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REVIEW ARTICLE

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Constraints and Research Needs in Forecasting and Prevention of Malaria Epidemics in India

M.A. ANSARI

INTRODUCTION

Malaria was considered as a major scourge in ancient time causing disastrous epidemics in many parts of India. In 1908, the explosive outbreaks of the disease have attacked 100 million people and caused about one million deaths in regions of unstable malaria.[1] Since then attempts were directed to develop elaborate system for forecasting of epidemics to prevent the morbidity and mortality caused by malaria. Earlier studies have shown a positive correlation with fever and rainfall. The spleen rates were found very low after the epidemics resulting in gradual decline of immunity in children. The epidemics have also shown a certain periodicity and used to occur at an interval of 7–9 years cycles depending upon local meteorological factors.[2] Later it was reported that four determinants were crucial for forecasting of epidemics. They were rainfall (July–August); average enlarged spleen in 2–12 years children for the last three years; an economic and human factor — average price of food grain; and epidemic potential factor calculated by multiplying the standard deviation by number of years of observations.[3] This system of epidemic forecasting adopted in Punjab state continued to provide useful predictions in regions of unstable malaria until the launching of National Malaria Eradication Programme (NMEP) in 1958.[4,5] The human economic factor was later deleted because of low stable price of food grain as it lost a value as an indicator of hunger.[6] A most comprehensive monitoring system was later proposed where inoculation rate, immune status, meteorological factors, environmental fac-

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tors and social variables were taken into consideration for forecasting and selection of intervention measures to prevent epidemics.[7] Recently historic epidemics in post-DDT era in Sri Lanka and India were explained by Bouma *et al.*[8] and Bouma and Van deKaay.[9] They reported that long range malaria epidemics in different parts of the world are related to climate fluctuations and *EL* Nino-Southern Oscillation (ENSO). This method may not help in developing an early warning system to prevent malaria epidemics in areas of unstable malaria because of rapid ecological changes associated with planned and unplanned developmental activities, human settlement, social and cultural diversity and rapid transportation. The epidemiological determinants keep on changing, therefore, determinants which are based on macro-ecosystem may not hold good at micro-ecosystem in a given space and time and failure of intervention measures without addressing risk factors at local level are not likely to prevent onset of epidemics. The present paper describes the currently adopted strategy by the programme for forecasting and prevention of epidemics, constraints in its implementation and basic applied research requirement to strengthen the basic health infrastructure.

Current strategy for forecasting and prevention of epidemics in India

The National Malaria Eradication Programme (NMEP, now National Anti Malaria Programme, NAMP) was launched in 1958, which has resulted in steep reduction in malaria cases. In 1965 there were only 0.1 million cases without any malaria death. However, the wide-spread resurgence of malaria in 1970 escalated the incidence to 6.4 million in 1976 which necessitated the introduction of modified plan of operation (MPO) emphasizing the need to establish fever treatment depot (FTD) and drug distribution centre (DDC) at community level. This has resulted in stabilization of malaria incidence around two million cases annually by 1984. However, in 1994 fulminating outbreaks of malaria were observed causing malaria deaths in Rajasthan and entire northeastern region. Consequently, an expert committee was appointed by the Govt. of India to identify high-risk groups and suggest short-term strategic measures for different malaria paradigms. In pursuance of the expert committee report, NMEP has prepared malaria action plan for epidemic prone-areas where guidelines have been given to monitor following indicators which usually signal a warning that may help in the early prediction and prevention of epidemics in regions of unstable malaria.[10]

Environment

Environment has profound effect in the propagation of parasite and vector species of mosquitoes. Average temperature (20–30°C) and relative humidity (>60 per cent) are ideal for the survival of both malaria parasites and vectors. Precipitation creates innumerable temporary water bodies which are conducive for the breeding of *An. culicifacies*, a principal vector of malaria in rural plain areas of India and is associated with major malaria epidemics in the country. In fact, it is the pattern and time of rainfall, which is most important to build-up vector population in epidemic prone areas along with

other climatic variations. Natural disasters associated with malaria epidemics are floods following heavy precipitation, seepage associated with intensified irrigation, etc. The concerned officer has to make arrangements for weekly collection of these data, analysis, interpretation for the early detection and prevention of malaria epidemics. Environmental indicators in combination with vector density and annual parasite incidence (API) determine the receptivity of the area.

Vulnerability

Movement of the population from endemic to non-endemic areas for economic gains, developmental activities or due to natural calamities is sensitive and a dependable indicator to determine the vulnerability of the area. It is estimated that there is about 15–20 per cent floating population in project areas or urban areas, which move every year from endemic to non-endemic or vice-versa.[11] Several epidemics in recent past were associated with movement of population particularly in urban slums and project areas like Goa state, National Thermal Power Project, Ghaziabad and Shankargarh and the rest. High morbidity and mortality was observed in indigenous non-immune population of the area.[12] Efforts to contain drug-resistant falciparum malaria failed due to movement of the population.[13]

Identification of high risk groups: In 1994, the expert committee on malaria recommended that high risk areas should be identified on the basis of 30 per cent *Pf* proportion, doubling of SPR, recorded malaria deaths in transmission period during the last three years and detection of 25 per cent RII and RIII level resistance foci in endemic areas. In urban areas about 15 towns (population around 40.3 million) and additional towns (population around 2.3 million) where SPR is ranging about 10 per cent for the last three years have been identified as high-risk urban areas in the country. Health officer is directed to monitor the SPR and SfR on monthly basis taking village/locality as a unit for the corresponding period of three years to detect species-wise rising trend of the disease and analyse the factors responsible for gradual or sizable increase. An indication of increasing trend of malaria particularly falciparum malaria should immediately be reported to the concerned district authority for further investigations. The density of mosquitoes is also monitored along with the susceptibility status of vector species with diagnostic doses of commonly used insecticides. Monitoring of drug-resistance through regional centres is also being done to revise the drug schedule.

Effective response mechanism

In view of increasing number of epidemics observed during the last five years, district epidemic response teams/mobile malaria epidemic control units have been established in epidemic prone areas by re-organising and training the existing staff. They are deployed in the district to contain malaria epidemics, standby equipments and materials required to deal with the situation are to be kept ready well before the transmission season. The Medical Officer in-charge is required to send the report immediately by fax to the State Malariologist, Regional Director,

Regional Officer, Health and Family Welfare and the Director, NAMP when the epidemic is suspected. He should also indicate any further assistance required to contain the epidemic. The report of follow-up action should also be sent to the aforesaid officials.

Delineation of affected areas

Delineation of affected areas is being done after ascertaining the epidemic in village/PHC's. The magnitude of the problem is assessed to know the intensity of the epidemic and delineate the epidemic area and to make arrangements for containment measures with the help of mobile malaria epidemic control team at district/zonal/ state level. Mobile malaria epidemic control team has to carry out rapid survey in every village of suspected PHC/Zone along with mass blood survey starting from areas with normal positivity rates. The multi-purpose workers, laboratory technicians should be drawn from adjoining PHC's/District to complete the survey within the stipulated period of 7–10 days or even less. All blood smears collected are to be examined within 24 hours and presumptive and radical treatment is to be given as per schedule. In case slide could not be examined due to manpower constraints, then 1500 mg chloroquine (three days) and single dose of 15 mg primaquine (on first day) are to be advocated to all fever cases. The population of the affected area is also to be estimated to calculate the requirement of insecticide spraying for anti-vector measures. Space spray is to be taken up immediately to kill the infected mosquitoes followed by indoor residual spraying or antilarval measures in urban areas.

Constraints

Inadequate health infrastructure

As per NAMP proposed guidelines epidemiological data, meteorological data, natural calamities, developmental activities, movement of population have to be recorded regularly and analyzed for delineation of affected area and identification of high risk group to forecast epidemic, early preparedness and timely intervention measures to prevent both mortality and morbidity associated with outbreak of the disease. However, poor or inadequate surveillance, information management system and lack of laboratory services and inadequate capacity building at district level results backlog of slides, accumulation of unanalysed data, as such vulnerability of the area and high risk group is not identified timely. Incidence keeps multiplying without notice of concerned health official and outbreak of the disease takes place. In addition to this many posts of different levels remain vacant for several years, therefore, it is practically impossible to cover the target population. There is complete break-down of communication among different public sector agencies and as such desired information is not automatically available to Health Officer.

In fact, current strategy of forecasting and prevention of malaria epidemics under NAMP requires an in-built mechanism of integrated disease surveillance, trained manpower to collect and analyse the data at peripheral level, upgraded laboratories for rapid diagnostic, drug resistance, strong entomological component for monitoring vector density, man-mosquito contact and in-

secticide resistance, adequate transport, financial resources and decentralised power to execute the plan of action. In addition to this, inter- and intra-sectoral coordination hardly exist resulting non-accessibility of environmental indicators — meteorological data, developmental plan and unplanned activities, natural calamities etc., which require at micro level for determining the receptivity and vulnerability of the area.

Inadequate motivation

Since the experienced health personnel are opting for incentive based health programme — National AIDS Control Programme, Family Planning etc. therefore, the incentives are required to motivate and sustain the interest of health officials associated with malaria control programme particularly at peripheral level. As there is much paper work for reporting at PHC level, multipurpose workers and technicians do not find adequate time to devote for surveillance and examination of slides. In the absence of strong health infrastructure at peripheral level, it will not be possible to detect true incidence of malaria particularly falciparum malaria and the cases will multiply as usual till an outbreak is reported by the newspapers. Primary health centres are located at remote and distant places where no facilities and resources are available to cater the basic health requirements. Medical Officers posted at peripheral level are only entrusted the responsibility and they get agitated and frustrated with poor health infra structure, inadequate civic amenities and lack of facilities for quality education to their children. Therefore, they take their job very casually and do not take interest in the supervision of field work. In the absence of transport facilities, it is impossible for any medical officer to visit villages or cross-check the performance of multipurpose workers who have to carry out several other assignments besides surveillance. They are also not paid TA/DA and asked to cover an area of 10–15 sq kms. The budget of PHC is about 30 paisa/year/person. This also includes establishment cost and with these meagre resources it is not possible to implement central guidelines issued for forecasting of epidemics at peripheral level.

