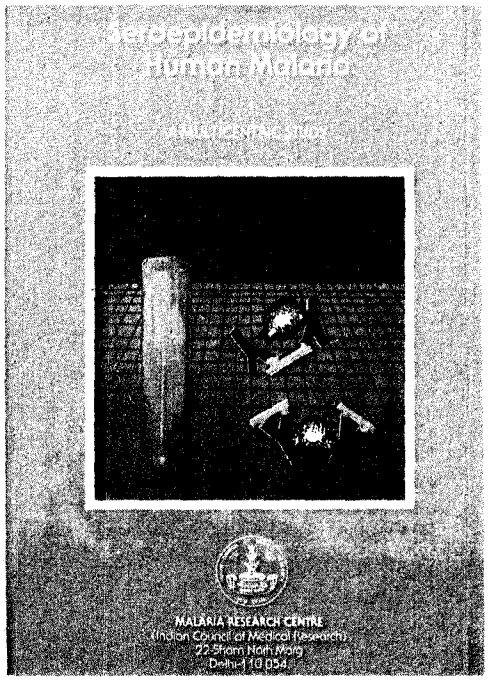

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