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Falciparum Malaria and Pregnancy

DHANPAT K. KOCHAR, INDU THANVI, ASHISH JOSHI, SUBHAKARAN, SUSHIL ASERI and B.L. KUMAWAT

Falciparum malaria in pregnancy is a significant health problem in India. Pregnant women constitute an important high risk group for malaria infection which may cause abortion, still births, intra uterine growth retardation (IUGR), and pre-mature labour. Two hundred eighty-eight admitted female patients of falciparum malaria were included in the study out of which 45 were pregnant. The mortality rate was highly significant in pregnant females (37.77%) in comparison to non-pregnant females (14.81%); ($p < 0.001$). The incidence of various pernicious syndromes including cerebral malaria, severe anaemia (Hb < 5 g%) hepatic and renal failure were more in pregnant females in comparison to non-pregnant females. The incidence of infection was higher among primigravida and second gravida 30/45 (66.66%) as compared to multigravida 15/45 (33.33%) and the greater incidence of infection was seen during 14-28 wk of gestation 23/45 (51.11%). Pregnancy related complications in the form of preterm live birth (20%). Intra uterine death (IUD 31.11%), still births (13.33%) and abortions (11.11%) were more pronounced in primiparous women as compared to multiparous. Weight of placenta in majority of patients ranged between 200-400 g (22/31; 70.96%). Normal pregnancy continued in only 11 out of 45 pregnant females, out of which seven had low birth weight baby (63.63%). As the pregnancy is associated with increased incidence and adverse outcome of falciparum malaria infection, chemoprophylaxis should be made an integral part of antenatal care along with antianaemic therapy to reduce the risk of serious maternal and fetal complications.

Keywords: Falciparum malaria, Pregnancy

INTRODUCTION

It has been recognized for nearly a century that pregnant women are especially prone for developing severe attacks of malaria and associated complications. Studies from various countries of Asia regarding impact of unstable malaria in pregnant women have

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shown it to be a frequent cause of maternal deaths[1,2]. The impact of malaria in pregnancy is quite significant in India but only a limited number of hospital-based studies have been carried out regarding the consequences of malaria infection in pregnant women[3-6]. Moreover these studies have been conducted in different geographical regions which differ in endemicity, climate, parasite species prevalence and socio-cultural attitude related to malaria.

Considering the severity of problem we planned to undertake an indepth prospective study of effects of falciparum malaria on pregnancy and its out come in comparison to non-pregnant female patients residing in same environment and community. These observations are of immense clinical significance and essential for the theme presented in implementation of global malaria control programme.

MATERIALS AND METHODS

This study was conducted on pregnant and non-pregnant female patients of *P. falciparum* malaria (confirmed by demonstration of asexual phase of parasite) admitted in classified malaria wards of PBM Hospital, Bikaner (Rajasthan). Only smear positive cases (*P. falciparum* or mixed *P. vivax* and *P. falciparum*) were included in the study. Thorough clinical and biochemical examination which included haemoglobin estimation, complete blood counts, packed cell volume, liver function tests, blood urea, serum creatinine and urine examination was performed in all patients. Special investigations like widal test, blood culture, Australia Antigen (HBsAg) (Positive HBsAg cases were not included), brucella antigen and chest X-ray were performed to rule out other causes of fever, whenever indicated. Patients were categorized in cerebral malaria group according to strict WHO definition, i.e. unarousable coma not attributable to any other cause and lasting for more than 30 min in a patient with falciparum malaria[7]. Similarly all patients were grouped under various syndromes according to strict definition of severe malaria by WHO (1990)[7]. Fundus and CSF examination was done in all patients of cerebral malaria.

All pregnant patients were subjected to regular obstetric checkup in antenatal clinic and cases were followed up till delivery. Sonography of abdomen was done in all pregnant patients, weight of the baby and placenta were recorded. Cord blood smear and baby's blood smear were taken and examined for evidence of malaria parasite. Histopathological examination of placenta was done in patients who had preterm labour or abortions.

All the patients of simple malaria and pernicious syndromes were treated with oral/IV quinine and IV dextrose according to WHO guidelines[7]. Mortality and morbidity trends were compared in pregnant and non-pregnant females as well as in cerebral malaria and non-cerebral pernicious syndrome pa-

tients by applying chi-square (χ^2) test and p-values were calculated.

RESULTS

The study was conducted on 288 female patients of falciparum malaria, which included 45 pregnant females and 243 non-pregnant females. There was no history of prior hospitalization and treatment in pregnant women. Thus for all these patients the present attack of malaria was first episode. Similarly, there was no prior history of attending any malaria clinics by pregnant women of both groups. Out of 45 pregnant *P. falciparum* patients, cerebral malaria was present in 34 patients (75.55%). The incidence of cerebral malaria in non-pregnant females was 80/243 (32.92%). Severe anaemia (Hb < 5 g%) was present in nine out of 45 pregnant patients and 8 (88.89%) of them expired. While severe anaemia was seen in only 10 out of 243 non-pregnant patients and 5 (50%) of them expired. The incidence of all other pernicious syndromes and related mortality were more in pregnant females in comparison to non-pregnant female (Table 1).

The mortality in pregnant females (37.77%) was statistically highly significant than in non-pregnant females (14.81%; $p<0.001$). The mortality in pregnant cerebral malaria

Table 1. Incidence and mortality trends of various pernicious syndromes observed among pregnant and non-pregnant patients

Pernicious syndrome	Pregnant (n=45)		Non-pregnant (n = 243)	
	Incidence (%)	Mortality (%)	Incidence (%)	Mortality (%)
Cerebral malaria	34 (75.55)	14 (41.17)	80 (32.92)	18 (22.50)
Severe anaemia (Hb<5 g%)	9 (20.00)	8 (88.89)	10 (4.11)	5 (50.00)
Hepatic failure (Serum bilirubin > 3 mg%)	6 (13.33)	4 (66.60)	22 (9.05)	8 (36.36)
Convulsions	5 (11.11)	3 (60.00)	7 (2.88)	3 (42.86)
Renal failure	9 (20.00)	8 (88.90)	15 (6.17)	6 (40.00)
Hypoglycaemia blood glucose < 40 mg% at time of admission	3 (6.66)	2 (66.66)	4 (1.64)	2 (50.00)
ARDS	2 (4.44)	2(100.0)	4 (1.64)	3 (75.00)
Multiple organ failure (≥ 3)	6 (13.33)	6(100.0)	20 (8.23)	16 (80.00)

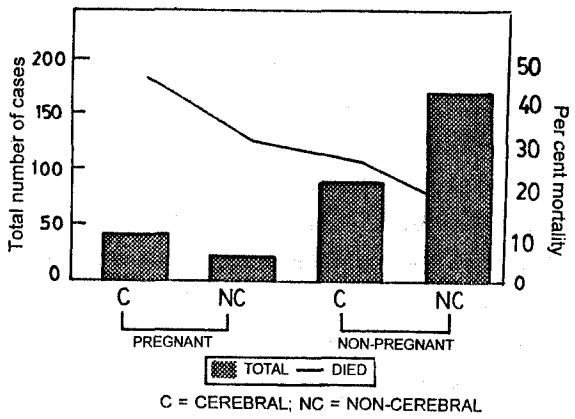

Fig. 1: Incidence and mortality rate in pregnant and non-pregnant women patients of malaria

patients (41.17%) was significantly more than in non-pregnant cerebral malaria (Fig. 1).

In pregnant group the incidence of infection was higher among primigravida and second gravida 30/45 (66.66%) as compared to multigravida 15/45 (33.33%). The greater incidence of infection was seen during 14-28 wk of gestation 23/45 (51.11%) followed by the period > 28 wk 15/45 (33.3%) and least in period of < 14 wk 7/45 (15.55%) of gestation.

The outcome of pregnancy was worse in primiparous women as compared to multiparous. Normal pregnancy continued in only one out of 16 primiparous (6.25%) and 10 out of 29 multiparous pregnant women (34.48%). Other complications of pregnancy, i.e. preterm live birth (20%), IUD (31.11%), still births (13.33%) and abortions (11.11%) were more pronounced in primiparous women as compared to multiparous (Table 2).

Greater number of foetus were born with birth weight < 2.5 kg (27/31; 87.09%). This included five aborted babies who had birth weight < 500 g. Fourteen patients died with

Table 2. Outcome of pregnancy in primiparous v/s multiparous patients of falciparum malaria

Pregnancy/ Complications	Primiparous (16)		Multiparous (29)	
	Number	Percentage	Number	Percentage
1. Normal pregnancy and labour	1	6.25	10	34.48
2. Complications during pregnancy				
(i) Preterm live birth	3	18.75	6	20.68
(ii) Intra uterine death	7	43.75	7	24.13
(iii) Still birth	3	18.75	3	10.34
(iv) Abortion	2	12.50	3	10.34

Table 3. Distribution of patients according to weight of foetus (n = 31)

Weight of foetus (g)	No.	Percentage
< 500	5	16.12
500-2500	22	70.96
> 2500	4	12.90

in utero death of foetus (Table 3). Out of 31 placentae examined, 22 had weight between 250-400 g (70.96%). Five patients who aborted had placental weight < 250 g.

DISCUSSION

Pregnant women are especially prone for developing severe and complicated malaria. As very limited studies are available in Indian literature [3-6] regarding the effect of falciparum malaria on pregnancy, we planned to undertake an indepth study of pregnant women suffering from falciparum malaria and compare the data with non-pregnant females residing in same environment and community. The study was conducted in Bikaner (Rajasthan) during epidemic of malaria in 1994-96. The possible causes of this outbreak are increase in the annual rainfall, collection of water around Indira Gandhi Canal, forestation of shrubs around them and migration of labour, adaptation of *Anopheles stephensi* to desert climate and favourable breeding of *An. culicifacies* in the areas under impact of irrigation[8].

The incidence of severity of infection and pregnancy related complications varies according to the level of acquired immunity of pregnant women against the infections and the parity[9]. In this study, the susceptibility to malaria infection was high in first and second pregnancies as 30 patients (out of 45; 67%) were either primigravida or second gravida. This is in accordance with various hospital based studies carried out in India[3,4]. These studies have shown that primigravida has higher clinical severity than multigravida in the areas where transmission rate of malaria was high.

In highly endemic areas the peak level of falciparum parasitaemia occurs between 9 to 16 wk of gestation and then decreases progressively until delivery. In our study 23 (51.11%) patients were having 14-28 wk of gestation at the time of infection. This shows a greater incidence of malaria infection during second trimester and is in accordance with earlier studies[3,4]. The mechanism of increased susceptibility during pregnancy has not been elucidated but increased cortisol level, depressed cell mediated immunity, altered immune functions of spleen, immune evasion by parasite and interactions between malaria parasite and human nutritional status are believed to play important role[10]. The synthesis of immunosuppressive factors mainly oestrogens, in placenta may be responsible for decreased cell mediated immune response in placenta in relation to peripheral blood[11], leading to increased number of parasite sequestration in placenta.

Malaria during pregnancy results in increased perinatal wastage in form of maternal deaths

and foetal wastage. In our study 17 out of 45 patients (37.77%) expired during course of illness and 14 patients died within utero death of foetus. Two patients expired just after delivery due to acute respiratory distress syndrome (ARDS) and one patient expired due to uncontrolled bleeding (postpartum haemorrhage) after delivery. Pregnant women are likely to have 2-10 times higher mortality than non-pregnant women[7]. Various studies in India have also shown high incidence of maternal deaths in cases of malaria[3,4,12].