Inadequate inter-sectoral coordination

There is no inter-sectoral coordination among agriculture, railways, works and housing and other concerned government departments or NGO's to exchange or share the knowledge for prevention of the disease. In the absence of functional partnership with allied agencies and community, 60–70 per cent malaria cases are associated with man-made breeding places, which are created during the gestation phase of the developmental project. There are no in-built mitigation measures to curtail the mosquitogenic conditions. Further, there is no provision to carryout preventive measures resulting in local and focal out break of the disease.

Insurgency and movement of population

Insurgency and illegal migration are serious problems in bordering states of northeastern region of India. The area is highly endemic to malaria transmitted by *An. minimus, An. fluviatilis* and *An. dirus*. The drug-resistance is wide-spread though vector species are still

susceptible to DDT. In normal situation, epidemics should not occur because of highly stable ecosystem, however, due to insurgency and illegal migrants the surveillance is totally collapsed. Health officials are scared to visit the insurgency areas resulting in failure of intervention measures, which may lead to unprecedented increase of malaria incidence. Non-immune indigenous or illegal migrants and drug-resistance may produce outbreak of the disease.

Research need

The epidemic cycle consists of a pre-epidemic, epidemic, post-epidemic and inter-epidemic waves. Of them, pre-epidemic phase (incubation period) is most important because during this phase epidemiological foci multiply and show gradual upward trend of malaria incidence. It may be due to either high density of vector species or increased ratio of gametocyte carriers. The combination of both events is responsible for the creation of epidemic foci in a given ecosystem. The impact of various epidemiological indicators may vary under different micro- and macro-ecosystems. Therefore, it is suggested that longitudinal studies should be carried out in different epidemiological paradigms to elucidate the functional relationship between different epidemiological determinants for the accurate prediction of epidemics.

In view of the advancements in computer and communication technology, geographical information system (GIS) should be explored to identify epidemic prone areas with the help of good quality topographical, political and thematic maps. A computer-based GIS at state/central level will allow the collection of data linked to geographical location from different sources and stores it in a form, which promotes subsequent analysis and integrated composite presentation in the map form. Remote sensing and GIS have been recently used to identify and map breeding places of mosquitoes and also to determine the malaria receptivity.[14–18] However, efficacy of GIS/RS depends upon generation of regular quality field data at desired intervals by technocrats and is essential for feeding into GIS based programme for integrated spatial temporal results.

In several developing countries entomological components are quite inadequate or non-existing at primary health centres. There is also no infrastructure for surveillance of vector borne diseases particularly in inaccessible areas. Operational studies are required to identify minimum epidemiological, social and environmental indicators using sampling methods to estimate true incidence of malaria for accurate and timely prediction particularly in high risk areas. There is also an urgent need to develop mathematical model to monitor inoculation rate, disease burden, immunity status, disability, adjusted life years and impact of intervention measures in epidemic prone areas.

Early detection and prompt treatment (EDPT) is one of the most important components of the revised global strategy for malaria control. Therefore, increased emphasis should be placed on rapid diagnostic techniques, which are highly sensitive, species-specific and cost-effective and could be produced with indigenous technology. Studies on seroepidemiological techniques

should also be undertaken to measure immune status and to assess the impact of intervention measures particularly during ascending trend of epidemic.

Studies are also required to monitor drug and insecticide resistance at district level to detect failures of intervention measures, selection of appropriate strategic measures particularly on onset of pre-epidemic period to prevent the epidemics in stable malarious areas.

Population movement for economic reasons is a serious problem in developing countries. The continuous influx of population from endemic areas or vice-versa create epidemic foci and also responsible for spread of drug-resistant strains of falciparum malaria. Studies should be carried out to study the migration pattern and containment of drug-resistant malaria strains. Molecular studies may be taken up to study the genetic diversity of falciparum strains, their virulence, susceptibility and periodicity for geographical mapping.

Because of ecological, genetic and social diversities, the techniques developed in particular ecosystem may not be applicable at national, regional and global level as malaria is a local and focal disease. Therefore, meta-analysis of studies is pre-requisite before recommending or adopting any technique in a global programme.

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A Randomized Controlled Trial Comparing Artemether and Quinine in the Treatment of Cerebral Malaria in Bangladesh

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A randomized controlled trial on 51 patients receiving artemether and 54 patients receiving quinine was undertaken to compare the effectiveness of intramuscular artemether and parenteral quinine in the treatment of cerebral malaria in adults in Bangladesh. Case fatality, fever and parasite clearance times were not significantly different in the two treatment groups. Coma resolution time was significantly delayed in artemether recipients. Results of the study suggest that treatment with artemether is as effective as parenteral quinine in the treatment of cerebral malaria in adults.

Keywords: Artemether, Cerebral malaria, Clinical trial, Malaria, Quinine

INTRODUCTION

Cerebral malaria is a leading cause of death among patients with severe and complicated malaria in Bangladesh.[1] Recently, there has been growing concern about the malaria situation in Bangladesh particularly in Chittagong area.[2] The World Health Organization (WHO) estimates that there are some 300-500 million clinical cases of malaria per year with 1.5–3.0 million deaths in the world.[3] More deaths due to *Plasmodium falciparum* occurs yearly world-wide.[4]

In Bangladesh, about 3,09,687 cases of malaria were reported in 1992.[5] It is apparent that in Bangladesh as well as in other parts of the world, cerebral malaria is the most important manifestation of falciparum malaria and also the

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leading cause of death due to malaria.[4] Cerebral malaria accounts for 10 per cent of cases of falciparum malaria revealed in a hospital based study in Bangladesh.[6] Despite adequate and prompt treatment, the case fatality rate of cerebral malaria remains high.[7]

In the national workshops held during preparation of "Malaria clinical case definition" emphasis was given to apply the new strategies for early diagnosis, prompt treatment and epidemiological surveillance of malaria so that malaria related mortality can be reduced by 25 per cent by the year 2000.[8] Three new clinical case definitions, namely uncomplicated malaria, treatment failure malaria and severe malaria have been adopted.[9] Quinine has been recommended for the treatment of treatment failure and severe malaria cases. Intravenous quinine is currently recommended by WHO as standard therapy for cerebral malaria,[9] as *P. falciparum* infection is reported to have resistance to chloroquine and pyrimethamine-sulfadoxine.[4] However, the efficacy of quinine has also been claimed to be declining.[10]

Quinine is a toxic drug. It has the potential side effects, like cardiac arrhythmia and hypoglycaemia.[4] Because of these limitations, artemether has been introduced recently for the treatment of falciparum malaria. Artemether is a sesquiterpene lactone, a derivative of artimisinin, obtained from the plant *Artemisia annua*.[11] It is formulated in peanut oil for intramuscular injection only. Its efficacy in cerebral malaria was reported first from China in 1979.[12] An intervention study conducted in Thailand showed that artemether is superior to quinine in severe falciparum malaria. It saved lives three times as many as quinine.[14] No significant side effects were observed in that study in the artemether treated patients compared with patients treated with quinine.[11] In Nigeria, a country where quinine remains effective, a similar study found no significant difference in response to treatment with artemether or quinine in children with cerebral malaria.[15] However, the sample size in that study was rather small and the 95 per cent CI of the difference was wide.

Quinine resistant falciparum malaria is not yet a major problem in Bangladesh, but there is clinical evidence of decreased quinine responsiveness in cerebral malaria.[16] Emergence of quinine resistant cases in the future is anticipated considering the situation in neighbouring countries. Artemether may be an effective alternative in this situation. No intervention study has been conducted in patients with strictly defined cerebral malaria using artemether in Bangladesh. Against this background, a randomized controlled trial was conducted to test the efficacy and safety of the artemether in patients with cerebral malaria in Bangladesh.

RESEARCH DESIGN AND METHODOLOGY

The study was conducted in the internal medicine department of Chittagong Medical College Hospital, a tertiary referral hospital, Chittagong, Bangladesh over a period of 18 months. The study was approved by the Bangladesh Medical Research Council. This trial was a randomized controlled study. It was considered impracticable to 'blind' the investigators as the test and control drugs were given by different routes.

Study site: Chittagong Medical College Hospital, situated about 265 km southeast of Dhaka, receives patients from a catchment area that includes hyper-endemic zones of malaria — Cox's Bazar, Banderban, Rangamati, Khagrachari and Chittagong district. The hospital is a tertiary level hospital, suitable for clinical research.

Inclusion criteria: Patients in the age group 14 to 50 years with unarousable coma and positive blood film for asexual forms of *P. falciparum* were included in the study. Patients with cereberal malaria who had received a single dose of quinine prior to hospitalization in Chittagong Medical College Hospital were also included in the study, but their data were recorded separately. It is national policy in Bangladesh that patients with cerebral malaria receive an initial dose of parenteral quinine at the primary level before referral.

Exclusion criteria: The criteria for excluding the patients are as follows: (i) presence of other complications of malaria, such as renal and hepatic insufficiency, pulmonary oedema, and hemoglobinuria; (ii) presence of other concurrent causes of coma like meningitis, encephalitis, hepatic coma, cerebrovascular disease, diabetic coma, epilepsy, typhoid encephalopathy, septicaemia; and (iii) pregnancy.

Sample size was calculated with EPI Info based on $\alpha = 0.05$, $\beta = 0.2$; and randomization = 1:1. The quinine treated group was expected to have a mortality of 37 per cent and the artemether treated group, a mortality of 12.2 per cent (RR = 0.33).

Codes for treatment were prepared before the trial started. After passing the selection criteria, patients were assigned to treatment according to the code in an envelope opened immediately before the treatment was given. Codes, A for artemether and Q for quinine, were prepared in blocks of eight. In each block there were eight codes, each will be put in an envelope containing A (4) and another Q (4). Envelopes were reshuffled. Immediately before the treatment is given to eligible patients, the envelopes were torn and treatment was given according to the code in the envelope.

Drug schedule

Test drug artemether (Paluther, Rhone-Poulenc Rorer, Antony Cedex, France) was given according to weight as follows:

Weight > 45 kg: Artemether 160 mg intramuscular (IM) initial dose (Day 1), followed by 80 mg/kg IM daily for subsequent four days.

Weight < 45 kg: Artemether 3.2 mg/kg I M as initial dose (Day 1), followed by 1.6 mg/kg IM daily for subsequent four days.

