

Studies on prevalence of anopheline species and community perception of malaria in Jaffna district, Sri Lanka

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Abstract

Background & objectives: Over two decades of civil unrest and the conflict situation have had detrimental effects on vector control activities and management of malaria in Jaffna district which is an endemic region for malaria in Sri Lanka. With the background that only a few small-scale studies on malaria and its vectors have been reported from this district, a study was designed to explore the current status of malaria in the Jaffna district in relation to vector and community aspects.

Methods: Adults and larvae of anopheline mosquitoes were collected monthly from selected endemic localities. Species prevalence of the collected mosquitoes was studied while the collected adults of *Anopheles subpictus*, a potential vector in the district, was screened for sibling species composition based on morphological characteristics and exposed to common insecticides using WHO bioassay kits. Knowledge, attitude and practices (KAP) of the community were tested using a pre-tested structured questionnaire in high-risk and low-risk localities in the district.

Results: The anopheline mosquito species distribution in the district was—*An. culicifacies* (0.5%), *An. subpictus* (46%), *An. varuna* (4%), *An. nigerrimus* (44%) and *An. pallidus* (5.5%). Among the collected larvae the percent prevalence of *An. culicifacies* was 13% and other species follows as: *An. subpictus* (71%), *An. varuna* (4%), *An. nigerrimus* (10%) and *An. pallidus* (2%). Sibling species B, C and D of *An. subpictus* were present in the district with the predominance of B in both coastal and inland areas, while all members showed both indoor and outdoor resting characteristics, they were highly resistant to DDT (4%) and highly susceptible to malathion (5%). KAP study in the district showed a reasonable level of knowledge, positive attitude and practices towards malaria.

Conclusion: *An. subpictus*, the reported major vector of Jaffna and a well-established secondary vector of malaria in the country, continues to be the predominant anopheline species. The distribution of sibling species of *An. subpictus* complex in the Jaffna district, revealed for the first time, has implications for future studies on its bionomics and malaria transmission pattern in this area and the planning of control strategies for this region. The community perception of disease, which revealed a satisfactory knowledge indicates the potential for better community participation in future malaria control activities in this region. As potential vectors are still present, health authorities need to be vigilant to prevent any future epidemics of malaria.

Key words *Anopheles subpictus* – insecticide resistance – Jaffna – malaria – personal protection – sibling species – Sri Lanka – treatment seeking behaviour

Introduction

Malaria has traditionally been an important public health problem and socioeconomic burden in Sri Lanka. North and eastern provinces, which lie in the dry zone of the country, were the most affected regions in terms of morbidity and mortality due to malaria¹. Over two decades of civil unrest, which has been mainly confined to northern and eastern parts of the country, had greatly affected the socioeconomic conditions of the people in Jaffna district with periodic internal displacements, restricted public movement to other parts of the country and limited medical facilities with inadequate number of health professionals. The conflict situation greatly hampered organized vector control activities and treatment of malaria. Although pyrethroids were introduced in Sri Lanka in 1994^{2,3}, vector control activities in Jaffna district were mainly restricted to indoor residual spraying (IRS) in selected endemic localities with malathion till 2002 and then replaced by pyrethroids [Personal communication with Regional Malaria Officer (RMO), Anti Malaria Campaign (AMC), Jaffna]. The prolonged conflict situation resulted only in few small-scale studies related to the disease, parasite and the vectors of malaria⁴⁻⁶. Results of a study carried out in 1995, during a peak transmission period, revealed that the predominant anopheline mosquito species was *Anopheles subpictus*⁵ (Diptera: Culicidae) and it played the major role in transmitting malaria⁶ compared to *An. culicifacies* which is the major vector of malaria in rest of the country⁷. With this background a study was designed to establish the mosquito species prevalence in the Jaffna district, and the knowledge, attitude and practices of the public towards this disease discussed in the context of the malaria incidence in this locality.