The mortality rate in pregnant females (37.77%) was statistically highly significant than non-pregnant females (14.81%); $p < 0.001$) Pregnancy is associated with severe falciparum malaria and increased prevalence of various pernicious syndromes. Multiple organ failure was more common in pregnant females and associated with grave prognosis (Table 1). The most important factor for increased mortality in pregnant females is the presence of anemia. Severe anemia (Hb < 5 g%) was more commonly seen amongst pregnant group (9 out of 45; 20%) as compared to non-pregnant females (10 out of 243; 4.11%) and consequently the mortality in pregnant group was also high (8 out of 9; 88.89%) as compared to non-pregnant group (5 out of 10; 50%). The mechanism of anemia is multi-factorial and complex involving hemolysis and inappropriate bone marrow response[7].

The incidence of cerebral malaria in pregnant women was quite high in comparison to various other studies reflecting low level of immunity in this sub-group of population as well as in this region. Mortality in cases of cerebral malaria with pregnancy was statistically significant ($p < 0.05$) than in non-pregnant cerebral malaria.

Various authors from India[13,14] have described hypoglycaemia in severe falciparum malaria. The incidence of hypoglycaemia (Blood glucose < 40mg%) is also increased during pregnancy and is associated with high mortality (2 out of 3 patients; 66.67%). The possible mechanism for hypoglycaemia include increased glucose consumption by host and parasite[15], glycogen depletion and impaired gluconegenesis[16]and treatment with quinine[17].

Malaria leads to significant reproductive wastage in areas of unstable transmission. In our study preterm live births were seen in 9 (20%) patients while the incidence of low birth weight babies was 22 (out of total 31; 70.96%). Placental malaria is a significant cause of pregnancy related complications in the form of abortions, still births, premature deliveries and low birth weight babies.

In our study placental weight ranged mainly between 250-400 g (22 out of 31; 70.96%). Several investigations have demonstrated an association between placental infection and low birth weight[18,19]. The biologic mechanism by which placental malaria infection leads to low birth weight is not fully established. *P. falciparum* infected placentae show thickening of basement membrane of placental trophoblast cells, potentially result-

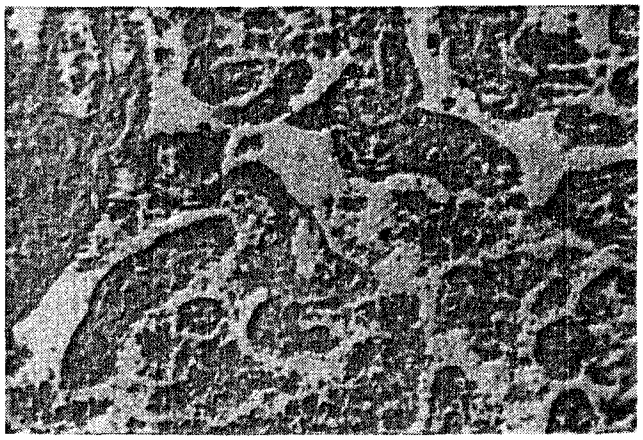

Fig. 2: Microphotograph of human placental villi showing intervillous spaces filled with malaria parasite and pigment containing RBC

ing in reduced nutrient transfer to foetus[20] (Fig. 2). Placental microinfarcts have been reported; transplacental passage of parasitised RBC to foetus does occur, and this may affect foetal nutrient use or stimulate early delivery.

In our study foetal cord blood was negative for *P. falciparum* parasite and no case of congenital malaria was observed in this series suggesting low prevalence of congenital malaria, an observation also confirmed by other Indian authors[3,5].

Limited data are available regarding spontaneous abortion as a complication of malaria infection. In our study five patients had abortion (11.1%). Although various authors from other parts of world have reported that malaria is not an important cause of spontaneous abortion in highly endemic areas[18]. Various hospital-based studies from India have also shown that incidence of abortion in malaria patients is not high[3,5]. As most of the reports are from highly endemic areas and have failed to document a direct association between maternal malaria and abortions, it seems that the evidence of abortions varies inversely with immunity of mother.

Thus based on our observation, we can safely conclude that falciparum malaria infection has an adverse effect on progress and outcome of pregnancy hence early diagnosis and proper initiation of therapy can reduce the risk of serious maternal and foetal complications. As the chances of acquiring malaria infection are increased during pregnancy, chemoprophylaxis should always be considered to protect pregnant women from hazards of malaria infection.

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Larval Ecology and Mosquito Fauna of Upper Brahmaputra Valley, Northeast India

S.A. KHAN, R. HANDIQUE[a], S.C. TEWARI[b], P. DUTTA, K. NARAIN and J. MAHANTA

Ninety-one mosquito species in 15 genera were collected from 22 habitat types of Upper Brahmaputra valley in Assam and bordering areas of Arunachal Pradesh. Nineteen species have been recorded for the first time in Assam including seven first records from the northeast region and three first country records. Jaccard's coefficient was used to quantify the similarity between 13 different geographical localities surveyed on the basis of their mosquito fauna. Group average sorting (UPGMA) cluster analysis was used to find out relationship between Jonai, Dhemaji, Dibrugarh, Tinsukia and north Lakhimpur (study zones) on the basis of their mosquito community. Immatures of three malaria vectors incriminated from the northeast India, viz. *Anopheles dirus, An. minimus* and *An. philippinensis* were collected exclusively from forest areas. Fourteen potential vectors of Japanese encephalitis incriminated from elsewhere in India were collected during the study.

Keywords: Japanese encephalitis, Malaria, Mosquito fauna, Northeast India, Vectors

INTRODUCTION

Interest in the mosquitoes of Assam and other adjoining states is mainly related to malaria and Japanese encephalitis (JE) transmission which are major public health problems in this region[1-9]. Christophers[4] and Barraud[5] were among the earliest workers to record mosquitoes from the Indian subcontinent including Assam. In 1957 and 1966, Zoological Survey of India and Defence Research Laboratory, Tezpur, conducted joint surveys

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of mosquito fauna in Assam and Arunachal Pradesh[6]. Nagpal and Sharma[7] reported a total of 59 species (spp) of mosquitoes from Assam. Sarkar *et al.*[9] studied mosquito fauna of Dibrugarh in 1978 and reported 27 spp in eight genera from this region. However from our earlier experience, it appears that some more genera and species are present.

In the present study, an extensive survey of mosquito fauna and their breeding habitats in the study area was undertaken keeping in view the greatly changed ecology during the last few decades of socio-economic developments such as deforestation, urbanization, exploitation of natural resources like oil and coal mining, local biotope changes and various other factors. The consequences of these changes is evident from the sudden upsurge of vector-borne diseases and increasing outbreaks of JE.

MATERIALS AND METHODS

Study area

The study area lies between 93°45' to 96°E longitude and 26°45' to 28°N latitude and comprises upper reaches of Brahmaputra valley in Assam and some bordering areas of Arunachal Pradesh (Fig. 1). The survey of mosquito fauna was conducted from four districts of Assam namely, Dibrugarh, Tinsukia, north Lakhimpur and Dhemaji and the contiguous areas of Arunachal Pradesh. Climate of the study area is of tropical monsoon type. Details of the climatological features has been given elsewhere[10]. The study area has extensive tea garden plantations, bamboo forests, reserve forests, paddy fields and most of the area is frequently inundated by flood waters of the River Brahmaputra and its tributaries resulting in ample mosquito breeding

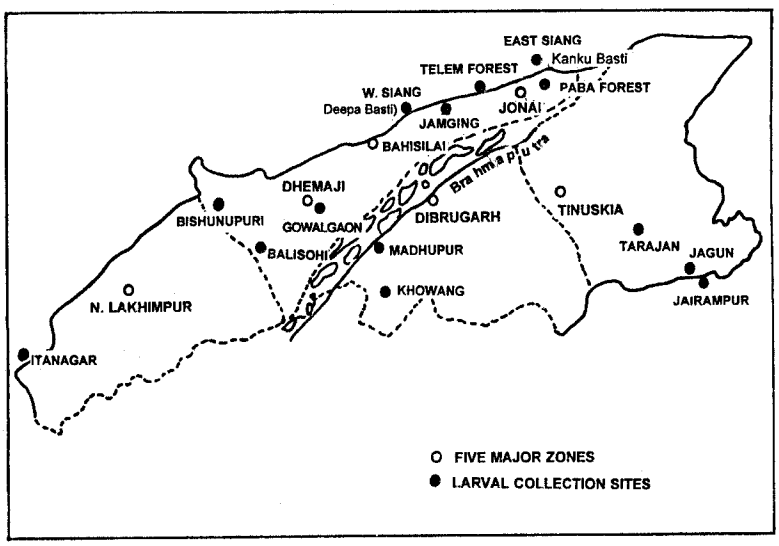

Fig. 1: Map of study area showing immature mosquito collection localities in five major zones

sites for mosquito proliferation and diversification.

Larval sampling

Larval and pupal collections were made from five major zones of the study area (Fig. 1). The period of collection was September to November 1991. A total of thirteen sites (Fig. 1) were randomly selected for sampling. The immature mosquitoes were collected from twenty-two types of habitats (Table 1). Standard size plastic dippers of 11 cm diam and 350 ml water holding capacity were used for collecting mosquito immatures from surface habitats. Collection from tree holes, bamboo stumps and leaf axils were made by glass pipettes fitted with rubber bulb for suction. The larvae and pupae thus collected were individually link-reared to adult stage in transparent plastic photo-film vials in the temporarily set up field laboratories. The mosquitoes were identified using both adult and associated immature characters. Altitude of the collection sites were recorded using portable altimeter. Identifications have been confirmed and voucher specimens have been deposited in insect museum of the Centre for Research in Medical Entomology (Indian Council of Medical Research), Madurai, India. Abbreviations of mosquito generic and subgeneric names used in the text is after Reinert[11]. Distribution of species in the world is according to Knight and Stone[12].

Adult sampling

Besides larval collections, adult mosquitoes were also collected during dusk hours (May 1991 to April 1992), whole nights off human and cattle baits (August 1991 to July 1992) and daytime outdoor and indoor resting collections (May 1992 to April 1993).

Statistical analysis

UPGMA hierarchical cluster analysis[13] was used to find out relationship between Jonai, Dhemaji, Dibrugarh, Tinsukia and north Lakhimpur on the basis of their mosquito community. The above analysis was done using similarity matrix generated by Jaccard's coefficient[13].

RESULTS

Larval collections

A total of 2283 immature mosquitoes were collected from 22 different habitat types during this study (Table 1). These represented 72 spp belonging to 13 genera. The genus *Anopheles* was most dominant and was represented by 23 spp followed by *Culex* (17 spp), *Aedes* (12 spp), *Armigeres* (5 spp), *Uranotaenia, Mimomyia, Malaya, Trypteroides, Toxorhynchites* and *Orthopodomyia* represented by two spp each and *Aedeomyia, Heizmania* and *Ficalbia* represented by one sp each.