Control group

A loading dose of 20 mg/kg of quinine dihydrochloride (Jasoquine, Jayson Pharmaceuticals Ltd., Bangladesh) dissolved in 10 ml/kg of five per cent dextrose solution over four hours. Loading dose was not be given if first dose of quinine has already been received. This was followed by eight hours later by 10 mg/kg quinine dihydrochloride dissolved in 10 ml/kg of

five per cent dextrose solution over four hours and was repeated every eight hours until the patient could take drug orally, when changed to oral quinine sulphate at a dose of 10 mg/kg given eight hourly for a total of seven days.

A medically qualified research fellow was employed full-time throughout the research period. For improved nursing care of patients three auxiliary nurses were also employed. All cases were subjected to the following examination and investigations. Data were collected and recorded in a preformed data collection sheet designed for the study:

(i) Identification of the patient including age and sex.

(ii) History and physical examination, including a full neruological examination, coma score chart (Glasgow coma scale) and fundoscopic examination, was done every six hours after using short acting mydriatics, one per cent tropicamide (OPSO Saline Ltd., Bangladesh).

(iii) Vital signs — temperature, pulse, blood pressure and respiratory rate were monitored every six hours during the period of coma and every 12 hours thereafter.

(iv) All adverse reactions during the study period were recorded with date and time using a check-list noting when they occurred and disappeared. All the abnormalities possibly attributable to artemether or quinine were recorded.

(v) Thick and thin blood films were prepared and examined by a microscopist and cross-checked by another microbiologist. Parasite counts were done every six hours during the period of coma and daily thereafter till Day 7 and then on Days 14, 21 and 28. Parasite densities were calculated by counting number of asexual parasites per 200 leucocytes considering the average white cell count to be 8000/mm^3 in all patients. Blood glucose content was estimated immediately after admission, later one hour before and after both quinine and artemether dosing to be continued for initial 24 hours.

(vi) Lumbar puncture was done in all the cases to exclude other CNS diseases, CSF pressure was measured and the CSF was analysed.

(vii) On admission, total white blood cell count, serum bilirubin, SGOT, SGPT, prothrombin time, urea, creatinine and electrolytes were measured. In cases of anaemia (Hb< 6 g/dl) the patient was given packed cells under frusemide cover (1 mg/kg IV); patients with hypoglycaemia: blood glucose< 40 mg/dl were given 2–4 ml/kg of 25 per cent glucose followed by 10 per cent dextrose solution infusion. Peritoneal dialysis was performed in patients who developed acute renal failure subsequently.

Convulsions were controlled with diazepam 0.5–1 mg/kg per rectum or 0.15 mg/kg intravenously.

(viii) Chest radiograph, urine analysis and Widal test were done in all cases, but blood culture was done only when septicaemia was suspected.

(ix) ECG was done on Day 0, before treatment, and then after starting treatment daily up to 3 days and on Days 7, 14 and 28.

Evaluation criteria

Fever clearance time is the time for the temperature to fall to $\leq 37^{\circ}C$ and to remain so for at least 48 hours. Parasite clearance time is defined as the period of time between the initiation of treatment and disappearance of plasmodium parasites from the blood. Parasitological treatment success is considered to have been achieved if parasitaemia disappears before Day 7, and remains absent until Day 14. Pyrexia is considered to be present if the temperature is $\geq$ 37.5°C. Time to regain consciousness, and to be able to sit, drink and eat unaided was also noted.

Data analysis: Data were double entered in EPI Info version 6 (Centres for Disease Control and Prevention, Atlanta, GA) database and analysed.

RESULTS

A total of 105 adults were randomly assigned to receive either artemether or quinine at Chittagong Medical College Hospital, Chittagong, Bangladesh between July 1996 and December 1997. Amongst the patients, 78 were male, 28 female (Male: Female 2.9:1) with 29 per cent female in artemether group and 28 per cent female in quinine group, three patients were excluded-one died after randomization but before treatment, two were found to have multiorgan failure. Fifty one patients were allocated to artemether (mean age 28 $\pm$ 10 years, mean weight 47.5 $\pm$ 8.7 kg), and 54 to quinine (mean age 30 $\pm$ 10 years, mean weight 48.1 $\pm$ 9.6 kg). The clinical features on admission are shown in the Table 1. There were no significant differences in any major variables between the

Table 1. Comparison of treatment groups (artemether vs quinine)

Clinical criteria	Artemether	Quinine
Total number of patients	51	54
Age (years) (mean $\pm$ SD)	28 $\pm$ 10	30 $\pm$ 10
Sex–Male	36 (71 %)	42 (72 %)
Weight in kg (Mean $\pm$ SD)	47.5 $\pm$ 8.7	48.1 $\pm$ 9.6
Pre-treatment parasitaemia range (n/cm)	500–500000	500–500000
Duration of coma at presentation (hrs)	17.7 $\pm$ 12.3	17.4 $\pm$ 12
Prior quinine n (per cent)	24 (57%)	25 (58 %)
H/O convulsion	22 (53%)	23 (42%)
Splenomegaly	11 (21%)	16 (29%)
Retinal haemorrhages	7 (14%)	8 (15%)
Papilloedema	5 (10%)	3 (6%)

n = No. of patients.

Table 2. Comparison of treatment groups (Important laboratory parameters)

Lab. characters	Artemether	Quinine
Blood glucose (mg/dl ± SD)	135 ± 91	152 ± 99
Blood urea (mg/dl ± SD)	68 ± 17.5	59.5 ± 35
S. Creatinine (mg/dl ± SD)	1.6 ± 1.3	1.5 ± 1
S. Sodium (mmol/l ± SD)	128.7 ± 7.3	129 ± 4.9
S. Potassium (mmol/dl ± SD)	3.6 ± 0.6	3.7 ± 6.0
S. Bilirubin (mg/dl ± SD)	2.3 ± 1.5	2.1 ± 1.2
CSF protein (mg/dl ± SD)	41.4 ± 24.6	40.8 ± 28.3
CSF cell (n ± SD)	8 ± 3	9 ± 7
CSF pressure raised	7 (15%)	13 (25%)
Hb < 9 g/dl	21 (42%)	19 (35%)

n = No. of observations.

Table 3. Comparison of outcome of treatment groups (artemether vs. quinine)

Outcome of treatment

Group	Complete recovery n (%)	Death n (%)	Recovery with sequelae n (%)
Artemether	39 (76)	9 (18)	3 (6)
Quinine	43 (79)	10 (19)	1 (2)
Prior quinine	43 (88)	6 (12)	0

Other outcome variables

Variables	Artemether	Quinine	p-value
Coma resolution time (hours ± SD)	74.2 ± 51.8	53.4 ± 36	0.0306
Parasite clearance time (hours ± SD)	52.1 ± 33.3	60.7 ± 39	0.28
Fever clearance time (hours ± SD)	58 ± 15.6	47 ± 31.5	0.18
Recrudescent parasitaemia [n (%)]	4 (10)	1 (2)	0.18

two groups of patients. Over half of the patients 24 (57 per cent) in the artemether group, and 25 (58 per cent) in quinine group had a history of receiving single dose of parenteral quinine before admission to the hospital. The duration of coma at presentation was about 17 hours in both the groups. The important laboratory variables such as anaemia, blood sugar, urea, creatinine, electrolytes, and serum bilirubin concentration, CSF-pressure, protein, cells in both the groups were also similar (Table 2).

Nine of 51 patients treated with artemether died in the hospital (18 per cent) compared to 10 out of 54 patients treated with quinine (19 per cent) ($p = 0.97$). Four patients had neurological sequelae (three in artemether group, one in quinine group — Table 3). Thus, there was no significant difference in both number of deaths and cases of neurologic sequelae between the groups. The cause of death was multifactorial in most of the cases. Of the 19 patients who died, six had acute renal failure and dialysis had been started in four of these patients, two had respiratory arrest, one pulmonary oedema and one gastrointestinal haemorrhage.

Artemether treatment was associated with a relatively quicker clearance of parasites from peripheral blood (52.1 and 60.7 hour in the artemether and quinine groups respectively; $p = 0.28$) but slow resolution of fever (58 and 47 hours in the artemether and quinine groups; $p = 0.18$). In the patients treated with artemether, recovery from coma was slower in comparison to that of quinine group (74.2 vs. 53.4 hour $p = 0.0306$).

Four patients treated with artemether and one with quinine recovered with neurological sequelae, which included monoparesis, cortical blindness. Adverse reactions like vomiting (35 vs. 39 per cent) and diarrhoea (16 vs. 13 per cent) were similar in both the groups. Convulsions (41 vs. 31 per cent) and neuropsychiatric side effects (27 vs. 15 per cent) were more common with artemether than with quinine (Table 4). No significant ECG changes were found in either group.

DISCUSSION

Results from this study showed that parenteral

Table 4. Comparison of adverse effects of treatment groups (artemether vs. quinine)

Adverse effects	Artemether n (%)	Quinine n (%)	p-value
Vomiting	18 (35)	21 (39)	0.703
Diarrhoea	8 (16)	7 (13)	0.690
Convulsion	21 (41)	17 (31)	0.301
Neuropsychiatric	14 (27)	8 (15)	0.111
Arrhythmia	0	0	

n = No. of observations.

artemether is equally effective as parenteral quinine in preventing death in adult patients with cerebral malaria. The baseline variables in arte-mether and quinine groups were similar in this study, and the included severe cases in the artemether group was by chance. When patients with a Glasgow coma score < 4 in artemether group were excluded from analysis, the mortality remained similar in both the groups.

Early non-randomised trials conducted in 138 patients from a military hospital, Bangladesh suggested that artemether was superior to quinine.[17] Clinical details, including coma scores and biochemical results were not mentioned by the authors but the severity of the disease was said to be similar in the treatment groups.

The artemisinin derivatives have been widely investigated in adults and children with cerebral malaria in countries of southeast Asia and Africa where primary end points (mortality) and principal secondary end points — fever clearance time (FCT), coma resolution time (CRT), and parasite clearance time (PCT) were investigated.