Material & Methods

Study area: This study was carried out from November 2005 to July 2006, in five coastal areas, namely Ampan (09° 72'N: 80° 28'E), Chempianpattu (09°

63'N: 80° 38'E), Kudathanai (09° 75'N: 80° 27'E), Mamunai (09° 66'N: 80° 36'E) and Mankumban (09° 63'N: 79° 93'E), and five inland, areas, namely Kachchai (09° 66'N: 80° 21'E), Tholpuram (09° 76'N: 79° 92'E), Kantharodai (09° 75'N: 80° 01'E), Uduvil (09° 73'N: 80° 01'E) and Vaddukoddai (09° 73'N: 79° 96'E) in Jaffna district (Fig. 1). Selection of the localities was based on the past malaria records. These localities had annual parasite index (API) >100 during the year 2000 (Source: AMC, Jaffna). Entire district is 1032.2 km² in area and having the population of 0.6 million.

Mosquito collection: The study period included the rainy and dry seasons of the district. Adult anopheline mosquitoes were collected from the localities mainly using cattle-baited net (CBN) and cattle-baited hut (CBH) collection techniques. Larval collections were carried out in the same localities during the same period, using standard dipper method. Larvae were collected from small ponds and ditches. Due to logistic reasons (transport and accessibility) adult anopheline mosquito collections were carried out once a month in each locality using CBN. Only a limited adults (using CBH) and larvae collections could

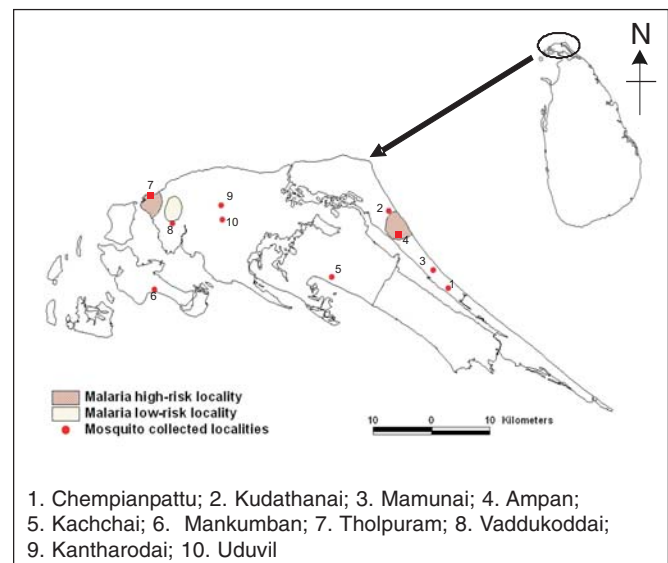


Fig. 1: Study areas in the district where mosquito collections and KAP studies were carried out

be performed. Field collected and reared mosquitoes were identified using standard keys⁸.

Identification of An. subpictus sibling species: Among the identified anopheline mosquitoes blood-fed females of *An. subpictus* were used to study sibling species composition. Individual females after reaching gravid stage were kept in small plastic cups (3.5 cm dimension and 6.5 cm height) with egg-laying surfaces and nylon net on top. From each batch of individual females, 5–10 eggs were examined under the light microscope for diagnostic morphological features (number of egg ridges) for sibling species as outlined by Suguna *et al.*⁹. Identified egg batches were reared as separate families in plastic bowls (4 × 17.5 cm, 750 ml capacity) with well water under similar laboratory conditions. Larvae were fed with finely ground fish meal pellets twice a day. Five to eight III and IV instar larvae of each batch were cross-checked for stage-specific diagnostic morphological features⁹ to ascertain the identification based on egg morphology. The emerged adult mosquitoes of same sibling species were pooled together and used for insecticide susceptibility tests.

Insecticide susceptibility was determined by exposing them to the WHO recommended dosage of DDT (4%) and malathion (5%) using WHO bioassay kit as described by Surendran *et al.*¹⁰.

Study of knowledge, attitude and practices (KAP) towards malaria: API at Grama Niladhari (GN) level was calculated using malaria incidence for the year 2000 at GN level (obtained from AMC, Jaffna) and population data at GN level (obtained from Department of Statistic, Jaffna). GN Division is the lowest administrative level in Sri Lanka. Based on API two high-risk localities (API >100) [Kudaththanai (09° 75' N: 80° 27'E) and Tholpuram-west (09° 76'N: 79° 92'E)] and one low-risk locality (API <10) [Pannakam (09° 77'N: 79° 95'E)] were selected for this study (shaded areas in Fig. 1). A pre-tested, struc-