Different habitat types harboured 1 to 22 types of mosquitoes. Bamboo stumps harboured maximum number of mosquito species (22 spp) followed by mud pools (18 spp), ponds (15 spp), irrigation channels (12 spp), spring pools (11 spp), rainwater

Table 1. List of immature mosquitoes collected from different habitats

Sl. No.	Species	Habitat types (No. of individuals)*	Total
1.	*An. (An.) barbirostris*	2(6), 6(6), 21(3), 22(1)	16
2.	*An. (An.) bengalensis*	7(1), 16(4), 22(1)	6
3.	*An. (An.) crawfordi*	6(1), 7(3)	4
4.	*An. (An.) gigas*	21(3)	3
5.	*An. (An.) hodgkini*	22(1)	1
6.	*An. (An.) lasteri paraliae*	6(9)	9
7.	*An. (An.) peditaeniatus*	2(26), 5(5), 6(3), 7(16), 12(2)	52
8.	*An. (An.) sinensis*	6(1)	1
9.	*An. (Cel.) aconitus*	22(2)	2
10.	*An. (Cel.) annularis*	2(3), 6(14), 7(11)	28
11.	*An. (Cel.) dirus*	5(2), 10(3), 15(5)	10
12.	*An. (Cel.) jamesii*	2(2), 4(4), 6(6)	12
13.	*An. (Cel.) karwari*	4(8), 7(3)	11
14.	*An. (Cel.) kochi*	2(15), 5(10), 9(8), 10(8), 21(4)	55
15.	*An. (Cel.) maculatus*	2(87), 5(1), 10(4), 12(18), 22(15)	125
16.	*An. (Cel.) minimus*	7(3), 14(7), 22(1)	11
17.	*An. (Cel.) nivipes*	5(32), 21(6)	38
18.	*An. (Cel.) philippinensis*	6(3)	3
19.	*An. (Cel.) pseudowillmorei*	12(13)	13
20.	*An. (Cel.) splendidus*	5(8)	8
21.	*An. (Cel.) tessellatus*	2(1)	1
22.	*An. (Cel.) vagus*	9(5), 21(45)	50
23.	*An. (Cel.) varuna*	22(2)	2
24.	*Ad. (Ady.) catasticta*	6(10)	10
25.	*Ae. (Adm.) alboscutellatus*	2(1)	1
26.	*Ae. (Fin.) caecus*	2(14), 9(30), 10(86), 15(9)	139
27.	*Ae. (Fin.) albolateralis*	1(66)	66
28.	*Ae. (Fin.) albotaeniatus*	20(4)	4

contd...

Table 1. *(contd.)*

Sl. No.	Species	Habitat types (No. of individuals)*	Total
29.	*Ae. (Fin.) dissimilis*	3(3)	3
30.	*Ae. (Fin.) harveyi*	20(10)	10
31.	*Ae. (Fin.) prominens*	3(2)	2
32.	*Ae. (Stg.) albolineatus*	1(3), 3(2)	5
33.	*Ae. (Stg.) albopictus*	1(98), 3(14), 8(3)	115
34.	*Ae. (Stg.) annandalei*	1(8)	8
35.	*Ae. (Stg.) craggi*	1(2)	2
36.	*Ae. (Stg.) pseudalbopictus*	1(8)	8
37.	*Ar. (Arm.) durhami*	1(221), 18(36)	257
38.	*Ar. (Arm.) subalbatus*	3(128), 16(107)	235
39.	*Ar. (Lei.) digitatus*	1(2)	2
40.	*Ar. (Lei.) flavus*	1(250)	250
41.	*Ar. (Lei.) longipalpis*	1(3)	3
42.	*Cx. (Cux.) alienus*	7(1)	1
43.	*Cx. (Cux.) bitaeniorhynchus*	6(3), 10(1), 11(3), 12(5), 21(8), 22(15)	35
44.	*Cx. (Cux.) fuscocephala*	2(6), 5(6), 7(11), 9(5), 10(1), 11(9), 12(18),21(42)	98
45.	*Cx. (Cux.) gelidus*	9(1)	1
46.	*Cx. (Cux.) mimulus*	2(12), 5(2), 9(4), 10(5), 11(1), 16(20), 19(13), 21(7), 22(1)	65
47.	*Cx. (Cux.) pseudovishnui*	2(11), 5(17), 6(1), 7(26), 21(16)	71
48.	*Cx. (Cux.) quinquefasciatus*	2(24), 9(6), 10(73)	103
49.	*Cx. (Cux.) tritaeniorhynchus*	2(5), 6(6), 7(7), 9(2)	20
50.	*Cx. (Cux.) vishnui*	2(3), 5(1), 11(2), 12(12), 21(6)	24
51.	*Cx. (Cux.) whitmorei*	1(1), 2(5)	6
52.	*Cx. (Cui.) fragilis*	10(2)	2
53.	*Cx. (Cui.) pallidothorax*	9(39), 10(1)	40
54.	*Cx. (Eum.) khazani*	7(3)	3
55.	*Cx. (Eum.) malayi*	1(2), 2(7), 6(34), 7(12), 9(1)	56
56.	*Cx. (Lop.) bengalensis*	1(3), 3(1)	4
57.	*Cx.(Lop.) minor*	5(20)	20

contd...

Table 1. *(contd.)*

Sl. No.	Species	Habitat types (No. of individuals)*	Total
58.	*Cx.(Lut.) halifaxii*	2(5)	5
59.	*Hz. (Hez.) chandi*	1(13), 3(2)	15
60.	*Fi. minima*	3(1), 6(2)	3
61.	*Mi. (Eto.) luzonensis*	1(1)	1
62.	*Mi. (Mim.) intermedia*	6(4)	4
63.	*Or. albipes*	1(4)	4
64.	*Or. anopheloides*	3(4)	4
65.	*Ml. genurostris*	8(18), 13(42)	60
66.	*Ml. jacobsoni*	13(3)	3
67.	*Tp. (Rah.) aranoides*	1(10)	10
68.	*Tp. (Trp.) tarsalis*	1(13)	13
69.	*Ur. (Ura.) edwardsi*	1(6)	6
70.	*Ur. (Ura.) orientalis*	1(1)	1
71.	*Tx. (Tox.) gravelyi*	1(3)	3
72.	*Tx. (Tox.) splendens*	1(9), 8(12)	21

*Habitat types: 1—Bamboo stump; 2—Mudpool; 3—Tree hole; 4—Paddy field; 5—Spring pool; 6—Pond; 7—Irrigation channel; 8—Plantain leaf axil; 9—Manure pit; 10—Rain water pool; 11—Fallow field; 12 — Sandy pool; 13—Pineapple leaf axil; 14—Well; 15—Elephant foot print; 16—Spring; 17—Plantain stump; 18—Papaya stump; 19—Abandoned boat; 20—Split bamboo; 21—Stream pool; 22—Slow flowing stream; Nos. 1 to 22 denote different habitats and figures in parentheses indicate numbers of individuals collected.

pools (10 spp), manure pits (10 spp), stream pools (10 spp), tree holes (9 spp), slow flowing streams (9 spp), sandy pools (6 spp), fallow fields (4 spp), plantain leaf axils (3 spp), elephant foot prints (2 spp), paddy fields (2 spp), pineapple leaf axils (2 spp), split bamboos (2 spp), wells (1 sp), plantain stumps (1 sp), papaya stump (1 sp), and abandoned boats (1 sp).

Fifty-four species were recorded from forest areas whereas non-forest localities yielded 43 mosquito species. Twenty-nine species were forest specialists as they were exclusively present in forests whereas 18 mosquito species were exclusively present in non-forest areas. Twenty-five mosquito species were generalists present in both forest and non-forest areas (Table 2).

Table 2. Classification of mosquitoes on the basis of their breeding site preference

Forest specialist	Non-forest specialist	Generalist
An. (An.) gigas	*An. (An.) bengalensis*	*An. (An.) barbirostris*
An. (Cel.) dirus	*An. (An.) crawfordi*	*An. (An.) peditaeniatus*
An. (Cel.) jamesii	*An. (An.) hodgkini*	*An. (An.) sinensis*
An. (Cel.) karwari	*An. (An.) lasteri paraliae*	*An. (Cel.) annularis*
An. (Cel.) minimus	*An. (Cel.) aconitus*	*An. (Cel.) kochi*
An. (Cel.) nivipes	*An. (Cel.) splendidus*	*An. (Cel.) maculatus*
An. (Cel.) philippinensis	*An. (Cel.) varuna*	*Ae. (Adm.) caecus*
An. (Cel.) pseudowillmorei	*Ad.(Ady.) catasticta*	*Ae. (Fin.) albolateralis*
An. (Cel.) tessellatus	*Ae. (Stg.) craggi*	*Ae. (Fin.) albotaeniatus*
An. (Cel.) vagus	*Ar. (Lei.) longipalpis*	*Ae. (Stg.) albolineatus*
Ae. (Adm.) alboscutellatus	*Cx. (Cux.) alienus*	*Ae. (Stg.) albopictus*
Ae. (Fin.) dissimilis	*Cx. (Cux.) quinquefasciatus*	*Ar. (Arm.) durhami*
Ae. (Fin.) harveyi	*Cx. (Cux.) tritaeniorhynchus*	*Ar. (Lei.) digitatus*
Ae. (Fin.) prominens	*Fi. minima*	*Ar. (Lei.) flavus*
Ae. (Stg.) annandalei	*Mi. (Eto.) luzonensis*	*Hz. (Hez.) chandi*
Ae. (Stg.) pseudalbopictus	*Or. albipes*	*Cx. (Cux.) bitaeniorhynchus*
Ar. (Arm.) subalbatus	*Ml. genurostris*	*Cx. (Cux.) fuscocephala*
Cx. (Cux.) gelidus	*Tx. (Tox.) gravelyi*	*Cx. (Cux.) mimulus*
Cx.(Cui.) fragilis		*Cx. (Cux.) pseudovishnui*
Cx. (Cui.) pallidothorax		*Cx. (Cux.) vishnui*
Cx. (Eum.) khazani		*Cx. (Cux.) whitmorei*
Cx. (Lop.) bengalensis		*Cx. (Eum.) malayi*
Cx. (Lop.) minor		*Tr. (Rah:) aranoides*
Cx. (Lut.) halifaxii		*Tr. (Trp.) trasalis*
Mi. (Mim.) intermedia		*Tx.(Tox.) splendens*
Or. anopheloides		
Ml. jacobsoni		
Ur. (Ura.) edwardsi		
Ur. (Ura.) orientalis		

Although mosquitoes were collected from altitudes ranging from 100 to 650 m above mean sea level, most of the species were collected between 300 and 400 m above sea level.

Of the five major zones studied (Fig. 1), three zones (north Lakhimpur, Dhemaji and Jonai) are located on the northern bank of Brahmaputra river and two zones (Dibrugarh and Tinsukia) on its southern bank. Comparison of the mosquito fauna of these areas using Jaccard's similarity coefficient revealed that mosquito fauna of Dhemaji and Jonai areas had maximum similarity (Jaccard's coefficient 0.33, i.e. 33% similarity) whereas Tinsukia and north Lakhimpur areas were least similar (Jaccard's coefficient 0.06). In order to find relationship between these geographical areas on the basis of their mosquito community, UPGMA hierarchical cluster analysis was done (Fig. 2). The analysis showed dichotomy between north Lakhimpur area and rest of the areas, i.e. Dibrugarh, Tinsukia, Jonai and Dhemaji formed one group and north Lakhimpur formed another group. Jonai and Dhemaji were more closely related to each other.

Adult collection

Besides larval collections, 31,420 adult mosquitoes were collected during dusk hours, whole nights (off human and cattle baits) and during daytime (outdoor and indoor resting). These represent 39 spp belonging to 6 genera (Table 3). Information on mosquito species collected for the first time in the study area is summarized in Table 4[14-42].