Results from this study show relatively early parasite clearance with artemether and early fever clearance with quinine (although not significant) despite this trend of early parasite clearance and a prolonged period of coma was observed in the artemether group. Similarly, a prolonged coma recovery time has been observed in adult patients with cerebral malaria treated with artemether in Vietnam and in children in Africa.[18,19]

Despite the use of higher dose of artemether in the Vietnam study, mortality (13 vs. 17 per cent) was not significantly different from that of quinine. Meta-analysis of studies conducted in African and southeast Asia in children and adults revealed similar case fatality rates (18 per cent) in artemether treated patients.[20]

In Thailand, an area of emerging quinine resistance, artemether apparently reduced the mortality of severe malaria (13 per cent).[21] In Bangladesh, similar case fatality rates with artemether and quinine suggested that the efficacy of quinine in falciparum malaria has been sustained. No significant difference in mortality between intramuscular artemether and quinine infusion were found in an archival study of adult cerebral malaria in the same hospital in Bangladesh.

An increased but statistically insignificant incidence of convulsions and neuropsychiatric manifestations found in this study was also reported in artemether-treated adult patients in Vietnam, and in the Gambia; and the neurological sequelae in patients treated with artemether and quinine in these studies and in the present one from Bangladesh.

Artemisinin derivatives were found to be neurotoxic in experimental animals when used in large doses for a prolonged period.[22] Whether the prolonged coma recovery time and relatively increased incidence of convulsions found in this study and others is attributable to such effects on the central nervous system needs to be resolved by further detailed and powerful clinical studies in depth by including large number of patients.

It may be concluded from this study that there is no evidence of clinical and laboratory difference between artemether and parenteral quinine in the treatment of cerebral malaria in adults.

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A Study on Clinical Profile of Falciparum Malaria in a Tertiary Care Hospital in South India

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Malaria continues to be a major problem in tropical countries. To study the clinical features and complications of malaria in a tertiary care hospital in south India, records of 183 patients were analysed. Among 86 patients with *P. falciparum* and mixed infection, 24 (28 per cent) had cerebral malaria and 32 (37 per cent) had hyperbilirubinemia. Twenty-three out of 32 (72 per cent) patients with jaundice had direct hyperbilirubinemia and elevated liver enzymes suggesting hepatocellular damage. Mortality of the order of 10 per cent was seen only in *P. falciparum* malaria. High incidence of hepatic involvement and hepatorenal failure were the unusual features observed in the study.

Keywords: Clinical complications, Hepatitis, Jaundice, Malaria, *P. falciparum*

INTRODUCTION

Malaria continues to be a major killer disease in tropical countries. The incidence of malaria worldwide is estimated to be 300-500 million clinical cases every year, with about 90 per cent of these occurring in Africa.[1] *Plasmodium falciparum* causes the most severe form of malaria and is known for its protean manifestations and high mortality. Between 1.1 and 2.7 million people die worldwide each year due to malaria.[2]

Malaria is endemic in most parts of India, especially in the northern areas of the country. Among the cases of malaria reported in India, only 12.3

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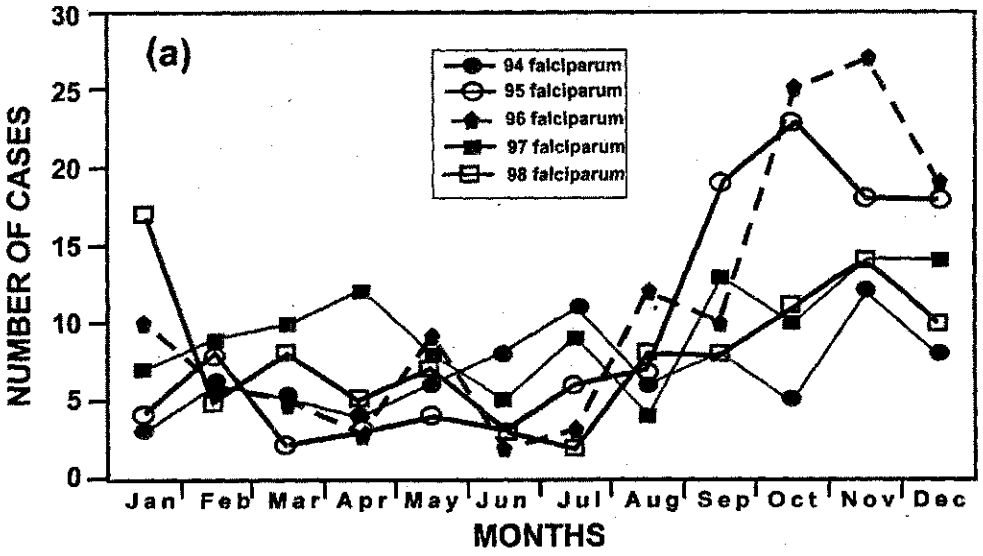

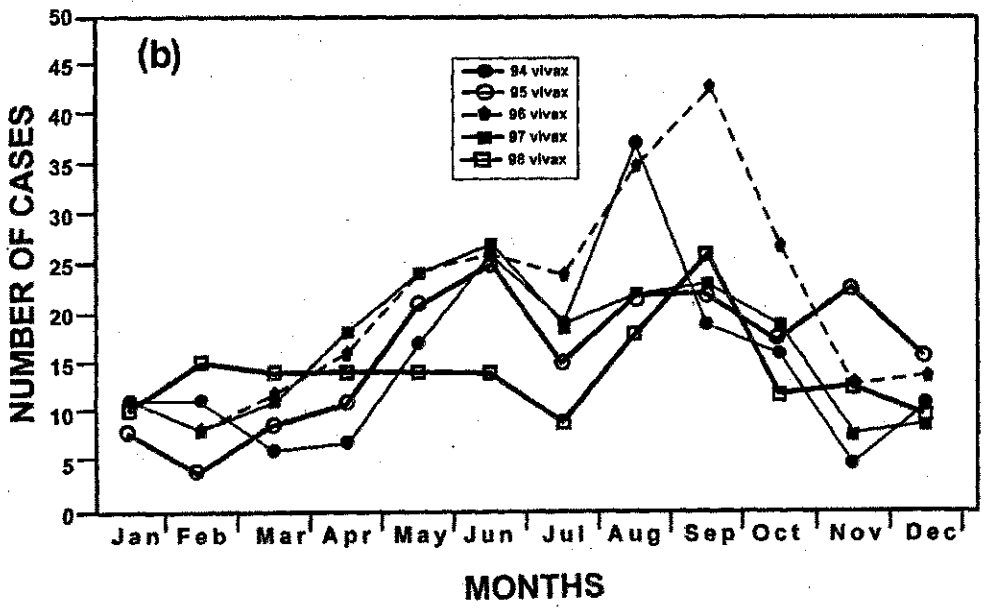

Fig. 1(a and *b)*: Monthly distribution of falciparum and vivax malaria diagnosed during 1994–1998

per cent of *P. vivax* and 6.8 per cent of *P. falciparum* malaria were from the southern states like Andhra Pradesh and Karnataka.[3] Tamil Nadu, where this study was carried out, has a low endemicity for *P. vivax* and has only pockets of *P. falciparum* transmission. *Anopheles culicifacies* is the main vector implicated.[3] Because of increased travel and increased number of migrant workers, hospitals in these low endemic states encounter cases in whom malaria was acquired elsewhere. Hence, a hospital-based study was undertaken to describe the complications encountered in malaria cases and may help in undertstanding the uncommon clinical manifestations in malaria.

MATERIALS AND METHODS

This study was conducted at Christian Medical College Hospital, Vellore, a 1700 bed tertiary care teaching hospital situated 140 km west to Chennai City in the state of Tamil Nadu.

The data regarding malaria positivity and the species involved were collected for the years 1994–98 from the records maintained in the diagnostic laboratory. Both thick and thin blood smears from the patients suspected to have malaria were stained by Leishman's stain and examined under oil immersion objective (100X) by qualified technical and medical professionals. The case records of 183 adults out of 255 diagnosed to have malaria in the year 1998 were analyzed in detail.

RESULTS

P. vivax and *P. falciparum* were identified throughout the year, during the period 1994–98. However, in all these years, an increase of both types of malaria was seen during the rainy season— August to December (Figs. 1a and 1b).

Of the 284 patients diagnosed to have malaria in the year 1998, 255 were adults and the remaining 29 were children below 12 years. The records of 97 adults with *P. vivax*, 70 with *P. falciparum* and 16 with both infections, were only available for analysis (Table 1). Only 91 of these 183 (50 per cent) patients were from Vellore and suburbs. Fifty-nine (32 per cent) were from the neighbouring states – Andhra Pradesh, Karnataka and Kerala and a further 18 (10 per cent) were from the northeastern states—West Bengal, Orissa, Assam and Tripura.

Though the age of the patients ranged from 12 to 88 years, 122/183 (67 per cent) of the patients were in 20 to 40 years age group with a mean of 31 years. Fever was the only common manifestation and was of varying duration ranging from two days to two weeks.

Jaundice (total bilirubin > 2 mg/dl) was observed in 29 (41 per cent) patients with falciparum malaria and in three (19 per cent) with mixed infection (Table 2). Fourteen of those with falciparum infection and all with mixed infection had total bilirubin levels >5 mg/dl. Twenty-three out of 32 (72 per cent) patients with jaundice had direct hyperbilirubinemia and elevated aspartate and alanine aminotransferases (AST > 40, ALT > 40) suggesting hepatocellular damage.

Cerebral malaria occurred as a complication in 24 individuals with either *P. falciparum* or mixed

Table 1. Characteristics and complications of adult patients with malaria (Jan–Dec 1998)

	P. vivax	*P. falciparum*	Mixed	Total
Cases detected	158	98	28	284
Records analysed	97	70	16	183
Male : Female	76 : 21	56 : 14	14 : 2	146 : 37
Residence				
Vellore	57 (59)	25 (36)	9 (57)	91 (50)
Rest of Tamil Nadu	10 (10)	4 (5)	1 (6)	15 (8)
Neighbouring states	20 (21)	34 (49)	5 (31)	59 (32)
Distant states	10 (10)	7 (10)	1 (6)	18 (10)
Complications				
Cerebral malaria	0	21 (30)	3 (19)	24 (13)
Jaundice	0	29 (41)	3 (19)	32 (18)
Anaemia (< 8 g%)	0	17/51 (33)	3 (19)	20/67 (30)*
Renal failure	0	5 (7)	0	5 (3)
Overall mortality	0	10** (10)	0	10 (3)

*Haemoglobin was done only for 67 patients with *P. falciparum* (alone or mixed) infection; **Cereberal malaria 3; Hepatorenal failure 3; Hepatic failure, ARDS, Aspiration pneumonia, Ventricular arrhythmia one each; Figures in the parentheses indicate percentage calculated.