tured-questionnaire prepared in Tamil (native language) was used in this study during December 2007. The questionnaire was mainly composed of: (a) demographic information such as age, sex and previous history of malaria; (b) knowledge about malaria parasite, clinical signs and symptoms of the disease, vector transmitting malaria, environmental conditions suitable for mosquito breeding and vector control measures; (c) attitude and practices for protection against mosquito bites; and (d) attitude towards treatment seeking behaviour to suspected malaria fever and completing the course of treatment for malaria. Questionnaire was administered to the head of every tenth household, if no male head of household was available a female head of household was interviewed from 0900 to 1300 hrs. The results were analyzed using SPSS (version 12) software.

Results

Anopheline mosquitoes: A total of 890 (824 from CBN and 66 from CBH) adults and 256 larvae of anophelines were collected during the study period. The percent prevalence of each adult species collected was as follows: *An. culicifacies* (0.5%), *An. subpictus* (46%), *An. varuna* (4%), *An. nigerrimus* (44%) and *An. pallidus* (5.5%) (Table 1). Among the larvae collected the percentage prevalence of *An. culicifacies* was 13% and other species follows as: *An. subpictus* (71%), *An. varuna* (4%), *An. nigerimus* (10%) and *An. pallidus* (2%). A difference in species composition in each locality was also observed.

Sibling species of An. subpictus species complex: A total of 409 blood-fed adults of *An. subpictus* were collected during the study period. Considerable number of blood-fed females died during laboratory processing and only 296 females laid eggs were screened for sibling species status. The identified sibling species were B, C and D. The predominant sibling species was B (67%) whilst species C and D constituted as 11 and 22% respectively (Fig. 2).

Table 1. Anopheline mosquito species collected during the study period

Period (Months)	Anopheline species					Total
	<i>An. culicifacies</i>	<i>An. subpictus</i>	<i>An. varuna</i>	<i>An. nigerrimus</i>	<i>An. pallidus</i>	
Nov 2005	0	106	5	45	6	162
Dec	1	171	21	189	22	404
Jan 2006	4	75	10	95	14	198
Feb	0	22	3	16	3	44
Mar	0	18	0	28	0	46
Apr	0	12	1	10	2	25
May	0	3	0	4	0	7
Jun	0	2	0	2	0	4
Total	5	409	40	389	47	890

Only 65 isofemale lines of *An. subpictus* established from adults collected using CBH could be identified to sibling species. Among these 74% were species B, 18% were C and 8% were species D, whereas out of 231 *An. subpictus* isofemale lines established from

mosquitoes collected in the CBN, species B were 65%, species C 8% and D 27% (Table 2).

Susceptibility to common insecticides: Sibling species B, C and D were highly susceptible to 5%