DISCUSSION

In 1994, Malhotra and Mahanta[8] compiled an updated list of mosquitoes present in the seven sister states of northeast India wherein 79 spp of mosquitoes in 10 genera are reported from Assam. However, in the present survey, a total of 91 taxa of mosquitoes in 15 genera were collected from the study area out of which 19 species of mosquitoes were reported for the first time in Assam (Table 4) including three species reported for the first time from India, viz. *Culex alienus* Colless, *Culex variatus* (Leicester) and *Tripteroides tarsalis* Delfinado and Hodges and seven species recorded for the first time from north-

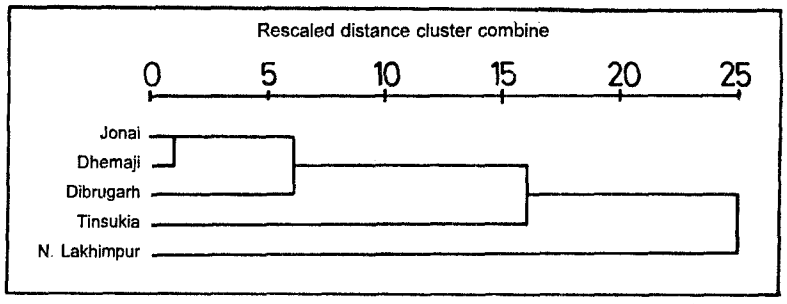

Fig. 2. Dendrogram showing interrelationship between five major zones of the study area based on their mosquito fauna (Based on UPGMA method)

Table 3. Comprehensive list of adult mosquitoes collected during different periods from the study area

Sl. No.	Species	Dusk hour collection	Day time		Whole night			Total	Per-cent-age
			Outdoor resting	Indoor resting	Outdoor human	Indoor human	Outdoor cattle		
1.	*An. (An.) barbirostris*	8	2	0	0	0	5	15	0.05
2.	*An. (An.) hyrcanus s.l.**	902	35	0	30	21	294	1282	4.08
3.	*An. (Cel.) annularis*	91	0	0	4	3	25	123	0.39
4.	*An. (Cel.) dirus*	0	0	0	10	21	11	42	0.13
5.	*An. (Cel.) jeyporiensis**	0	0	0	1	1	0	2	0.006
6.	*An. (Cel.) karwari*	4	0	0	1	0	0	5	0.016
7.	*An. (Cel.) kochi*	21	0	0	0	0	52	73	0.23
8.	*An. (Cel.) maculatus*	1	0	0	0	0	4	5	0.016
9.	*An. (Cel.) philippinensis*	0	20	0	16	8	380	424	1.35
10.	*An. (Cel.) vagus*	153	0	47	4	2	454	660	2.10
11.	*Ae. (Adm.) nigrostriatus**	1	2	0	1	0	0	4	0.013
12.	*Ae. (Adm.) pipersalatus**	0	3	0	0	0	0	3	0.009
13.	*Ae. (Adm.) vexans**	501	0	0	19	23	152	695	2.21
14.	*Ae. (Fin.) pseudotaeniatus**	0	7	0	0	0	0	7	0.02
15.	*Ae. (Neo.) lineatopenis**	8	0	0	0	0	0	8	0.025
16.	*Ae. (Stg.) aegypti**	0	112	0	0	0	0	112	0.36
17.	*Ae. (Stg.) albopictus*	4	0	0	1	2	14	21	0.07
18.	*Ae. (Stg.) mediopunctatus**	0	8	0	0	0	0	8	0.025
19.	*Ae. (Stg.) anandalei*	0	1	0	0	0	0	1	0.003
20.	*Ar. (Arm.) kuchingensis**	138	4	0	4	0	0	146	0.46
21.	*Ar. (Arm.) obturbans**	20	0	0	0	0	0	20	0.064
22.	*Ar. (Arm.) subalbatus*	2	0	0	0	0	0	2	0.006
23.	*Cx. (Cux.) bitaeniorhynchus*	147	2	0	95	55	58	357	1.14
24.	*Cx. (Cux.) fuscocephala*	2686	294	1	29	39	333	3382	10.76
25.	*Cx. (Cux.) gelidus*	250	0	0	15	9	25	299	0.95
26.	*Cx. (Cux.) pseudovishnui*	1710	40	0	257	173	501	2681	8.53
27.	*Cx. (Cux.) quinquefasciatus*	128	3	1	178	120	22	452	1.44

contd...

Table 3. (*contd.*)

Sl. No.	Species	Dusk hour collection	Day time		Whole night			Total	Per-cent-age
			Outdoor resting	Indoor resting	Outdoor human	Indoor human	Outdoor cattle		
28.	*Cx. (Cux.) tritaenior-hynchus*	2676	103	0	58	79	593	3509	11.17
29.	*Cx. (Cux.) vishnui*	2632	147	0	232	199	1263	4473	14.23
30.	*Cx. (Cux.) whitmorei*	438	0	1	16	26	24	505	1.61
31.	*Cx. (Lop.) cinctellus**	0	4	0	0	0	0	4	0.013
32.	*Cx. (Lop.) variatus**	0	4	0	0	0	0	4	0.013
33.	*Cx. (Lop.) bengalensis*	0	2	0	0	0	0	2	0.006
34.	*Cx. (Lut.) fuscanus**	0	1	0	0	0	0	1	0.003
35.	*Cq. crassipes**	0	4	0	0	0	0	2	0.013
36.	*Ma. (Man.) annulifera**	501	5	0	196	140	149	991	3.15
37.	*Ma. (Man.) indiana**	2569	8	0	294	62	138	3071	9.77
38.	*Ma. (Man.) uniformis**	3880	768	13	615	542	1723	7541	24.0
39.	*Ma. (Man.) dives**	318	8	9	17	62	72	486	1.55
	Total	19789	1587	72	2093	1587	6292	31420	100.00

*Immatures not found.

eastern India, viz. *Anopheles hodgkini* Reid, *Anopheles lasteri paraliae* Sandoshan, *Anopheles pseudowillmorei* Theobald, *Aedes pipersalatus* Giles, *Aedes harveyi* Barraud, *Culex khazani* Edwards and *Heizmania chandi* Edwards. Five genera, viz. *Aedeomyia, Ficalbia, Heizmania, Orthopodomyia* and *Uranotaenia* were additions to the checklist of mosquitoes of Assam and three of them, viz. *Aedeomyia, Ficalbia* and *Orthopodomyia* were additions to the mosquito list of northeast India published by Malhotra and Mahanta[8].

Three malaria vectors incriminated from this region, viz. *An. dirus, An. minimus* and *An. philippinensis* were collected from forests in the study area corroborating the fact that malaria transmission in this region is mostly observed in forest fringe areas, hills and foothills having forest terrains. Of the 16 potential vectors of JE incriminated elsewhere in India[43,44], 14 have been collected during the present study. More extensive surveys of the northeast region of India mostly in container habitats would definitely yield much more unreported as well as unknown mosquito

Table 4. Checklist of mosquito taxa recorded for the first time from Assam

Species	Present study		Distribution
	Site (Ecotype)	Habitat (No. of individuals)	
Anopheles (An.) hodgkini	Jairampur (Hilly forest)[14]	Slow flowing stream (1)	India, Malaya
*An. (An.) lasteri paraliae**	Madhupur (Forest fringe)[15,16]	Pond (1)	India, Borneo, Thailand, Malaya
*An. (Cel.) pseudowillmorei**	Telem forest (Plain forest)[17]	Sandy pool (13)	India
*Aedes (Aed.) pipersalatus**	Telem forest (Plain forest)[5,18]	Outdoor resting (3)	India, Cambodia, Srilanka, Thailand, Pakistan
Ae. (Fin.) albotaeniatus	Tarajan (Forest fringe)[5,19]	Split bamboo (4)	India, Malaya, Sumatra, Srilanka
*Ae. (Fin.) harveyi**	Tarajan (Forest fringe)[5,20,21]	Split bamboo (10)	India, China Srilanka, Thailand, Sumatra, Vietnam, Malaya, Taiwan
Ae. (Fin.) prominens	Telem forest (Plain forest)[5]	Tree hole (2)	India, Indo-China, China, Celebes
Ae. (Stg.) pseudalbopictus	Kanku Basti, Bahisilai (Rural area)[5,22,23]	Bamboo stump (8)	India, Burma, Java, Vietnam, Malayasia, Thailand
Armigeres (Lei.) digitatus	Itanagar (Urban area)[24-28]	Bamboo stump (2)	India, Malaya, Java, Sumatra, Thailand Formosa, Philippines
*Culex (Cux.) alienus***	Gowalgaon (Rural area)[29,30]	Irrigation channel (1)	Borneo, Thailand, Singapore
Cx. (Cui.) fragilis	Telem forest (Plain forest)[5,30-32]	Rain water pool (2)	India, Philippines, Srilanka, Thailand, Malaya, Indonesia, New Guinea, Solomon Island, Borneo

contd...

Table 4. (*contd.*)

Species	Present study		Distribution
	Site (Ecotype)	Habitat (No. of individuals)	
*Cx. (Eum.) khazani**	Bahisilai (Rural area)[5,33]	Irrigation channel (3)	India
Cx. (Lop.) bengalensis	Deepa Basti (Rural area)[5,30,34]	Bamboo stump (3), Tree hole (1) Outdoor resting (2)	India, Malaya, Hainan Island, Thailand
Cx. (Lop.) cinctellus	Deepa basti, Balisohi (Rural area)[5,30]	Outdoor resting (4)	India, Malaya, Java, Singapore, Sumatra, Borneo, Hainan Island Srilanka, Ryukyu Retto, Philippines, Thailand
*Cx. (Lop.) variatus***	Balisohi (Rural area), Khowang (Semi urban)[30,34]	Outdoor resting (4)	Malaya, Thailand
Cx. (Lut.) halifaxii	Bahisilai (Rural area)[5,30,35-38]	Mud pool (5)	India, Malaya, Japan Srilanka, Thailand, China, Indonesia, Nepal, Philippines New Guinea, Bismark Archipelago, Solomon Island, USSR (Maritime Province), Australia
*Heizmannia (Hez.) chandi**	Kanku Basti (Rural area), Telem forest, Jamzing (Plains forests)[5]	Bamboo stump (13), Tree hole (2)	India
Mimomyia (Eto.) luzonensis	Itanagar (Urban area)[39-41]	Bamboo stump (1)	Oriental region
*Tripteroides (Trp.) tarsalis***	Kanku Basti, Deepa Basti (Rural area)[42]	Bamboo stump (13)	Malaya

*First records from northeast India; **First record from India.

species due to the excellent mosquitogenic environment and ecological set up of this region.

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A System for Studying Vector Competence of Mosquitoes for Japanese encephalitis Virus

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A method to infect mosquitoes with Japanese encephalitis virus (JEV) and to demonstrate virus transmission after an extrinsic incubation period is described. Using per oral feeding method infection rate as high as 90% could be achieved. Demonstration of transmission of the virus was achieved by allowing the infected mosquitoes to probe a suitable serum medium and testing the probed serum for virus. Both infection and transmission were demonstrated by using insect-bioassay.