Table 2. Clinical profile of 32 patients with jaundice and total bilirubin > 2 mg%

Hyperbilirubinemia	Direct		Indirect	Indeterminate
	Pf	Mixed	*Pf*	*Pf*
Number	20	3	4	5
Mean AST	121	67	57	102
Mean ALT	55	61	42	99
Hepatomegaly	2	0	0	1
Splenomegaly	2	0	0	1
Hepatosplenomegaly	5	0	1	1

AST— Aspartate aminotransferase; ALT— Alanine aminotransferase.

infection. Of the 67 individuals with either *P. falciparum* or mixed infection tested for haemoglobin, 32 (48 per cent) had haemoglobin levels < 10 g/dl of which 20 (30 per cent) had levels < 8 g/dl. Reticulocyte count was done only in 20 patients with anemia and only three showed reticulocytosis of more than three per cent.

The overall mortality due to malaria was four per cent, and was 10 per cent in the patients with *P. falciparum* infection. Of the 10 patients who died, three had cerebral malaria. All the three developed multi organ dysfunction syndrome (MODS). Two patients had pre-existing chronic renal failure of which one died of MODS

and the other patient died of aspiration pneumonia. Six patients had total bilirubin > 5 mg/dl, of which two had cerebral malaria.

DISCUSSION

Although the number of cases diagnosed per year is not very high as compared to other parts of India, malaria continues to be a problem in our study area.[4] Despite an increase in antimalarial measures all over the country, there has not been significant reduction in the number of cases diagnosed over the years. As reported by Gupta,[5] we also observed an upsurge of malaria during rainy season. Patients seeking treatment for malaria at hospital were also predominantly males, as reported by Verma and Srivastava.[6]

As expected, most of the patients with *P. vivax* malaria had an uncomplicated course. Although *P. falciparum* accounted for about 44 per cent (126/284) of the total cases seen, this infection is not known to be transmitted in this area. Fifty-two out of eighty six patients (60 per cent) with falciparum malaria came from other regions for the diagnosis or for the management. The remaining, probably acquired the infection from other areas with malaria transmission. Although Gopinathan and Subramanian[7] reported cerebral and haemopoietic involvement as the commonest form of pernicious syndromes in patients with *P. falciparum* malaria in India, the most common complication observed in this study was liver damage. Jaundice is a known manifestation of malaria in adults. In Thailand 19 per cent of falciparum malaria cases had jaundice and it was predominantly due to unconjugated bilirubin.[8] However, in this series conjugated bilirubin was responsible for hyperbilirubinemia in 72 per cent cases. AST, ALT enzymes were also elevated in these patients, indicating hepatocellular damage. Jaundice in malaria can be due to intravascular haemolysis, associated septicemia, disseminated intravascular coagulation and malarial hepatitis (hepatocellular jaundice).[9] However, severe hepatic dysfunction presenting as acute hepatic failure is uncommon.[10]

Six patients who died had jaundice where three died of heart failure, one of hepatic failure and two had cerebral malaria as described earlier. In this series 6/32 (19 per cent) of patients with jaundice had a fatal outcome compared to 4/54 (8 per cent) without jaundice. Since jaundice in malaria is associated with a higher fatality and this appears to be a frequent complication of falciparum malaria, malaria should be considered as a differential diagnosis in the patients presenting with fever and jaundice. Haemolysis was probably the cause of jaundice in the remaining 28 per cent of the patients. Out of 24 patients with cerebral malaria, 17 (70 per cent) had jaundice (11-direct, 1-indirect and 5-indeterminate) and 9 (38 per cent) had anaemia. Association of jaundice with other serious complications is also previously described.

Anaemia was another common complication. Reticulocyte response was demonstrated only in 3/20 (15 per cent) of the patients tested, indicating that anaemia in most of the patients was due to hypoproliferative or ineffective erythropoiesis rather than haemolysis.[11] Adult respiratory distress syndrome (ARDS), the most common pulmonary complication of fulminant *P. falciparum* infection accounted for one

death.[12] Cerebral malaria, reported to be the most common cause of death contributed to 30 per cent fatality.[7]

A recent report from Kolkata also showed complications similar to that of seen in this study.[13] Complications and mortality observed in this series were high as compared to other published data.[7] The main reason may be the nature of cases seen in a referral centre. There is a need to create more awareness especially about uncommon manifestations and complications of *P. falciparum* malaria among doctors in peripheral health centres in order to institute prompt therapy.

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Epidemiological Observations on Malaria in Some Parts of Darrang District, Assam

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A study on malaria conducted in tribal villages of Darrang district, Assam during April 1994 to March 1995 revealed that the malaria incidence due to *Plasmodium falciparum* was considerably high. Slide positivity rate (SPR) ranged between 2.3 to 45.67 per cent with transmission from May to October. *P. falciparum* was the dominant species (91.7 per cent) followed by *P. vivax* (7.25 per cent) and mixed infection (*Pv* +*Pf* = 1.05 per cent). Malaria cases were recorded throughout the year in all the age groups including infants, however, age groups between 0–1 and 21–30 years were more affected. Among 17 anophelines collected, *Anopheles vagus, An. jamesii, An. crawfordi* and *An. minimus* were the most abundant species. Known vectors of malaria like *An. annularis, An. culicifacies, An. minimus, An. philippinensis* and *An. varuna* were detected. Perennial transmission of malaria was attributed to low socio-economic conditions, poor surveillance and inadequate intervention measures.

Keywords: *Anopheles minimus*, Malaria, *Plasmodium falciparum*

INTRODUCTION

Malaria is one of the most formidable and serious public health problem in northeastern region in general and Assam in particular.[1] The situation is getting worse because of the resurgence of chloroquine resistant strain of *Plasmodium falciparum*. *P. falciparum* infection is showing a steady increase in the number as well as in proportion with respect to total cases in this region.[2-4] *P. falciparum* contributes about 35 per cent of total malaria cases in the country. It is worth mentioning that, the northeastern region alone contributes about one-

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eighth of the entire *P. falciparum* load of the country. Although *P. vivax* was the predominant species earlier, in recent years a greater prevalence of *P. falciparum* is observed.[5] Among all the northeastern states, Assam has the history of persistence of malaria.[6] The persistent malaria transmission is due to poor surveillance, difficult terrain and favourable climatic conditions (high humidity, heavy rainfall and moderate temperature) for mosquito breeding and survival and low socio-economic status.[7]

Many areas of Assam, particularly those which either neighbour hill states or share an international border with other countries, experiences persistent transmission of malaria. Darrang district is one such region which shares an international boundary with Bhutan. So far, there are very few published reports on the malaria situation of this region. Therefore, a study was conducted in some parts of Darrang district, Assam to know the incidence of malaria, the seasonal prevalence of parasite species, anopheline fauna and vector species. Data included in this paper are for the study period from April 1994 to March 1995.

MATERIALS AND METHODS

Study area

Darrang district covers an area of 3,481 sq km. The district headquarter is located at Mangalodoi. It is surrounded by Kamrup district on western side, Nowgong and Sonitpur districts on southern side and shares an international border with Bhutan on eastern side. The present investigation was restricted to rural areas of the district particularly those situated near the Bhutan border. Four villages namely Ulubari, Bhutiamari, Bengali Basti and Ghaghra Bazaar under Tangla PHC, covering a population of 4400 were selected for the present investigation. These villages were selected for the present investigation because of high incidence of malaria as evidenced by published and unpublished reports; and low socio-economic condition of the local inhabitants. The area was plain and forested. In these villages the houses are sparsely distributed. Most of the houses are made up of split bamboo walls, having mud plaster and thatched roof with mud floor. The villages were surrounded by tea-gardens, paddy fields, forest and other low-lying areas with numerous streams and streamlets. These villages are dominated by 'Bodo' tribes (also known as 'Kachries') and Bengalis with some settlers from Nepal. Some of the tribal groups from south Bihar and Orissa have also migrated to this area in search of job. Most of these people are very poor with low socio-economic status. They are either engaged in agriculture (mainly paddy cultivation) or working in tea-gardens. The climate is hot and humid for most part of the year except from November to February which marks the cold season.

Methods

To know the malaria situation of the district, epidemiological data were also collected from local National Malaria Eradication Programme (NMEP) unit (now NAMP) and analysed for the period from 1986 to 1995. To determine the malaria incidence and transmission pattern active fever surveillance was conducted at

monthly intervals in all the study villages from April 1994 to March 1995. Blood smears were collected from the fever cases including infants, stained with giemsa and examined under the microscope for the presence of malaria parasites. Malaria positive cases were treated with antimalarial drugs as per NAMP drug policy. Epidemiological parameters such as SPR, SfR, *Pf* per cent, age and sex-wise distribution of malaria cases were calculated and analysed.

For entomological surveillance, morning indoor resting collections of anophelines were carried out between 0500 to 0700 hrs from fixed catching stations at monthly intervals in the study villages. Searches were made for 15 min in each dwelling using a flash light and mosquitoes were collected with the help of oral aspirator. The collected mosquitoes were transported to the field laboratory, anaesthetised and identified to species using standard identification keys.[8-9] Man hour density (MHD) was calculated for individual anopheline species and recorded.

RESULTS

Epidemiological situation

The epidemiological data of Darrang district collected from the local office of the NAMP for the period from 1986 to 1995 revealed high malaria endemicity with *P. vivax* dominance contributing > 80 per cent of the malaria cases and raise in *P. falciparum* cases with increase in annual parasite incidence (API) 6.25 per cent in 1988 to 20.9 per cent in 1995.