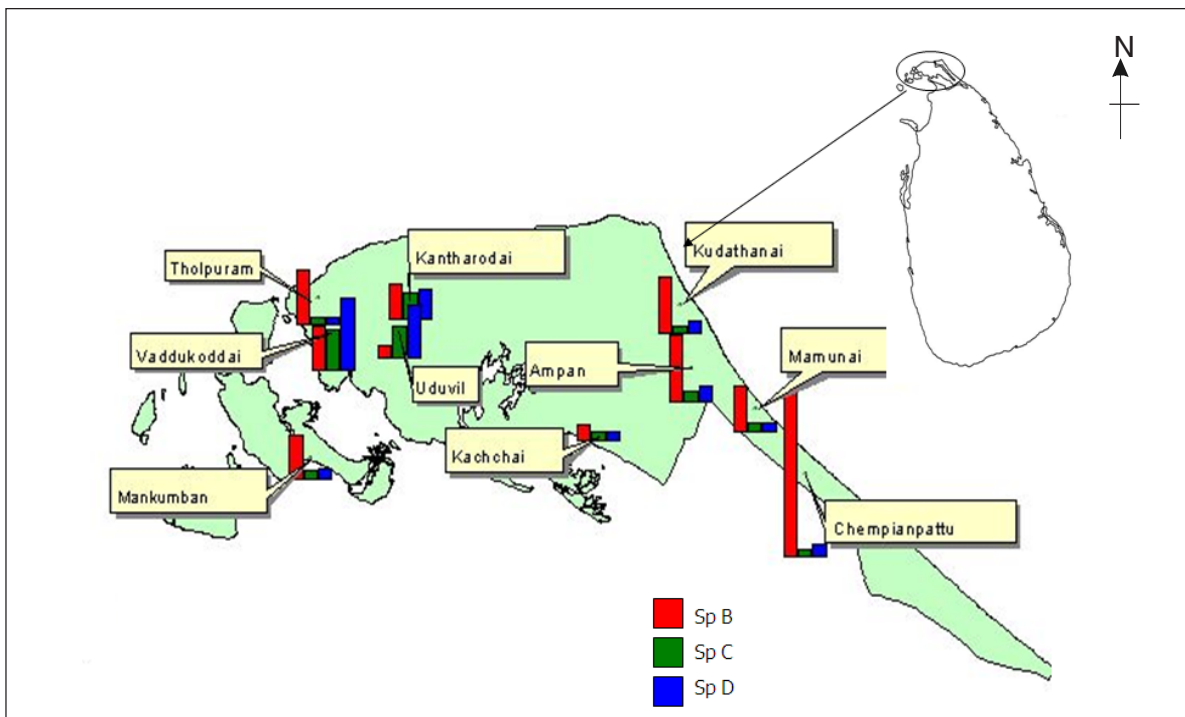


Fig. 2: Prevalence of sibling species in *An. subpictus* species complex in study areas

Table 2. Sibling species composition of *An. subpictus* collected using CBH and CBN techniques

Collection technique	<i>An. subpictus</i> sibling species			Total
	B	C	D	
CBH	48 (74)	12 (18)	5 (8)	65
CBN	151 (65)	20 (8)	60 (27)	231

*Numbers in parentheses denote percentages.

malathion (83–93%), species B and D were highly resistant (86–92%) to 4% DDT. Due to limited number of samples species C could not be exposed to DDT. No significant association was observed between sibling species and resistance to malathion (5%) ($p > 0.05$).

Knowledge, attitude and practices towards malaria (KAP study): The questionnaire was administered to heads of 157 households (106 in the high-risk area and 51 in the low-risk area) representing 10% of the total households in each locality. On an average, 54% of the households in high-risk localities had previous history of malaria and none of them had such a history in the low-risk area. Eighty-nine percent of the households in the high-risk area had only primary education (below Grade 5) compared to 59% in low-risk area.

Knowledge of the respondents on the ability to name the malaria parasite was 9% in the high-risk area and 14% in the low-risk area. Knowledge on mosquitoes as vectors was 95% in the high-risk area and 96% in the low-risk area. Table 3 shows the knowledge on vector breeding sources, the attitude towards mosquito control activities and the personal protection measures practiced by the respondents in high-risk and low-risk area. Fifty-nine percent of respondents in high-risk areas used mosquito nets while 59% in low-risk area used mosquito coils.

Treatment seeking behaviour for suspected malaria fever cases, favoured Government hospitals and at-

Table 3. Respondents' knowledge, attitude and practice towards malaria

Particulars	High-risk area (%)	Low-risk area (%)
<i>Knowledge on actual cause for malaria</i>		
Do not know	9	10
<i>Plasmodium</i>	9	14
Mosquito	82	76
<i>Knowledge on mosquito as vector</i>		
Do not know	5	4
Mosquito	95	96
<i>Knowledge on mosquito breeding</i>		
Do not know	0	4
Bushes and unclean environment	9	26
Water bodies	91	70
<i>Attitude towards mosquito control</i>		
Do nothing	0	4
Cleaning the environment (1)	9	25
Removing the water bodies (2)	67	54
(1) + (2)	24	17
<i>Personal protection measures</i>		
Mosquito coils	13	59
Mosquito nets	59	18
Smoke (traditional fumigants)	19	10
Combination of more than one	9	13

titude towards completing the full course of treatment for malaria was significantly higher among households living in the high-risk area than in the low-risk area ($p < 0.05$). A significant difference ($p < 0.05$) was observed in the knowledge on clinical signs of malaria between high-risk and low-risk areas (Table 4).