Keywords: Japanese encephalitis, JE virus, Mosquito, Vector competence

INTRODUCTION

In order to incriminate a species of mosquito as vector of a given virus, it is essential to determine its competence to support multiplication and successful transmission of the agent. The lack of research in this area of vector competence resulted from the general lack of interest in studying non-vector mosquito species. Furthermore, the knowledge was not thought to be essential to the control of mosquito vectors of medically important arboviral diseases[1]. It is not always possible to achieve a favourable feeding response even when a satisfactory host animal is available[2]. The primary aim of vector competence study is to determine whether or not a particular mosquito species which ingested a blood meal containing Japanese encephalitis virus (JEV) is competent enough to transmit the virus orally by bite (horizontal transmission) after an extrinsic incubation period. The objective of this study was to develop a system for studying vector com-

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petence of mosquitoes by a simple method by which more number of mosquitoes at a time can be screened without employing any vertebrate host in the laboratory. Antigen capture enzyme immuno assay (EIA) and insect-bioassay described for Japanese encephalitis virus detection is employed for this purpose and the better method is suggested. This system has not been reported before.

MATERIALS AND METHODS

Lyophilized JE virus P (20778) strain provided by the National Institute of Virology, Pune was reconstituted as per directions and passaged twice in mice and stored as 20% brain suspensions at –80°C. This 20% mouse brain suspension was serially diluted 10 fold (10^{-3}) with goose blood and used for feeding.

Mosquito species

Culex tritaeniorhynchus mosquitoes were collected from Cuddalore and Madurai (Tamil Nadu) and Alleppey (Kerala) as immatures and reared up to adults.

Method of oral infection

Starved, 3-5 days old females of *Culex tritaeniorhynchus* were allowed to imbibe virus-blood mixture (defibrinated goose blood and JE virus 10^{-3}) sweetened with 1% sucrose soaked in cotton pledgets and kept in one foot mosquito cage. Soon after feeding, six full-fed mosquitoes representing Day 0 and a sample of the feeding suspension from each experiment were frozen for subsequent viral assay. Viraemic blood fed mosquitoes were held for 12-14 days at 29°C. During this period they were fed with glucose and soaked raisins. Mosquitoes fed on normal blood were kept as controls.

Transmission technique

After 12-14 days of incubation, test mosquitoes starved for 16-18 h were allowed to probe/feed on normal animal serum (50 µl) placed in Eppendorf tubes (one mosquito per tube). In order to avoid accidental escape of infective mosquitoes all manipulations were carried out inside a specially designed one foot cage placed inside a laminar-flow. After probing/feeding, each mosquito and the corresponding serum specimen were frozen for subsequent viral assay.

Viral assay

Antigen capture enzyme immuno assay (EIA) and insect-bioassay (inoculation into *Toxorhynchites splendens* larva and identification by Indirect immunofluorescence assay using JE virus-specific monoclonal antibody) were carried out as described before[3].

Statistical analysis

The infection rate (percentage of total mosquitoes that become infected) and transmission rate (percentage of infected mosquitoes that transmitted virus to the serum) were

calculated[4]. Chi-square values were calculated using the Epi Info software package (Centre for Diseases Control, Atlanta, GA, USA).

RESULTS AND DISCUSSION

Three experiments were carried out with 10^{-3} dilution of JEV (Table 1). Infection rates by EIA ranged from 63-79% and transmission rates ranged from 0-15.8%. Infection rates in two experiments by insect-bioassay was 71 and 96% whereas transmission rates in three experiments ranged from 32-74%. Chi-square test showed that both infection rates by EIA and insect-bioassay were comparable. But, transmission rates were significantly higher by insect-bioasay in two experiments and comparable in one, which indicated that insect-bioassay was more sensitive than EIA for transmission studies. This may be due to fact that in

Table 1. Vector competence of *Culex tritaeniorhynchus* for JE virus

Species	Infection						χ^2
	EIA			Insect-bioassay			
	Total no. tested	No. infected	Infection rate (%)	Total no. tested	No. infected	Infection rate (%)	
Cx. tritaeniorhynchus (Cuddalore)	26	17	65.4	Not done		Not done	
Cx. tritaeniorhynchus (Madurai)	24	19	79.0	24	23 ($p > 0.05$)	95.8	1.71
Cx. tritaeniorhynchus (Alleppey)	33	21	63.6	14*	10 ($p > 0.05$)	71.4	0.03
Species	Transmission						χ^2
	EIA			Insect-bioassay			
	Total no. tested	No. transmitted	Transmission rate (%)	Total no. tested	No. transmitted	Transmission rate (%)	
Cx. tritaeniorhynchus (Cuddalore)	19	3	15.8	19	6 ($p > 0.05$)	31.6	0.58
Cx. tritaeniorhynchus (Madurai)	24	1	4.3	23	17 ($p < 0.05$)	73.9**	21.32
Cx. tritaeniorhynchus (Alleppey)	14	0	0.0	14	5 ($p < 0.05$)	35.7**	3.90

EIA — Antigen capture enzyme immunoassay; *19 dead; **Significant at 5% level (EIA and Insect-bioassay).

infection experiments the total virus content in the whole mosquito is tested by EIA but in transmission studies only a small quantity of viable virus excreted[5] in the saliva to the medium is tested. In bioassay, the initial virus content multiplies in the mosquitoes. Thus insect-bioassay can be used uniformly to find out infection and transmission rates.

For infection experiments viraemic vertebrates hosts like chicks[6-9], pigeon[9], ducklings, white leghorn chicks[10], pigs[5], hamsters[11,12] were employed. Peroral feeding was carried out by different methods like membrane feeding[9,13,14], blood droplet method[15], capillary feeding[12], pledget feeding[4] and also by allowing the mosquitoes to feed on the viraemic suspensions[16]. By the parenteral inoculation method, intracerebral inoculation[17] and intrathoracic inoculation[14] of virus was carried out for infecting the mosquitoes. For transmission of JEV, mosquitoes were allowed to feed on the vertebrate hosts like suckling mice, chicken, duckling[7,8,13] and ardeid birds[18]. Similarly lightly anaesthetized mosquitoes placed in a 12 x 75 mm test tube with 1 ml of BAPS (Bovine albumine phosphate saline) allowed to excrete virus[5], capillary tube method[2,4,12,14], blood droplet method[15] and induced salivation method[19] were employed for the artificial transmission experiments.

The system described here is relatively simple and suitable for testing a large sample size. We could test more mosquitoes simultaneously in transmission experiments without the use of vertebrate hosts. The pledget feeding technique for oral infection has been described before,[20] but the technique using Eppendorf tubes for transmission studies developed by us is the first report. The use of heparin or other anti-coagulants has been found to reduce virus titres[21]. Therefore, we used defibrinated goose blood for preparing blood-virus mixture.

The system described may be useful to study vector competence of other mosquito-virus combinations also.

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Investigation on Malaria Vectors and Mosquito Fauna in South Tripura District, Tripura State

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In an entomological study in South Tripura district of Tripura state during June 1998, a total of 31 species of mosquitoes belonging to eight genera were recorded. Among the known malaria vectors in India, *Anopheles annularis, An. jeyporiensis, An. minimus, An. philippinensis/ nivipes* and *An. varuna* were collected but none of them could be incriminated. During the survey, *Aedes pallidostriatus* and *Malaya genurostris* were recorded for the first time in the north-eastern region and Tripura state respectively. Presence of host seeking *An. minimus* in light-trap collections indoors and its absence during morning indoor resting collections indicated the probability of some degree of exophilic behaviour of *An. minimus* population in Tripura.

Keywords: Malaria vectors, Mosquito fauna, Tripura state

INTRODUCTION

Tripura is a small constituent state of the north-eastern region of India. It is situated between 22°35' to 22°95'N and 91°10' to 92°20'E and is surrounded by Bangladesh from three sides except for a narrow neck in the northeast direction where it borders Assam and Mizoram states. The terrain of the state is hilly with undulating plains. Malaria is a major mosquito-borne disease in Tripura with annual parasite incidence averaging 3.1/1000 during 1991-95 and over 75% infection caused by *Plasmodium falciparum*[1]. However, the existing knowledge on the abundance of malaria vectors and

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mosquito fauna in Tripura is scanty and largely based on the works of Misra and Dhar[2] and Das *et al.*[3] The entomological information needs to be updated considering the possible influence of rapidly changing ecological conditions such as deforestation, population movement, developmental activities etc. on mosquito distribution and vector bionomics. In view of this, the present study was carried out in South Tripura district of Tripura state to update the existing entomological information on mosquito abundance, malaria vectors and their role in malaria transmission.

MATERIALS AND METHODS

Study area

The study was carried out during the month of June 1998 in Amarpur and Udaipur subdivisions of South Tripura district. Mosquito collections were made from three villages of Amarpur sub-division, viz. Chandipara (in Amarpur notified area), Govindtila and Nripendranagar and two villages, viz. Matarbari and Phulkumari of Udaipur subdivision. The study villages were compact and inhabited mainly by Bengalis, and were located approximately one km away from the dense forest.

Entomological techniques

Collections of adult mosquitoes: Adult mosquitoes were collected from human dwellings and cattlesheds in all the villages with the help of battery operated CDC miniature light-traps between 1800 and 0500 hrs. A total of 12 traps were operated, six in human dwellings and six in cattlesheds, during the study. Indoor resting mosquitoes were collected with the help of suction tubes and flashlights in the morning between 0500 and 0800 hrs from both human dwellings (total 330 min collection in 35 houses) and cattlesheds (total 165 min collection in 18 cattlesheds) in the villages Chandipara, Govindtila and Nripendranagar. Outdoor day resting mosquitoes were collected between 0630 and 0730 hrs by a drop net (total five attempts) from ground level vegetation and small bushes surrounding human dwellings and cattlesheds in the village Chandipara. Mosquitoes were also collected during dusk (1800-1930 hrs) in and around cattlesheds (total 75 min collection) in Govindtila and Nripendrangar villages.

Collections of mosquito immatures: Mosquito immatures were collected from different breeding habitats in and around all study villages with the help of dippers, ladles, pipettes etc. Immatures collected were link-reared individually in plastic photo vials. The adults emerged were identified using the standard mosquito identification keys.

Mosquito dissections: Adult mosquitoes collected by different methods were brought to the camp laboratory and identified using the standard keys. The salivary glands and midguts of the suitable specimens of the malaria vectors, viz. *An. minimus, An. philippinensis/nivipes, An. jeyporiensis* and *An. varuna* were dissected for the presence of

sporozoites and oocysts respectively.

RESULTS

Details on mosquitoes collected by different methods in South Tripura district during the study are given in Table 1. A total of 2206 adult mosquitoes and 346 immatures of mosquitoes were collected during the study. The fauna consisted of 31 species belonging to eight genera which included three *Aedes* spp, 13 *Anopheles* spp, one *Armigeres* sp, eight *Culex* spp, one *Coquillettidia* sp, one *Malaya* spp, three *Mansonia* spp and one *Uranotaenia* sp. Adult collections yielded 29 species of mosquitoes belonging to seven genera whereas 18 species belonging to five genera emerged from collections of immatures.

A total of 1960 mosquitoes belonging to 27 species and seven genera were collected in 12 trap-nights of collections with a mean density of 163.3 mosquitoes per trap per night (Table 2). Total density and numbers of mosquitoes species collected from cattlesheds (26 species, seven genera, density 299.2 mosquitoes per trap per night) were over ten times higher than of those collected in human dwellings (14 species, four genera, density 27.5 mosquitoes per trap per night). Overall, the proportions of anopheline and culicine mosquitoes in the light-trap collections were 31.2 and 68.8% respectively. *An. minimus*, the well-known vector of malaria in the north-eastern region, constituted about 10% of the anophelines and 3.2% of the total mosquitoes collected in light-traps.