Parasitological observations

Results of the active surveillance conducted in the study are given in Table 1. Of the total 1122 blood smears collected from fever cases, 193

Table 1. Age wise prevalence of malaria cases in Darrang District of Assam during 1994–1995

Age group (yrs)	BSCE*	No. (+) ve	*Pf*	*Pv*	Mixed (*Pf*+*Pv*)	SPR	SfR	*Pf* per cent
<1	70	23	23	–	–	32.85	32.85	100
1–10	372	62	60	2	–	16.66	16.12	96.77
11–20	239	35	32	3	–	14.64	13.38	91.42
21–30	128	28	25	2	1	21.87	19.53	89.28
31–40	155	28	25	3	–	18.06	16.12	89.28
41–50	103	12	9	3	–	11.65	8.73	75.00
>51	55	5	3	1	1	19.09	5.45	60.00
Total	1122	193	177	14	2	17.20	15.77	91.70

*Blood slides collected and examined ; Population – 4400.

were found positive for malaria parasites giving a slide positive rate (SPR) of 17.2 per cent for the entire period. Of these, 177 cases were positive for *P. falciparum* infection (91.7 per cent), giving a slide falciparum rate (SfR) of 15.77 per cent and 14 for *P. vivax* (7.25 per cent). The number of mixed infections (*Pf* and *Pv*) detected in this study was extremely low (1.05 per cent). About eight per cent of the total cases were found positive for gametocytes of *P. falciparum*, acting as a reservoir of malaria infection. It is evident from the study that the incidence of *P. vivax* was very low in comparison to that of *P. falciparum*.

Malaria positive cases were recorded in all age groups including infants in all months. However, maximum number of positive cases was recorded in the age groups 0–1 year (SPR = 32.85 per cent) and 21–30 years (SPR = 2.187 per cent). On the other hand, the least number of cases was recorded in the age group of 51 and above (Table 1). Analysis of the data revealed very little difference in malaria incidence between the male and female population. Both males and females suffered almost equally with SPR of 18.08 and 16.19 respectively.

Among the four villages, SPR was highest in Bengali Basti (31.04) followed by Ulubari (20.47), Bhutiamari (16.48) and Ghaghra Bazaar (5.95). Similarly, *P. falciparum* cases were highest in Bengali Basti (96.5) followed by Ulubari, Ghaghra Bazaar and Bhutiamari 92.3, 87.5 and 80.64 per cent respectively (Table 2). It was observed that the villages situated nearer to low-lying areas/streams reported maximum number of cases than those situated away from these areas.

During the entire study period, malaria cases were recorded in all months of the year indicating perennial transmission. However, in general there was a notable increase in the malaria cases beginning in May till October. This period of high rise was mainly due to *Pf* cases and it corresponded very well with the monsoon season. Thereafter, certain degree of transmission continued till February, but there was a clear decline in number of cases.

Entomological observations

A total of 17 anopheline species were collected from the study area. Among these *An. vagus* was the predominant species followed by *An. jamesii, An. crawfordi* and *An. minimus*. In human dwellings, *An. minimus* was the predominant species (29.68 per cent), whereas *An. jamesii* was the predominant species (20.28 per cent) in cattlesheds (Table 3). Among the known malaria vector species *An. annularis, An. culicifacies, An. minimus, An. philippinensis* and *An. varuna* were encountered in the study area. *An. minimus* was recorded in morning resting collections in houses which were relatively darker inside. The density of *An. minimus* was found to be high from May to September. During this period, the malaria transmission was also high as evident from high slide positivity rate.

DISCUSSION

The data provided by state NAMP authorities indicate that in Darrang district, *P. vivax* is the

Table 2. Malaria incidence in four villages of Darrang district of Assam during 1994–1995

Villages surveyed	Population	BSCE*	No. (+) ve	*Pf*	*Pv*	Mixed (*Pf* + *Pv*)	SPR	SfR	*Pf* per cent
Ghaghra Bazaar	900	403	24	21	3	–	5.95	5.21	87.50
Bengali Basti	1000	277	86	83	3	–	31.04	29.96	96.50
Ulubari	1100	254	52	48	4	–	20.47	18.89	92.30
Bhutiamari	1400	188	31	25	4	2	16.48	13.29	80.64
Total	4400	1122	193	177	14	2	17.2	15.77	91.7

*Blood slides collected and examined.

Table 3. Indoor resting density of anophelines from different sources during 1994-1995

Species	Human dwellings			Cattlesheds		
	No. collected	MHD	Percentage*	No. collected	MHD	Percentage*
An. aconitus	54	0.65	7.45	101	1.35	7.32
An. annularis	30	0.36	4.14	75	1.00	5.44
An. barbirostris	9	0.10	1.24	34	0.45	2.46
An. crawfordi	109	1.32	15.05	149	1.98	10.80
An. culicifacies	12	0.14	1.64	18	0.24	1.30
An. gigas	0	0.00	0.00	1	0.01	0.08
An. jamesii	55	0.67	7.59	280	8.73	20.28
An. kochi	9	0.10	1.24	8	0.10	0.57
An. maculatus	82	1.00	11.32	53	0.70	3.85
An. minimus	215	2.62	29.68	133	1.77	9.63
An. peditaeniatus	0	0.00	0.00	1	0.01	9.63
An. philippinensis	9	0.10	2.34	80	1.06	0.08
An. ramsayi	0	0.00	0.00	4	0.05	0.29
An. splendidus	17	0.20	2.34	117	1.56	8.48
An. tessellatus	0	0.00	0.00	4	0.05	0.29
An. vagus	109	1.32	15.05	262	3.50	18.98
An. varuna	16	0.19	2.20	60	0.80	4.35

*Percentage out of total anophelines collected; MHD — Man hour density.

dominant parasite species contributing > 80 per cent of the total positive cases. On the contrary, in the light of the present data, it is evident that this area is endemic for malaria and morbidity due to *P. falciparum* is alarming. Our study reveals that *P. vivax* cases were found no doubt, but less as compared to *P. falciparum*. This finding is in conformity with those reported earlier that initially most of the malaria cases were due to *P. vivax* but during the last few years *P. falciparum* has increased considerably.[10-12] Malaria positive cases were recorded in infants for all months which suggest heavy active transmission throughout the year. On the other hand, some researchers reported extremely low infant parasite rate.[13]

From the monthly parasite incidence it is apparent that period of May to October represents the bulk transmission, the rest being the low transmission period. There appeared to be a positive correlation between rainy months and high malaria incidence (correlation co-efficient r = 0.64989). High rainfall during this period was responsible for increased breeding places of the vector species, resulting in build-up of its density which coupled with favourable temperature and humidity amounted in heavy transmission. High densities of *An. minimus* were recorded from human dwellings in our study villages. A preliminary observation on vector incrimination from this region had already been reported. It was observed that malaria incidence was high in villages situated near low-lying areas/streams as the breeding places are nearer to the human habitation, man-vector contact will be high and even a low breeding potential can contribute to sufficient number of vectors transmitting malaria. Our finding is in conformity with those of earlier investigators who also reported reappearance of *An. minimus* as a major malaria vector in this region.[14-16]

High morbidity and persistent transmission of malaria could be attributed to poor surveillance, inadequate intervention measures and difficult terrains coupled with low socio-economic conditions. Practically, there were no anti-malarial measures being carried out by the state/district health authorities in this region. Persistent transmission of malaria has also been documented in other parts of Assam.[17-19] Although, *An. minimus* the major vector is highly sensitive to DDT, the poor population coverage coupled with technical and financial constraints and socio-cultural practices of tribal people, malaria transmission remains uninterrupted. Therefore, reasonable coverage and methodical spray of residual insecticides coupled with personal protection measures (like insecticide impregnated bednets) will be effective in combating malaria in this region.[20]

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An Outbreak of *Plasmodium falciparum* Malaria due to *Anopheles minimus* in Central Assam, India

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Epidemiological investigations were conducted in Nellie subcentre, PHC Jhargaon, under Morigaon district (Assam). The results of fever cases revealed 68 per cent slide positivity rate (SPR) and 40 per cent slide falciparum rate (SfR). The *Pf* proportion was >87 per cent and remaining cases were *P. vivax* infections. *An. minimus* was incriminated as a malaria vector during the study period. The sporozoite rate was 3.08 per cent. The indoor man mosquito contact was 35 per bait/night as against 23 in outdoors. Results of susceptibility test revealed that the vector was still susceptible to both DDT and malathion at discriminating dosages. The study revealed that inadequate surveillance and vector control measures were contributing factors for malaria outbreak. In view of this, insecticide treated nets may be introduced to provide cost-effective control of malaria.

Keywords: *An. minimus*, Central Assam, Malaria outbreak, Morbidity, Mortality, *P. falciparum*

INTRODUCTION

Malaria is endemic in Assam state. *Plasmodium falciparum* constitutes bulk of malaria cases (>60 per cent) and the remaining are *P. vivax* infections. Malaria transmission is perennial in the forest ecotype under the influence of *Anopheles minimus*, *An. dirus* and *An. fluviatilis*.[1,2] Morbidity and mortality due to malaria is alarmingly high commencing from April (the onset of rains) till September/October (the cessation of rainy season). Malaria outbreaks are more pronounced along inter-state/inter-country border areas due to inadequate and insufficient health

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services.[3] In 1995, focal outbeaks of malaria swept across the state affecting about 1.45 million population spread across 21 districts (of the total 23 districts) in the state. As many as 202 deaths were reported due to *P. falciparum* in all age groups. After a lapse of four years, series of focal outbreaks were witnessed beginning in May 1999 across the state reporting fever related deaths. We investigated the malaria outbreak in Nellie subcentre, Jhargaon PHC (District Morigaon) from July to August 1999 and results of the study are reported in the paper.

MATERIALS AND METHODS

Study area

Assam is a land of hills and valleys located between 24 to 28°N and 90 to 96°E. It is bounded by its sister states and has an international border with Bhutan to the north and Bangladesh to the south. The valleys are marked by major river systems and are flood prone during the monsoon season. Precipitation is in general two metres or above. Much of it occurs during monsoon season (July to September) with pre-monsoon showers beginning in March or April. The relative humidity varies between 60 to 80 per cent and most part of the year is hot and humid (22 to 33°C) except November to February (minimum temperature 9°C) which marked the winter season. The environment is conducive for both mosquito proliferation and active malaria transmission.

The Nellie health subcentre of Jhargaon Primary Health Centre (District Morigaon), is located on the south bank of Brahmaputra river. The subcentre has the population of about 8000 distributed in several villages. This area is a tribal dominated, located along with foothill bordering Karbi-Anglong district. The area is predominantly inhabited by low socio-economic groups, who are mostly engaged in paddy (jhoom) cultivation. The other means of subsistence included handlooms, forest products and daily wage jobs.