Discussion

Jaffna district has traditionally been perceived as an endemic area for malaria. *Plasmodium vivax* is the predominant malarial parasite followed by *P. falciparum* with few mixed infections¹¹. In recent times two peaks in malaria incidences were observed during 1994–95 and 1997–98 periods. However, there was a drastic reduction in malaria incidence in this district with only two cases reported in 2006 and no

Table 4. Respondents' health education and treatment seeking behaviour

Particulars	High-risk area (%)	Low-risk area (%)
<i>Awareness on mobile clinic</i>		
Not conducted	3	41
Conducted	97	59
<i>Knowledge on clinical signs</i>		
Do not know	5	41
Chill, rigors and fever	74	59
Chill, rigors, fever and other signs	21	0
<i>Attitude towards treatment seeking behaviour for suspected malaria</i>		
Pharmacy	0	4
Private hospital	0	84
Government hospital	100	12
<i>Attitude towards completion of the full course of malaria treatment</i>		
Tablets should be taken till the fever stops	3	88
Tablets should be finished	97	12

case was reported during the year 2007. This might be due to the combined result of the control activities carried out by the AMC and the closure of the A-9 highway since August 2006 after the renewed violence. The closure of the highway completely restricted public movement between Jaffna and Vanni mainland which is the most affected region for malaria and thus prevented the importation of malaria. However, malaria prevalence has been very low for the last few years in the country¹¹.

An. culicifacies s.l. is the established major vector of malaria in Sri Lanka⁷. *An. subpictus s.l.* and *An. varuna* are among the reported secondary vectors in Sri Lanka¹². Among the secondary vectors, *An. subpictus* is considered as a potential vector since its role in malaria transmission has been reported from many parts of the country^{12,13}. A study carried out, during a peak transmission period in 1995, indicated the role of *An. subpictus* in the local transmission of

malaria in the district, which was shown to be greater than that of *An. culicifacies*⁶. Another study in the same period showed that *An. subpictus* (56%) as the predominant anopheline species in the district⁵. The present study also indicates *An. subpictus* (46%) as the predominant anopheline species followed by *An. nigerrimus* (44%). *An. culicifacies* density is very much low in comparison with other anopheline species in the district. This trend was seen in previous reports as well^{5,6,14}. Anopheline larvae were collected from small freshwater ponds located close to agriculture fields in inland areas and small ditches in coastal areas. The water that fills in ditches along the coastal area are slaine and not used for human consumption or agricultural purposes. The collection of *An. subpictus* larvae from these ditches indicates their salinity tolerance. Since these breeding sources can be exploited, during rainy season, by vector mosquitoes for their breeding larvicides or larvivorous fish could be introduced into these breeding sources to control the vector species.

The low prevalence of *An. culicifacies* might be attributed to the geography and environmental conditions of the district since this species mainly breeds in rock pools and sand pools along river margin in the dry and intermediate zones of Sri Lanka¹⁵. The absence of natural reverine system in the district might have been a negative impact on the propagation and abundance of this vector species. Even though this species can breed in small water bodies and wells¹⁵, another aspect that might have contributed for the low density of the adult forms is the effectiveness of IRS with pyrethroids. The IRS is considered to be effective against *An. culicifacies* since it is mainly an indoor resting species¹⁶. A detailed study of species composition of anopheline mosquitoes with larger number of samples is indicated in order to gain a better insight into the malaria vector prevalence in this area.

In Sri Lanka, *An. culicifacies* and *An. subpictus* are reported to exist as species complexes. In *An. culicifacies* complex two sibling species B and E¹⁴, and in

An. subpictus complex sibling species A, B, C and D^{17,18} have been reported. The sibling species of *An. subpictus* are reported to show different ecological and biological characteristics which influence susceptibility to parasites and insecticides in Sri Lanka¹⁸, and in India^{19,20}. Although the prevalence of potential malaria vector species E of *An. culicifacies* species complex has been reported from eastern coast of Jaffna district¹⁶, there has been no detailed studies on the prevalence of sibling species of *An. subpictus* from the district. A previous small-scale study which was confined to Vadamarchchi-East, a high endemic coastal locality for malaria in the district, revealed the presence of species B of the *An. subpictus* complex²¹. The present study reveals for first time, the predominant presence of sibling species B in coastal and inland areas along with C and D in the district.