In indoor day resting collections, the eight species of mosquitoes belonging to three genera were collected (Table 2) and their per man hour densities (PMHD) were 10.4 in human dwellings (six species) and 16.4 in cattlesheds (eight species). *Culex quinquefasciatus* (PMHD 4.5) and *An. vagus* (PMHD 3.8) were the most common indoor resting mosquitoes in human dwellings in comparison to *An. barbirostris* (PMHD 5.5) and *An. subpictus* (PMHD 3.6) in the cattlesheds.

Outdoor day resting collections yielded a total of 123 mosquitoes (9 males and 114 females) of five species belonging to four genera (Table 1) with the predominance of *Ar. subalbatus* (94.3%). Interestingly, in day resting collections, both indoors and outdoors, not a single *An. minimus* could be collected.

In dusk collections, eight mosquito species belonging to four genera were captured (Table 1). *Cx. pseudovishnui* (42.9%), *Cx. fuscocephala* (14.3%) and *Ar. subalbatus* (19.0%) were predominant in these collections.

Streams, fish rearing ponds and large village ponds (called *Dighi* locally) were the perennial mosquito breeding habitats, whereas ditches, drains, paddy fields, tree holes and ground pools were the temporary breeding habitats in and around the villages. From

Table 1. Mosquitoes collected in South Tripura district, Tripura State

Species	Adults collection (%)						Emergence from collection of immatures (%)							
	Light-traps		Day resting			Dusk collect-ions	Ditches	Ponds	Drains	Paddy fields	Fish rearing ponds	Streams	Tree holes	Leaf axils
	HD	CS	Indoors		Outdoors									
			HD	CS										
Aedes albopictus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40.0	0.0
Ae. sp*	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Ae. pallidostriatus***	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anopheles aconitus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
An. annularis	0.0	0.0	0.0	4.4	0.0	0.0	5.4	23.3	0.0	0.0	0.0	0.0	0.0	0.0
An. barbirostris	1.8	8.5	10.5	33.3	0.0	4.8	39.3	45.0	0.0	0.0	25.6	0.0	0.0	0.0
An. hyrcanus gr	0.0	0.2	1.8	4.4	0.0	0.0	32.1	0.0	76.0	18.6	60.5	23.5	0.0	0.0
An. jeyporiensis	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
An. karwari	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
An. kochi	0.0	4.5	0.0	0.0	0.0	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
An. minimus	1.2	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	76.5	0.0	0.0
An. philippinensis/ nivipes	0.6	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	32.4	0.0	0.0	0.0	0.0
An. pseudojamesi	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
An. subpictus	0.0	0.0	3.5	22.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
An. vagus	1.8	0.9	36.8	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
An. varuna	0.6	1.3	0.0	0.0	0.8	0.0	0.0	13.3	0.0	0.0	0.0	0.0	0.0	0.0
Armigeres subal-batus	40.6	5.3	0.0	2.2	94.3	19.0	0.0	0.0	0.0	0.0	0.0	0.0	60.0	0.0

contd...

Table 1. (*contd.*)

Species	Adults collection (%)						Emergence from collection of immatures (%)							
	Light-traps		Day resting			Dusk collect-ions	Ditches	Ponds	Drains	Paddy fields	Fish rearing ponds	Streams	Tree holes	Leaf axils
	HD	CS	Indoors		Outdoors									
			HD	CS										
Culex bitaenior-hynchus	1.2	10.6	0.0	0.0	0.0	4.8	0.0	0.0	0.0	17.6	0.0	0.0	0.0	0.0
Cx. fuscocephala	3.0	16.7	0.0	0.0	2.4	14.3	0.0	0.0	0.0	12.7	13.9	0.0	0.0	0.0
Cx. gelidus	1.8	0.6	0.0	0.0	0.0	0.0	23.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cx. fuscanus	0.0	0.1	0.0	0.0	0.0	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0
Cx. pseudovishnui	27.9	26.5	3.5	17.8	0.0	42.9	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
Cx. quinque-fasciatus	14.5	0.1	43.9	11.1	0.0	0.0	0.0	0.0	24.0	0.0	0.0	0.0	0.0	0.0
Cx. tritaenior-hynchus	0.0	1.8	0.0	0.0	0.0	4.8	0.0	0.0	0.0	5.9	0.0	0.0	0.0	0.0
Cx. vishnui	0.0	1.4	0.0	0.0	0.8	0.0	0.0	0.0	0.0	12.7	0.0	0.0	0.0	0.0
Coquillettidia crassipes	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
*Malaya genuro-stris****	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100
Mansonia annulifera	2.4	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ma. dives	0.6	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ma. uniformis	1.8	0.5	0.0	0.0	1.6	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Uranotaenia sp*	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total mosquitoes	165	1795	57	45	123	21	56	60	50	102	43	17	10	8
Percentage	100	100	100	100	100	100	100	100	100	100	100	100	100	100

*Species could not be identified; **New northeast regional record; *** New Tripura state record; HD — Human dwellings; CS — Cattlesheds.

Table 2. Densities of mosquitoes captured in light-traps and by indoor resting collections in South Tripura district, Tripura state

Species	Density per trap night*			Indoor resting (PMHD)**		
	CS	HD	Total	CS	HD	Total
Aedes sp***	0.3	0.0	0.2	0.0	0.0	0.0
Ae. pallidostriatus	0.7	0.0	0.3	0.0	0.0	0.0
An. annularis	0.0	0.0	0.0	0.7	0.0	0.2
An. barbirostris	25.3	0.5	12.9	5.5	1.1	2.5
An. hyrcanus gr	0.5	0.0	0.3	0.7	0.2	0.4
An. jeyporiensis	3.5	0.0	1.8	0.0	0.0	0.0
An. karwari	10.3	0.0	5.2	0.0	0.0	0.0
An. kochi	13.3	0.0	6.7	0.0	0.0	0.0
An. minimus	10.0	0.3	5.2	0.0	0.0	0.0
An. philippinensis/nivipes	30.0	0.2	15.1	0.0	0.0	0.0
An. pseudojamesi	0.5	0.0	0.3	0.0	0.0	0.0
An. subpictus	0.0	0.0	0.0	3.6	0.4	1.5
An. vagus	2.6	0.0	1.6	0.7	3.8	2.8
An. varuna	4.0	0.0	2.1	0.0	0.0	0.0
Armigeres subalbatus	16.0	11.2	13.6	0.4	0.0	0.1
Culex bitaeniorhynchus	31.8	0.3	16.1	0.0	0.0	0.0
Cx. fuscocephala	49.8	0.8	25.3	0.0	0.0	0.0
Cx. gelidus	1.8	0.5	1.2	0.0	0.0	0.0
Cx. fuscanus	0.2	0.0	0.1	0.0	0.0	0.0
Cx. pseudovishnui	79.3	7.7	43.5	2.9	0.4	1.2
Cx. quinquefasciatus	0.3	4.0	2.2	1.8	4.5	3.6
Cx. tritaeniorhynchus	5.5	0.0	2.8	0.0	0.0	0.0
Cx. vishnui	4.2	0.0	2.1	0.0	0.0	0.0
Coquillettidia crassipes	0.3	0.0	0.2	0.0	0.0	0.0
Mansonia annulifera	0.3	0.7	0.5	0.0	0.0	0.0
Ma. dives	6.7	0.2	3.4	0.0	0.0	0.0
Ma. uniformis	1.5	0.5	1.0	0.0	0.0	0.0
Uranotaenia sp***	0.2	0.0	0.9	0.0	0.0	0.0
Total	299.2	27.5	163.3	16.4	10.4	12.4

* Total 12 trap-night collections were made from six human dwellings and six cattlesheds in five villages; ** Total 330 min collection in 35 human dwellings and 165 collection in 18 cattlesheds in three villages; *** Species could not be identified; HD — Human dwellings; CS — Cattlesheds; PMHD — Per man hour density.

mosquito immatures collected from these habitats, 17 species of mosquitoes emerged (Table 1). *An. barbirostris* and *An. hyrcanus* group mosquitoes emerged from all perennial breeding habitats surveyed. *An. minimus* was found breeding in streams only.

An. annularis, An. jeyporiensis, An. minimus, An. philippinensis/nivipes and *An. varuna* were the known malaria vectors collected in the study. Salivary glands and midguts of *An. minimus* (n=53), *An. philippinenesis/nivipes* (n=33), *An. jeyporiensis* (n=8) and *An. varuna* (n=6) were dissected, however, none was found positive for sporozoites or oocysts.

In the present study two mosquito species, viz. *Ae. (Aedimorphus) pallidostriatus* and *Malaya (Maorigoeldia) genurostris* were recorded for the first time in Tripura state. Occurrence of *Ae. pallidostriatus* has not been reported earlier from the north-eastern region, thus, constituting the new northeast regional record. Four adults of *Aedes pallidostriatus* (one male and three females) were captured in the light-trap from a cattleshed in the village Matarbari in Udaipur subdivision. In the north-eastern region *Malaya genurostris* has been reported from Arunachal Pradesh (District Tirap), Assam (Districts Dibrugarh and Tezpur) and Mizoram (District Aizawl) but not from Tripura[4]. This makes a new state record from Tripura. Immatures of this mosquito were collected from the leaf axil of *Colocasia* (Kochu/Arbi) plant in village Matarbari in Udaipur sub-division.

DISCUSSION

Present study recorded 31 species of mosquitoes belonging to eight genera, including one new regional and one new state record in South Tripura district. Earlier, 33 species of mosquitoes (six genera) were reported from Maharani PHC area of South Tripura district[3]. *Malaya* and *Uranotaenia* were the two additional genera recorded in our study over that of Das *et al.*[3] The species of *Uranotaenia* could not be identified in the present study as the only adult specimen collected was damaged for proper species identification.

The north-eastern region is under the influence of three major malaria vectors, viz. *An. minimus, An. dirus* and *An. fluviatilis*[5]. Amongst these, in Tripura only *An. minimus* was incriminated for malaria sporozoites[2] way back in 1954. We collected this species in good numbers but it was not incriminated. It is noteworthy that not a single mosquito of *An. minimus* could be found in human dwellings or cattlesheds during day resting collections whereas light-trap catches demonstrated night activity of this mosquito indoors. This indicated the possibility of exophily in *An. minimus* in the study area. However, our limited outdoor day resting collections failed to collect *An. minimus* from bushes and ground level vegetations. Though *An. minimus* is considered primarily endophilic in the north-eastern region[5,6], suspicion on circumstantial evidence about some degree of exophily in this mosquito in Assam has been raised[7,8]. Therefore, this aspect gains im-

portance in view of its relevance to malaria transmission and vector control and needs confirmation by further studies. *An. dirus*[3] and *An. fluviatilis*[2,9], though recorded earlier in Tripura state, could not be collected in the present study.

An. philippinensis/nivipes was collected in good numbers in the present study. This species is found abundantly in the north-eastern region and was incriminated as a malaria vector in Burnihat area of Meghalaya[10]. *An. philippinensis* is also a vector in the plains of the neighbouring country Bangladesh[11] and it is probable that it might be playing some role in malaria transmission in Tripura also. *An. philippinensis* resembles very closely with *An. nivipes* and it was opined by Nagpal and Sharma[12] that in the north-eastern region *An. philippinensis* was in fact *An. nivipes*. Distribution of *An. philippinensis* and/or *An. nivipes* and elucidation of its vectorial status in Tripura need further study.