Parasitological investigations

To determine the prevalence of malaria and proportion of parasite species, door-to-door active surveillance was initiated in affected villages under Nellie health subcentre. Blood smears were collected from fever cases and examined under microscope for malaria parasites. Malaria positive cases were administered with antimalarial drugs as per NAMP drug policy—chloroquine at the total dose of 25 mg/kg of body weight in three divided doses (10 mg/kg on Day 0 and 1, and 5 mg/kg on Day 2). As per the state health data the malaria is endemic in this subcentre for the past several years and a total of 11 deaths due to malaria were recorded from July to August 1999.

Entomological techniques

To determine the prevalence and relative abundance of anopheline species, day resting catches were made from human dwellings (indoor) between 0700 and 1100 hours. Mosquito adults resting on the walls, clothes and other articles inside the houses were collected with the aid of torch light and a suction tube and were identi-

fied in the laboratory. To determine the man/mosquito contact and biting behaviour, overnight collections were made over human bait both inside and outside human dwellings between 1800 and 0500 hours. Landing mosquito collections on the exposed human body were made at hourly intervals and identified vector species were collected. Anophelines from day resting catches and human bait collections were dissected for salivary glands in 0.9 per cent saline for the detection of sporozoites. Adult susceptibility tests were carried out against DDT (4 per cent) and malathion (5 per cent) by using standard WHO procedures.

RESULTS

Malaria prevalence

Of the 69 blood slides collected from fever cases, 47 (68 per cent) were malaria positive. *P. falciparum* constituted 87 per cent and the remaining were *P. vivax* cases (Table 1). In view of high positivity among fever cases, it was decided to carryout mass and contact surveillance in the target population. Of 561 blood slides collected, 227 (40 per cent) were malaria positive and *P. falciparum* constituted over 90 per cent of malaria cases. Malaria was prevalent in all age groups in varying percentage. Children in between 5 and 14 yrs age groups have shown 71 per cent parasite rate as against 70 per cent in 15 yrs and above age groups. However, in mass blood survey parasite rate was 44, 45 and 38 per cent in the age groups of 1–4, 5–8; and 15 yrs and above respectively. It is evident from the data that *Pf* infection may be the likely casue of deaths occurred in the subcentre.

Entomological observations

Relative density

Results of indoor daytime resting collection from human dwellings revealed the prevalence of *An. minimus*, *An. culicifacies*, *An. annularis* and *An. vagus*. Of these, *An. vagus* was predomi-

Table 1. Malaria prevalence during 1999 focal outbreak in Nellie subcentre, Jhargaon PHC, District Morigaon, Assam

Age group (yrs)	Active case detection				Mass and contact survey			
	Fever cases	+(ve) for malaria	*Pf* cases	Parasite rate (%)	Blood slides collected and examined	+(ve) for malaria	*Pf* cases	Parasite rate (%)
0–4	4	1	1	25	80	32	25	44
5–14	21	15	11	71	170	77	70	45
15 and above	44	31	29	70	311	118	112	38
Total	69	47	41	68	561	227	207	40

Table 2. Relative prevalence of anopheline species and their abdominal condition in day resting collections in indoor human dwellings in Nellie subcentre (Jhargaon PHC, District Morigaon), Assam

Species	Abdominal conditions			Total collected	MHD (Man hours 38)	Per cent species composition
	UF	FF	SG			
An. annularis	3	2	10	15	0.39	1.84
An. culicifacies	5	0	3	8	0.21	0.98
An. minimus	8	12	89	109	2.87	13.39
An. vagus	—	—	—	682	17.95	83.78
Total	16	16	102	814	21.42	100.00

UF—Unfed; FF—Fully-fed, SG—Semi gravid; MHD—No. of mosquitoes collected per man per hour.

nant species and comprised 83 per cent of the total collection (Table 2). The density of *An. minimus* was 2.87 per man per hour, while that of *An. culicifacies* was quite low (0.21). Most of *An. minimus* collected were either fed or semi-gravid suggesting thereby that the species predominantly rest indoors.

Biting behaviour

Results of indoor and outdoor human bait collections of study villages are presented in Table 3. In these catches, *An. minimus* was the most predominant species and man biting rate (MBR) per person per night was as high as 35 and 23 in indoor and outdoor catches respectively. *An. dirus* was encountered only in outdoor and MBR was only four per person per night. *An. nivipes* was the only other dominant species which was collected both in outdoor and indoor and MBR was 10 and 7 respectively. In all these species peak biting activities were observed during mid-night suggesting thereby that insecticide treated mosquito nets (ITMN) will be most appropriate in such ecotypes.

Vector re-incrimination

Mosquitoes collected from human dwellings and human baits were dissected for sporozoites in the salivary glands. Of 130, *An. minimus* dissected, four specimens were found gland positive. The sporozoite infection rate was 3.08 per cent (Table 4). Other species like *An. dirus*, *An. annularis* and *An. culicifacies* were also dissected but none was found positive for gland infection. The study revealed that active transmission was carried out by *An. minimus* in the study area.

Insecticide susceptibility status

The result of susceptibility test against *An. minimus*, which was found in inadequate numbers, revealed that the species is completely susceptible to both DDT (4 per cent) and malathion

Table 3. Records of whole night man biting (indoor and outdoor) collections in DDT unsprayed area during focal outbreak in Nellie subcentre (District Morigaon), Assam

Species	Locality	No. collected per person (hours)											Total collected/ MBR (man night = 1)
		1800-1900	1900-2000	2000-2100	2100-2200	2200-2300	2300-2400	0-0100	0100-0200	0200-0300	0300-0400	0400-0500	
An. annularis	Outdoor	0	0	0	0	0	0	0	0	0	0	0	0
	Indoor	0	0	0	0	0	0	0	0	2	0	0	2
An. dirus	Outdoor	0	0	1	0	0	1	0	0	1	1	0	4
	Indoor	0	0	0	0	0	0	0	0	0	0	0	0
An. jeyporiensis	Outdoor	0	0	0	0	0	0	0	0	0	0	0	0
	Indoor	0	1	0	0	0	0	0	0	1	0	0	2
An. minimus	Outdoor	0	0	0	0	0	1	4	4	2	6	6	23
	Indoor	0	0	1	0	3	7	4	6	8	3	3	35
An. nivipes	Outdoor	0	1	0	1	1	2	2	1	1	1	0	10
	Indoor	0	0	2	2	1	0	0	0	2	0	0	7
An. varuna	Outdoor	0	0	0	0	0	2	1	0	0	0	0	3
	Indoor	0	0	0	0	0	0	0	0	0	0	0	0
Total	Outdoor	0	1	1	1	1	6	7	5	4	8	6	40
	Indoor	0	1	3	2	4	7	4	6	13	3	3	46

MBR—Mosquito biting rate per person per night.

Table 4. Dissection record of vector species for re-incrimination in malaria affected villages of Nellie subcentre (Jhargaon PHC, District Morigaon), Assam

Species	Total dissected	Gland positive	Sporozoite rate (%)
An. annularis	17	0	0.00
An. culicifacies	8	0	0.00
An. dirus	4	0	0.00
An. minimus	130	4	3.08

(5 per cent) as 100 per cent corrected mortality were observed in *An. minimus* with diagnostic dosages of respective insecticides.

DISCUSSION

Malaria is a major killer disease in Assam impairing the socio-economic development of the state. Deaths due to malaria is a common feature of any local and focal outbreaks depending upon the transmission potential and proximity to the malariogenic situation.[4,5] As per the state health record, a total of 111 deaths were confirmed due to *P. falciparum* malaria in the year

1999 throughout the state spread over 15 districts.

In Nellie subcentre, Morigaon district, eleven deaths were attributed due to malarial infection. In view of this, it was evident that malaria was rampant and *P. falciparum* was the predominant species. All age groups were affected supporting active transmission of the diesease and there appeared to be a great parasite reservoir in the community to the extent that 40 per cent of the population was morbid due to malaria. From the entomological observations, it was evident that *An. minimus* was abundant and its eco-biological characteristics were intact as documented.[1] Sporozoite infection rate of 3.08 per cent in *An. minimus* along with man/vector contact ranging from 23 to 35 per man per night could be contributory factors for active indigenous malaria transmission. Focal outbreaks in other parts of Assam were also attributed to *An. minimus*, the major carrier of the killer parasite.[6,7] The role of *An. dirus* and the suspected vector species — *An. nivipes* and *An. annularis* could not be clearly established in the present study, yet *An. dirus* has been implicated with the spread of drug-resistant malaria in the north-eastern India and adjoining countries.[8,9] DDT spray operations were not conducted as per schedule and spraying was very irregular in the past resulting built-up of vector density due to heavy precipitation and rendered the environment conducive for heavy transmission. It may also be appropriate to point out that there is a high refusal rate for DDT spray in the communities coupled with incipient resistance to commonly used antimalarials—chloroquine as per the existing drug policy of NAMP.[3] This amounts to drug failure and multiplication of drug resistant strains of *P. falciparum* in the presence of efficient vectors in the region.[9,10] Malaria outbreaks owing to the proliferation of drug-resistant strains and neglected surveillance have been established in other parts of India.[11,12]

Though *An. minimus* by and large is susceptible to DDT but due to the operational constraints and insurgency, insecticide treated mosquito nets are being introduced as an alternate strategy for vector control in the N.E. region and would be the main stay as one of the key elements under Roll Back Malaria (RBM) initiatives.[13] The strengthening of the health infrastructure particularly at the peripheral level will ensure early detection and prompt treatment.[14]

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SHORT NOTES

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Childhood Malaria and Use of Bednets in Kolkata

D.K. RAUT[a] and G.K. PANDEY[a]

Keywords: Bednets, Childhood fever, Childhood malaria

Construction of metro-railway in Kolkata resulted in resurgence of malaria. Hati and Mukhopadhyay[1] incriminated *An. stephensi* as vector species. Use of bednets as a protection from mosquitoes during night has been practiced from very early times.[2] A study was carried out to ascertain the incidence of childhood fever and malaria and use of bednets among children in Kolkata. This study was carried out in the Chetla and Kalighat slums of south Kolkata. A house-to-house mass survey was carried out to include all the children under nine years of age residing in these two localities. A total of 179 families were present during the survey, out of which 133 families had children under nine years of age. A structured questionnaire was developed and pretested, which was used to collect quantitative and qualitative data. Mothers of the children were respondents. Information was collected on name of household, socio-economic data, details about children's date of birth, history of fever in preceding one month, reports of blood slide examination for malaria parasite etc. A case of childhood malaria was confirmed

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Table 1. Frequency distribution of variables and attributes

Variables	Attributes	Frequency No. (%)	Significance
Residency	Chetla	114 (100)	
	Kalighat	57 (100)	
Monthly family income	<1500	101 (59.06)	$\chi^2 = 24.24$; $p < 0.001$ Kalighat families are poor.
	1500 and above	70 (40.93)	
Childhood fever	0–9 years	42 (24.56)	
Childhood malaria	0–9 years	21 (21.28)	$Z = 2.97$; $p < 0.01$ Childhood malaria is more in Kalighat.
Bednet use	Chetla	61 (53.50)	
	Kalighat	30 (52.63)	
Repellent use	Chetla	41 (35.96)	
	Kalighat	8 (14.03)	

when blood slide for malaria parasite was positive. Majority of the families, 101 (59.1 per cent) had monthly income less than Rs. 1500 per month. The families of Kalighat were poor as compared to Chetla ($\chi^2 = 24.4$; $p < 0.001$) (Table 1). There were total 171 children under nine years of age. About 114 (66.7 per cent) children were from Chetla and 57 (33.3 per cent) belonged to Kalighat. Ten (5.8 per cent) children were infants.