The mosquitoes collected using CBN and CBH are generally interpreted as outdoor and indoor resting respectively²². The indoor and outdoor resting traits are shown by all sibling species and this should be taken into consideration in the application of adulticide as IRS would not be effective against outdoor resting siblings. The present study falls in line with other reports from India²⁰ and Sri Lanka¹⁸ to confirm the predominance of sibling species B in coastal areas. However, species C was reported to be predominant in inland areas of Sri Lanka based on a study reported from northwestern province, which also reported the presence of sporozoites¹⁷. On the contrary, in India species B is incriminated as vector in coastal areas²⁰.

In addition to the resting preference, the role of sibling species of *An. subpictus* in the transmission of malaria in the district is yet to be established—an important aspect that could only be possible during a transmission period or else based on laboratory feeding studies.

In Jaffna district malathion was replaced by pyre-

throids in 2003 (Personal communication with RMO, AMC, Jaffna). The malaria vectors such as *An. culicifacies s.l.* and *An. subpictus s.l.* are reported to be highly resistant to malathion in many parts of the Island^{3,23}. However, the present study reveals that members of *An. subpictus* complex though resistant to DDT are highly susceptible to malathion. This might be due to the less selection pressure owing to irregular spraying attributed by civil unrest. Continuous monitoring on the susceptibility status of pyrethroids is essential since resistant mechanisms for DDT and pyrethroids are more or less similar²⁴. The susceptibility to pyrethroids was not carried out in this study due to the limited sample size of mosquitoes available for testing. A detailed analysis based on resistance data reported from many parts of Sri Lanka (1991–2003) revealed a stratified (coastal and inland) resistance pattern for *An. subpictus s.l.* that could be attributed to the prevalence of sibling species³. A detailed study on the susceptibility and resistance mechanism of sibling species in *An. subpictus* complex is warranted.

At present, in Sri Lanka there is greater emphasis on educating the public to use personal protection measures against mosquito bites as an effective control measure against vector-borne diseases¹. Except a study carried out by Surendran and Kajatheepan²⁴, no reports are available with regard to the knowledge, attitude and practices towards malaria after the health education programme carried out by the AMC during 1998–99 in selected localities (Personal communication with RMO, AMC, Jaffna). The results of the present study indicate that the respondents in the KAP study have substantial level of knowledge on mosquito as vectors of malaria irrespective of their educational level. The historical exposure to malaria for generations may have contributed to this outcome.

It is also interesting that a good proportion of respondents were aware of at least one breeding source of mosquitoes and undertook measures to keep a cleaner environment by removing mosquito breeding sources

(e.g. stagnant water bodies) and environments conducive for mosquito propagation (e.g. bushes and grasses).

The contrasting responses received from households living in high-risk and low-risk areas may be due to their previous experience in contracting the disease and the health education conducted by the health authorities in high-risk areas. Health education and disease burden had positive impacts on knowledge regarding the clinical signs and symptoms, treatment seeking towards Government hospitals and the importance of the complete treatment in high-risk areas irrespective of their educational level. High usage of mosquito coils as the major personal protection measure against mosquito in the low-risk area may be due to its low cost and its availability for purchase at any given time. The high usage of bednets in high-risk area could be associated with free issue of nets by various Non-Governmental Organizations and the health authorities, is an encouraging sign because a study from a village in northcentral Sri Lanka revealed that the use of bednets²⁵ and insecticide-treated bednets²⁶ was strong factor that reduced malaria transmission. Substantial level of knowledge, and encouraging attitudes and practices towards malaria by local community are the factors that could positively be considered by the health authorities in planning future malaria control measures in order to better accommodate the public to play an active part in community-oriented malaria control programmes, in Jaffna district.

Although there is a continuous decline in malaria cases in the country since 2002¹¹, apart from the closure of A-9 highway, the other reasons for a drastic decline in malaria incidence in Jaffna in recent years could be attributed to one or more of the following: early diagnosis (mobile clinics) and prompt and ensured complete treatment (by home visits of volunteers) eliminated the parasite, free issue of bednets, health education to prevent human-vector contact

and vector control measures to reduce vector population (by the activity of the AMC, Jaffna). However, considering the cyclic nature of malaria in Sri Lanka and prevalence of potential vector species in the district, health authorities need to be vigilant to prevent any future epidemics of malaria.

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