Tripura has largely remained unexplored as far as its mosquito fauna and malaria entomology is concerned. Therefore, there is an urgent need for taking up more systematic entomological studies in order to establish the prevalence and distribution of various mosquito species, to incriminate/re-incriminate the malaria vectors and to study their bionomics in space and time. This will help in understanding the dynamics of malaria transmission in Tripura in a better way and to formulate appropriate vector control strategies.

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Trial with ParaSight-F in the Detection of *Plasmodium falciparum* Infection in Chennai (Tamil Nadu), India

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Efficacy of *Plasmodium falciparum* histidine-rich protein (HRP-II) based diagnostic test ParaSight-F, was evaluated for diagnosis of *P. falciparum* malaria at the Malaria Clinic in Malaria Research Centre (Field Station), Chennai, Tamil Nadu. A total of 93 febrile patients were screened in parallel by microscopy and by ParaSight-F. The sensitivity and specificity of the test were 100% for the detection of *P. falciparum* infection.

Keywords: Diagnosis, Malaria, *P. falciparum*, ParaSight-F

INTRODUCTION

P. falciparum constitutes about 40% of the total 2.5 million presently reported malaria cases in the country[1]. *P. falciparum* infection can produce all sorts of complications like cerebral malaria, anaemia and even death particularly among pregnant women, children and non-immune population[2]. The rising trend of *P. falciparum* foci in many tribal belts and forested regions of the country[3] is alarming and cause of concern for public health experts.

The current WHO Global Malaria Control Strategies emphasize early case detection and treatment and use of selective vector control measures. In this context, there is an urgent need to improve early diagnosis, treatment, case management and application of new diagnostic tools in order to reduce the avoidable mortality from *P. falciparum*. We have

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evaluated, dipstick antigen-capture assay ParaSight-F (Becton Dickinson, Sparks, MD) for *P. falciparum* detection at a Malaria Clinic in Chennai (Tamil Nadu) during the months of September to November 1996. Results of this study are reported in this paper.

MATERIALS AND METHODS

Malaria transmission in Chennai is perennial and peak transmission of *P. falciparum* take place during August to January. All *P. falciparum* cases are treated with 1500 mg of Chloroquine (600 mg Day 0, 600 mg Day 1 and 300 mg Day 2) and single dose of 45 mg of primaquine. RI and RII levels of chloroquine resistance in *P. falciparum* have also been reported in Chennai city[4,5]. The efficacy of the ParaSight-F in detection of *P. falciparum* infection was evaluated in the Malaria Clinic at Malaria Research Centre premises located at the north-western part of Chennai. The patients attending the clinic were examined on reporting, and history of fever, symptoms and drugs taken, if any, were recorded. Thick and thin blood smears from patients were prepared by finger prick, stained with JSB stain[6] and examined for the presence of malaria parasite under oil immersion lens. Simultaneously, blood from these patients were also subjected to dipstick diagnostic test procedure[7]. The diagnosis of *P. falciparum* by this method is based on antigen capture dipstick using a monoclonal antibody to the *P. falciparum* histidine-rich protein (HRP-11)[8,9]. The antigen/antibody reaction is revealed by the addition of detector reagent. A solid pink line on the test strip indicates a positive test results against a thin pink line in negative patient. To check the sensitivity of the test kit, density of asexual/sexual parasitic stages in all *P. falciparum* positive smears were recorded against 1000 WBC[10]. All negative smears were rechecked by another microscopist.

RESULTS AND DISCUSSION

Both conventional microscopic examination and ParaSight-F test were carried out on ninety-three febrile patients. Under microscopic examination, 65 smears were found positive for malarial parasites, of which 45 and 20 smears were positive for *P. falciparum* and *P. vivax* infections respectively. Interestingly, 45 test results obtained were positive for *P. falciparum* by ParaSight-F test thereby indicating 100% efficiency detection of *P. falciparum* cases.

Parasite density of all *P. falciparum* cases were recorded. It was found less than 100/µl in three; between 100-1000/µl in eight and more than 1000/µl in 34 patients. All microscopically identified *P. falciparum* cases including those three with less than 100/µl parasite density were detected positive by ParaSight-F test indicating 100% sensitivity of the test. All *P. vivax* cases were detected as negative. Although lesser sensitivity of this test has been reported[11,12] in samples with lower parasitaemia, in the present study three patients with low parasitaemia (<100/

μl) were also detected by this test.

ParaSight-F is rapid, simple, very sensitive and does not need any trained personnel. The dipstick can be stored at room temperature between 2-37°C and will be extremely useful in *P. falciparum* containment programmes in inaccessible areas. However, since both *P. vivax* and *P. falciparum* are present in almost all malarious areas a diagnostic kit for detection of both *P. vivax* and *P. falciparum* will be more useful.

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Distribution and Feeding Efficacy of Larvivorous Fishes of Goa

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Fifteen species of larvivorous fishes, viz. *Aplocheilus blockii, Aplocheilus lineatus, Rasbora daniconius, Puntius vittatus, Puntius melanampyx pradhani, Puntius amphibius, Macropodus cupanus, Danio aequipinnatus, Oreochromis mossambicus, Heteropneustes* sp, *Therapon jarbua, Etroplus suratensis, Mugil cephalus, Osteochilichthys nashii* and *Hypselobarbus dobsoni* were collected from different parts of Goa. Their habitat wise distribution was studied and density was recorded. Daily average consumption of mosquito larvae varied between 62.5 and 736 per fish. On the basis of their larvivoracity and extensive distribution, *Aplocheilus blockii* and *Rasbora daniconius* are recommended for use in malaria control operations in Goa.

Keywords: Goa, Larvivorous fish, Malaria control

INTRODUCTION

The state of Goa has witnessed resurgence of malaria since 1986 with its capital city Panaji as epicentre. Since then many other coastal areas of Goa have also experienced malaria outbreaks that have been linked with accelerated construction activities. To combat malaria transmission, indigenous larvivorous fishes, viz. *Aplocheilus blockii* and *Rasbora daniconius* formed the most important component of the bioenvironmental strategy of malaria control implemented in Panaji from 1989-1992[1] and in the malaria endemic villages of the Candolim Primary Health Centre from 1994-1995 (unpublished data). The useful role played by these larvivorous fishes has resulted in their persistent demand from different parts of Goa for vector control in the wells, masonry tanks, fountains, etc.

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Lloyd[2] and Choudhuri[3] are considered as pioneers in postulating mosquito control by different indigenous fish species in India. Fishes have been used as mosquito bio-control agents in India as early as in 1902 in Bombay[4]. *Poecilia reticulatus* (guppy) and *Gambusia affinis* (mosquito fish) were introduced in India from South America and Italy in 1908 and 1928 respectively and since then they have been extensively used for malaria control in many parts of India. In 1938, Hora and Mukerji[5] published a book on the probable larvivorous fishes of India. The interest in larvivorous fishes, however, waned at the arrival of DDT as a magic chemical tool in the mosquito control operations with collateral benefits against other vectors and nuisance insects. However, resurgence of malaria during mid-1970s, mainly due to the development of resistance to DDT and later on to other insecticides in mosquitoes, has rekindled interest in exploring the potential of indigenous larvivorous fishes in India[6]. Although larvivorous fishes were earlier being used primarily in urban malaria control in India, for the first time, they were extensively used for rural malaria control as a major component of bioenvironmental strategy from 1983 in Nadiad in Kheda district of Gujarat[7]. Since then, many field studies on many indigenous and exotic larvivorous fishes have been carried out in different parts of India with encouraging results[6].

In Goa, except for some preliminary studies by De Mello and Noronha[8], and Noronha[9], no systematic survey has been carried out on the distribution of larvivorous fishes, their larvivoracity and availability in different habitats. Thus due to the gradual spread of malaria along the coast in the recent years a need was felt for an extensive survey of larvivorous fish fauna of Goa that could yield information on the distribution of promising species suitable for malaria control operations in the state. The present study was undertaken from May to August 1996.

MATERIALS AND METHODS

The study area included all the talukas (District sub-divisions) of the north and south Goa districts. Five to six villages representing common physico-geographical features of the study areas were selected from each Taluka for the survey of larvivorous fishes. All the possible natural habitats, viz. backwaters, lakes, streams, ponds, paddy fields, swamps and drains were surveyed for the presence of fishes. Since larvivorous fishes are generally surface and column feeders, the survey was mostly restricted to such species. However, the bottom feeder species in shallow water bodies, where they could be easily observed were also collected.

The densities of each fish species was graded into four categories based on their comparative abundance in various habitats, viz. + = low density, ++ = moderate density, +++ = high density, and ++++ = very high density.

Samples of fishes were collected using hand nets of 45 x 45 x 100 cm size and brought to the laboratory in 100 litre plastic buckets for identification and also for assessing their

larvivoracity. Unknown fishes were given code numbers for later reference. Fish specimens were preserved in 5% formalin and identified using the Key's of Hora and Mukherji[5]; and Menon[10,11]. For the confirmation of identification fish samples were sent wrapped in 5% formalin soaked cotton in plastic airtight boxes to Zoological Survey of India, Chennai.

For assessing the larvivorous efficacy of fishes in laboratory glass aquaria of 45 x 22 x 15 cm were used. The tanks were half filled with water and after starving test fishes for 24 h, five fishes were introduced in the tank. They were offered counted numbers (n=5000) of III instar *Culex quinquefasciatus* larvae. After 24 h the remaining larvae in the tanks were recovered and counted. The experiment was repeated for five days in three replicates. At the end of the experiment, average number of larvae consumed/fish/day was calculated.

For the convenience of comprehensive presentation of the data, the distribution of larvivorous fishes has been presented separately for the coastal talukas and sub-coastal talukas of Goa.