There were 42 (24.6 per cent) children aged 0–9 years who had fever in preceding one month of the survey. The proportion of fever cases was higher 22 (52.4 per cent) in 1–4 years followed by 18 (42.8 per cent) in 5–9 years. Only two (1.2 per cent) infants had fever. In both the areas, fever rate was higher 27 (64.3 per cent) in males, compared to females 15 (35.7 per cent). However, proportion of fever cases in Kalighat 19 (33.3 per cent) was higher than Chetla 23(20.2 per cent). The childhood malaria was found in 21 (12.3 per cent) out of total 171 children. Out of 42 (24.56 per cent) fever cases 21 (50 per cent) had malaria which gives an incidence of childhood malaria as 12.3 per cent. Proportion of malaria cases at Kalighat was 13 (68.4 per cent), which is higher than that of Chetla 8 (34.8 per cent) ($Z = 2.97$; $p < 0.01$).

Among the personal protective measures, bednets were used by 91 (53.2 per cent) and mosquito repellents — coil, mats and smoke (Dhuno in Bengali) by 49 (28.65 per cent) families. Significantly higher proportion of families with monthly income above Rs. 1500 in Chetla 47 (41.96 per cent) compared to 6 (8.95 per cent) in Kalighat used bednets ($\chi^2 = 32.27$; $p < 0.001$), while no family having monthly income below Rs. 500 used bednets. It is found that 39 (21.8 per cent) families with monthly income above Rs. 1500 uses mosquito repellents com-

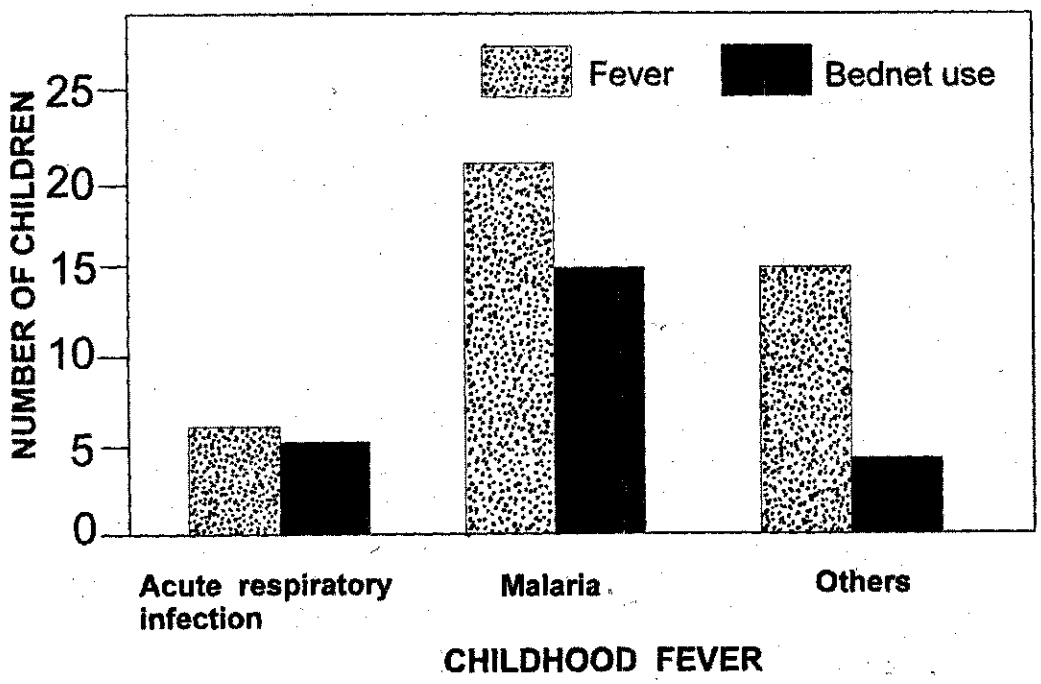

Fig. 1: Distribution of childhood fever according to bednet use

pared to 12 (6.7 per cent) families below Rs. 1500 ($\chi^2 = 1.61$; $p > 0.01$). Out of 42 fever cases, 24 (57.14 per cent) children use bednets. About 12 (57.14 per cent) children out of 21 (50 per cent) childhood malaria cases were using bednets (Fig. 1).

In the present study 42 (24.56 per cent) children suffered from febrile episode. Half of these, 21 (50 per cent) episodes were on account of malaria, which is higher than 14 (19.44 per cent) out of 72 school children (5–15 years) who were having history of pyrexia in old city of Hyderabad.[3] The proportion of childhood fever was 19 (33.3 per cent) and childhood malaria 13 (68.4 per cent) in Kalighat, which is higher than that of Chetla. The Kalighat, families are poorer than Chetla ($p < 0.001$). Wessen[4] also observed that the vicious cycle of malaria was a matter of great significance as low socio-economic status not only contributed to creating the precondition for malaria but the syndrome of poverty also facilitated its spread.[5]

Among the personal protective measures against malaria, bednets were used by 53.2 per cent families, whereas 28.6 per cent of families used mosquito repellents. A strong relationship between income and bednet usage was observed. Families with income less than Rs. 500 per month did not use bednets. There was a proportionate increase in bednet usage with rise in the income. The use of bednets with monthly income less than Rs.

1500 was 43.9 per cent and 56.05 per cent above Rs. 1500. However, use of mosquito repellents did not have any relation with income. Similarly, 57.1 per cent of childhood malaria cases use bednets. The childhood malaria is more in Kalighat where families are poor and usage of bednets and repellents is less compared to Chetla. A survey in the Farafenni area of the Gambia found parasitaemia in 53 per cent of people who did not use bednets as compared to 31 per cent who used well-maintained nets.[6]

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A Simple Method for Dissecting the Salivary Glands of Mosquitoes

D.T. MOURYA[a]

I report here a simple method for the dissection of salivary glands of mosquitoes. Salivary gland dissections are needed routinely for sporozoite detection studies on malaria transmission. Dissection of salivary glands in *Anopheles* is fairly easy as compared to *Aedes* and *Culex* mosquitoes. Routinely, a large number of gland dissections are not required for studying vectors of arboviruses but in experimental situations, studies are carried out on a limited number of laboratory mosquitoes where data of different organs of respective individual mosquitoes matters.[1] Basic techniques for the dissection of glands from mosquitoes have been explained since a long time.[2,3] Basically there are two ways by which the glands are dissected out on a slide containing a drop of saline. In the first method head is slowly pulled off from the thorax and in the second method the head is cut off and the thorax is gently pressed so that the glands are protruded out from the body.

The present paper describes a modification in the first technique for convenient dissection of the glands in case of *Aedes* and *Culex* mosquitoes where normally isolating the complete gland is generally difficult on account of strong musculature and fat bodies.

Procedure: Put the anaesthetised mosquito on a filter papter. Cut it into two parts exactly from the middle of the thorax (Fig.1). Take a drop of saline containing 0.3 per cent Triton-X 100 (Sigma Chemical Co., USA). Keep the cut-off portion of the thorax on the drop of saline. After about 30-40 sec slowly pull off the head from the thorax. The glands come out with ease without any difficulty, which can then be immediately transferred to saline without Triton-X to avoid further exposure to the detergent.

In our laboratory *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi* mosquitoes were dissected successfully using this technique. The success rate of dissecting out the complete glands in these species was 97, 96 and 97 per cent respectively (n = 50). Indirect immunofluorescence technique used to detect dengue

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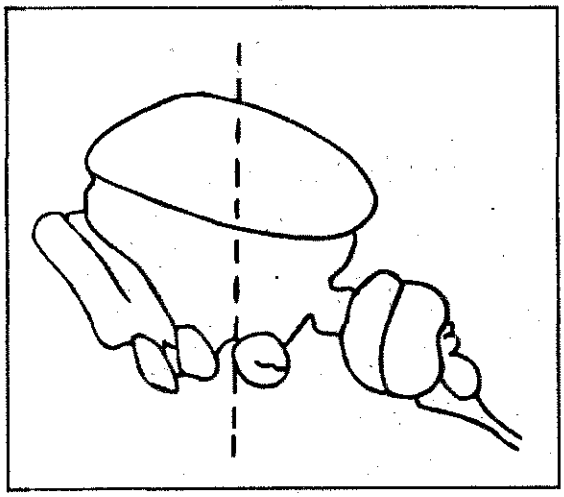

Fig. 1: Diagrammatic sketch of mosquito body (the dotted lines show the plane of cutting the thorax of mosquito)

virus antigen in these glands of *Ae. aegypti* did not show any difference in the staining of the glands dissected in saline with and without Triton-X (n = 25, in each case). The procedure was also found to be useful in dissecting out glands from the stored mosquitoes, which is otherwise very difficult. In this situation the cut portion of the thorax should be kept on the drop of saline for about 60-90 seconds. If the dissected glands are kept in the saline containing Triton-X the fat cells attached to the gland get detatched due to the detergent effect. Due to this process glands get cleaned automatically without any effort.

Merits of this method: (i) It is very useful in dissecting a large number of salivary glands for routine examination; and (ii) under experimental situations where a small number of mosquitoes are available and the data on salivary glands of each individual mosquito are needed with high success rate, it can easily be achieved with this method.

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