RESULTS AND DISCUSSION

Distribution

The Taluka-wise distribution of the larvivorous fish species in different habitats are given in Tables 1 to 3. Fifteen species were collected from all over Goa, viz. *Aplocheilus blockii, Aplocheilus lineatus, Rasbora daniconius, Puntius vittatus, Puntius melanampyx pradhani, Puntius amphibius, Macropodus cupanus, Danio aequipinnatus, Oreochromis mossambicus, Heteropneustes* sp, *Therapon jarbua, Etroplus suratensis, Mugil cephalus, Osteochilichthys nashii* and *Hypselobarbus dobsoni.* Among these species only *Aplocheilus lineatus* and *Rasbora daniconius* were found all over Goa. *Aplocheilus blockii* species was widely distributed except in Sanguem taluka where it could not be found. These fishes were observed in moderate to high densities in Pernem, Bardez, Tiswadi, Ponda, Mormugoa and Salcete talukas in different habitats from where they could be conveniently collected and used in malaria control operations (Tables 1 and 2). It's allied species *Aplocheilus lineatus* was present in low to moderate densities in Salcete, Ponda, Quepem and Sanguem. This species was detected in high densities only in the paddy fields of Tiswadi taluka. In the remaining parts of Goa, the species was found in low densities and may not be available for mass application. On the other hand, the densities of *Rasbora daniconius* were sufficiently high throughout Goa to make it a candidate larvivorous fish in conjunction with *Aplocheilus blockii. Puntius vittatus* fishes have limitation as they were observed in good densities only in Tiswadi and Salcete talukas. Among the remaining fish species *Puntius melanampyx pradhani* were observed in good numbers in Sanguem taluka, and *Danio aequipinnatus* in Sattari, Sanguem and Quepem talukas (Tables 2 and 3). *Oreochromis mossambicus*

Table 1. Habitat wise prevalence of larvivorous fishes in coastal talukas of South Goa

Fish species	Back waters			Lakes	Streams			Ponds			Paddy fields			Swamps	Drains
	A	B	C	C	A	B	C	A	B	C	A	B	C	B	B
Aplocheilus blockii	+	++	+++	+++	++	+	+	+	a	++++	+++	+	a	+++	a
Aplocheilus lineatus	+	a	++	a	+	+	++	+	+	+	+	+	+++	a	a
Rasbora daniconius	a	a	a	++	++	+	++++	+	+	++	a	a	a	a	++
Puntius vittatus	a	a	++	a	a	a	+++	a	+	++	a	+	++	a	a
Puntius amphibius	a	a	a	a	+	a	a	a	a	+	a	a	a	a	a
Macropodus cupanus	a	a	+	a	a	a	+	a	+	+	+	+	a	a	a
Danio aequipinnatus	a	a	a	a	a	a	+	a	a	a	a	a	a	a	a
Oreochromis mossambicus	a	a	+++	a	a	a	+	a	a	+	a	a	a	a	a
Heteropneustes sp	a	a	a	a	a	a	+	a	a	+	a	a	a	a	a
Therapon jarbua	a	+	++	a	a	a	a	a	a	a	a	a	a	a	a
Etroplus suratensis	a	+	+	a	a	a	a	a	a	a	a	a	a	a	a
Mugil cephalus	+	++	a	a	a	a	a	a	a	a	a	a	a	a	a

A — Pernem taluka, B — Bardez taluka, C — Tiswadi taluka; +Low density; ++Moderate density; +++High density; ++++Very high density; a — Absent.

although found in good numbers in Tiswadi taluka may not be preferred as only the young fries of this species are larvivorous and the food preference changes from mosquito larvae to algae and other plant matter or detritus as the fish grows[12]. Though *Heteropneustes* species showed the highest larvivoracity, this species is not suitable for large scale anti-larval programme due to its limited distribution, meagre density and difficulty in catching the species being a bottom dweller. None of the other species were

Table 2. Habitat-wise prevalence of larvivorous fishes in coastal talukas of south Goa

Fish species	Back water			Lake		Streams				Ponds				Pady fields				Rivulets
	A	B	D	A	B	A	B	C	D	A	B	C	D	A	B	C	D	B
Aplocheilus blockii	++	++	a	+++	++	a	+++	+	++	+	++++	a	++	++	a	++	++	+++
Aplocheilus lineatus	a	++	a	a	a	a	+	++	+	+	+	a	+	+	a	+	++	a
Rasbora daniconius	a	a	a	++	a	+	+++	++	++	a	+++	++	+++	a	++	a	++	a
Puntius vittatus	a	++	a	a	a	a	a	+	+	a	++++	a	a	a	a	+	+	a
Puntius amphibius	a	a	a	a	a	a	a	+	a	a	a	a	a	a	a	a	a	a
Macropodus cupanus	a	a	a	a	a	a	a	a	a	a	+	a	a	a	a	a	a	a
Danio aequipinnatus	a	a	a	a	a	a	a	+++	++	a	a	a	+	a	a	a	a	a
Oreochromis mossambicus	a	a	a	a	a	a	a	a	a	+	a	a	a	++	a	a	a	a
Heteropneustes sp	a	a	a	a	a	a	a	a	a	a	+	a	a	a	a	a	a	a
Therapon jarbua	a	a	++	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Etroplus suratensis	a	++	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Mugil cephalus	++	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Puntius melanampyx pradhani	a	a	a	a	a	a	a	+	a	a	a	a	a	a	a	a	a	a

A — Marmugao taluka, B — Salcete taluka, C — Quepem taluka; D — Canacona taluka; +Low density; ++Moderate density; +++High density; ++++Very high density; a — Absent.

Table 3. Distribution of larvivorous fishes of sub-coastal talukas of Goa

Fish species	Back water		Lake	Streams				Ponds				Paddy fields		Rivers		Irrigation canal	
	A	C	A	A	B	C	D	A	B	C	D	A	C	B	D	A	B
Aplocheilus blockii	+	+++	a	+	+	+++	a	a	a	+++	a	++	+++	+	a	+	a
Aplocheilus lineatus	+	a	+	+	+	a	++	+	+	++	+	+	+	+	+	+	a
Rasbora daniconius	a	a	+	+++	++	+++	++	+++	++	++	+	++	a	++	+++	a	a
Puntius vittatus	a	a	a	a	a	a	a	+	a	a	a	a	a	a	a	a	a
Puntius amphibius	a	a	a	+	a	a	++	+	a	a	a	a	a	a	+	a	a
Puntius melanampyx pradhani	a	a	a	a	++	+	++	+	++	a	a	a	a	++	+++	a	a
Macropodus cupanus	a	a	a	a	a	++	a	a	a	+	a	a	a	a	a	a	a
Danio aequipinnatus	a	a	a	+	+++	++	++	a	a	a	a	a	a	++	+++	a	+
Osteochilichthys nashii	a	a	a	a	a	a	a	a	a	a	a	a	a	+	a	a	a
Hypselobarbus dobsoni	a	a	a	a	a	a	a	a	a	a	a	a	a	+	a	a	a
Mugil cephalus	++	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

A — Bicholim taluka, B — Sattari taluka, C — Ponda taluka; D — Sanguem taluka; +Low density; ++Moderate density; +++High density; a — Absent.

found in sufficient densities to be useful in antimalarial operations. Interestingly all the talukas had atleast one species of larvivorous fish with high density.

Larvivorous efficacy

Among the thirteen species tested for larvivorous efficacy, *Heteropneustes* species consumed the highest average number of larvae per day (n = 736) and *Etroplus suratensis* the least (n = 62.5). The per day larval consumption of the remaining fishes ranged from 102.6 to 464.3 (Table 4). *Aplocheilus blockii* and *Rasbora daniconius* which are well distributed in Goa consumed 102.6 and 464.3 larvae per day respectively and are fairly efficient bio-control agents. The larvivorous efficacy of *Therapon jarbua* and *Mugil cephalus* on the other hand, could not be determined due to their mortality in laboratory conditions but they have been well-documented as larvivorous from elsewhere in the country[13,14].

Earlier De Mello and Noronha[8] reported four species of larvivorous fishes from Goa, viz. *Aplocheilus panchax* (as *Haplocheilus*

Table 4. Feeding efficacy and water preference of larvivorous fishes of Goa

Fish species	Water preference		Av. size (length x bredth) of the fish species tested (cm)	Daily av. larval consumption/ fish
	Fresh	Brackish		
Aplocheilus blockii	+	+	3.8 x 0.37	102.6
Aplocheilus lineatus	+	+	4.8 x 1.0	225.4
Rasbora daniconius	+	–	6.8 x 1.2	464.3
Puntius vittatus	+	+	3.7 x 0.7	154.4
Puntius amphibius	+	–	7.4 x 1.5	237.4
Puntius melanampyx pradhani	+	–	2.7 x 0.73	164.8
Danio aequipinnatus	+	–	6.1 x 1.8	191.8
Oreochromis mossambicus	+	+	5.9 x 0.7	356.1
Heteropneustis sp	+	–	12.0 x 1.35	736
Therapon jarbua	–	+	*	*
Etroplus suratensis	+	+	3.1 x 1.1	62.5
Mugil cephalus	+	+	*	*
Macropodus cupanus	+	+	3.6 x 1.2	189.4
Osteochilichthys nashii	+	–	6.0 x 1.4	289.8
Hypselobarbus dobsoni	+	–	4.7 x 1.0	270.4

*Fish did not survive in the laboratory conditions; + Preferred; – Not preferred.

panchax), *Rasbora daniconius, Danio* species and *Ophiocephalus punctata* and Noronha[9] reported *Aplocheilus lineatus* (as *Haplocheilus lineatus*). During the present survey we did not come across *O. punctata,* probably due to its bottom dwelling habits. Since not even a single specimen of *A. panchax* was collected during present surveys it is suspected that *A. blockii* was wrongly identified as *A. panchax* by these pervious workers due to apparent morphological similarity. This assumption is further supported by the fact that *Aplocheilus blockii* was originally described as *Haplocheilus panchax* var. *blockii* by Menon[10]. The remaining eleven species are new records from Goa. The present study is likely to be of great value in the bio-control of mosquitoes especially the disease vectors in Goa.

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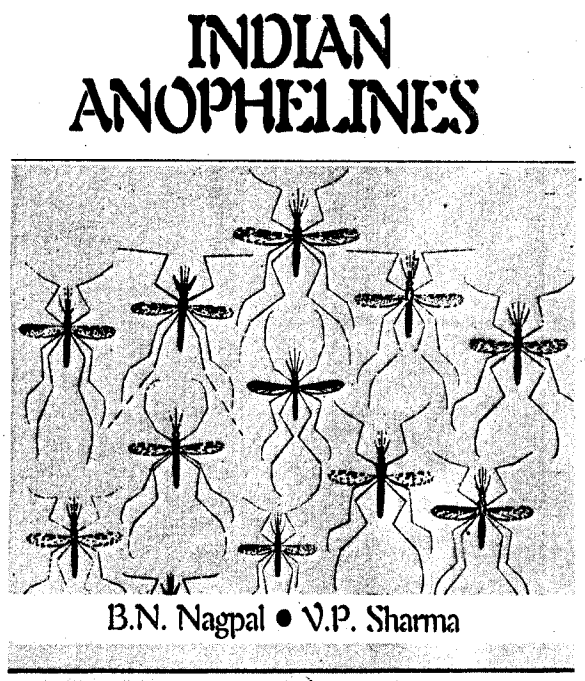

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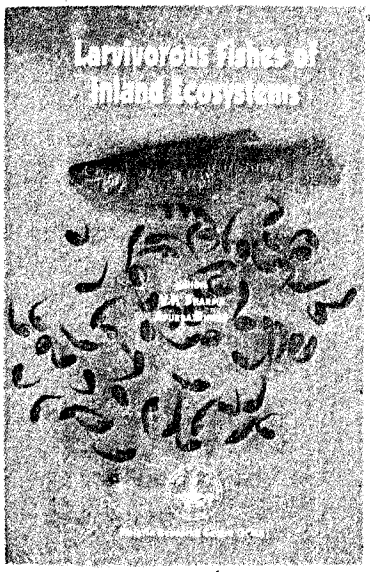

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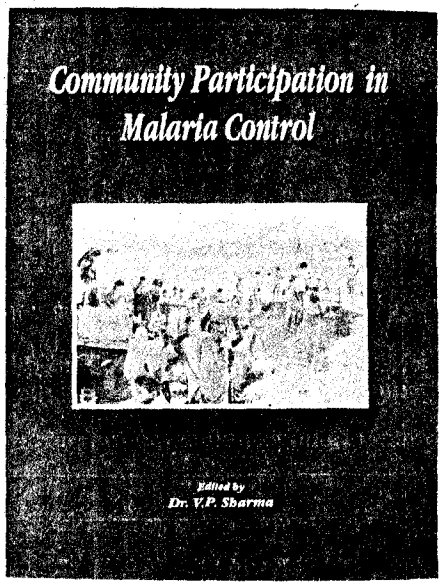

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