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Original article

Prevalence and insecticide susceptibility of dengue vectors in the district of Batticaloa in eastern Sri Lanka

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Abstract: Unprecedented incidences of dengue have been reported in Sri Lanka in recent years. The district of Batticaloa, which was devastated by the 2004 Asian tsunami, is one of the districts affected by dengue. One option to curtail this disease is to implement appropriate vector control measures. A nine-month study was carried out within the Batticaloa Municipal Council limit from April to December 2008. Larval collections were conducted fortnightly using conventional ovitraps for nine months covering the dry and wet seasons. Ovitraps (indoor and outdoor) were placed in 15 randomly selected houses. The collected larvae were brought to the laboratory and reared under laboratory conditions. The larval forms and emerged adults were identified on the basis of reported morphological descriptions. The identified adults of 2–3 d old were exposed to common insecticides following the WHO protocol. During the study period, a total of 10,685 *Aedes aegypti* and *Ae. albopictus* mosquitoes were collected, with the former constituting 57% of the total sample. Both species were collected from indoor and outdoor ovitraps, and their prevalence was recorded throughout the study period. A seasonal shift was observed in the density, with *Ae. aegypti* predominating during the dry season and *Ae. albopictus* during the wet season. Both species were highly resistant to 4% DDT and susceptible to 0.25% permethrin. The continuous presence of potential dengue vectors may have contributed to the dengue prevalence in the district. Since both species can oviposit in indoor and outdoor ovitraps, public awareness and participation should be promoted in the vector control programme of the Ministry of Health along with continuous vector surveillance.
Key words: *Aedes aegypti*, *Ae. albopictus*, dengue, insecticide resistance, ovitrap, vector, Sri Lanka

INTRODUCTION

Dengue remains a major public health problem in Sri Lanka. A dramatic increase in the reported cases of dengue fever (DF) and dengue hemorrhagic fever (DHF) in Sri Lanka has been observed in recent years (Table 1). During the past few years, the characteristics of dengue in terms of clinical manifestation (change in relative proportions of serotypes) and morbidity (high infection rates in children to adults) have changed [1]. In the year 2009, a total of 34896 dengue cases were reported in the country [2]. The country also experienced an epidemic of chikungunya during 2006–2007 [3].

Aedes aegypti Linnaeus and *Ae albopictus* Skase, the established vectors of dengue and chikungunya in many countries [4, 5], are present in Sri Lanka [6]. The eastern coastal district of Batticaloa, which was devastated by the 2004 Asian tsunami and lies in the dry zone of the country, is one of the districts badly affected by dengue fever in recent years (Table 1). Data on the bionomics of dengue vectors in the district are scanty owing to over two decades

Table 1. Reported cases of DF/DHF during the period 2004–2009 (source: Regional Director for Health Services, Batticaloa)

Year	Sri Lanka	Batticaloa District
2004	15463	101
2005	5994	06
2006	11980	63
2007	7314	74
2008	6555	89
2009	34896	660

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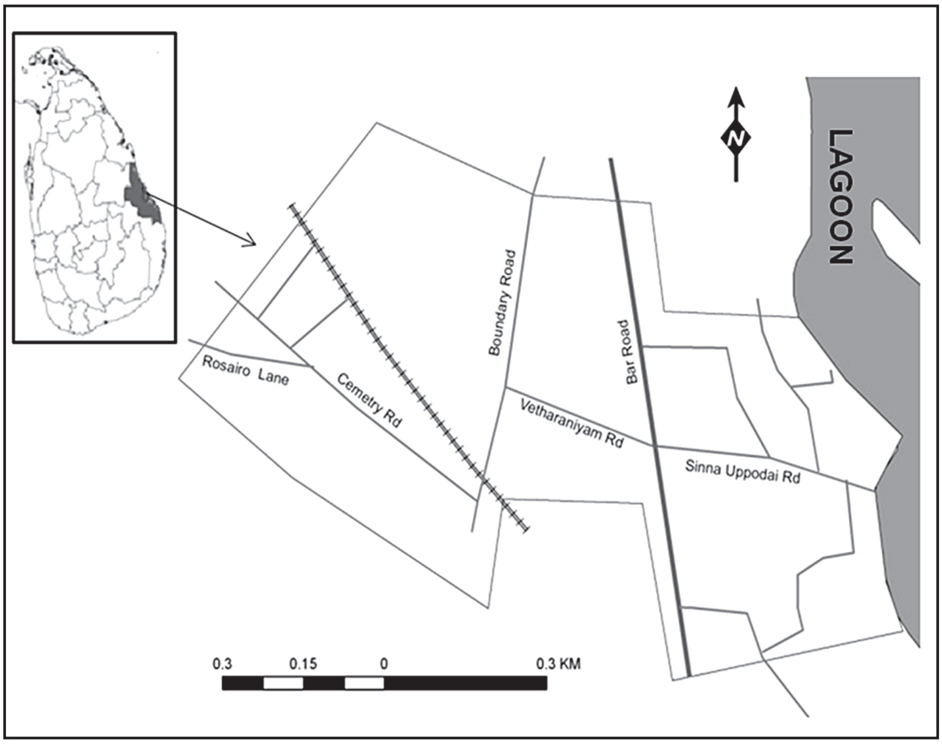

Fig. 1. Map showing the sample collected area in the Batticaloa District

of civil disturbances, which have exerted a great influence on the day-to-day life of the people and the deliveray of health services to them. Knowledge regarding the ecology and biology of vector mosquitoes is essential to devise appropriate control measures to combat dengue in the country. Under the present conducive environment, a study was carried out to assess the prevalence of *Aedes* mosquitoes and their susceptibility to common insecticides and the outcome of this study is expected to be useful for health officers hoping to devise appropriate vector control measures in the district.

MATERIAL AND METHODS

Study site

The dengue-affected Batticaloa Municipal Limit (BML) (7°43'35.81"N: 81°42'4.04"E) area in the Batticaloa district (Fig. 1) was selected for a nine month study (covering the dry and the wet seasons) between April and December 2008. The area is inhabited by over 500 families. The elevation is 10 ft mean sea level. The climate is warm and dry throughout the year except during the monsoon rainy season from November to February. The climatic and weather conditions of the area are mainly determined by the northeast monsoon and atmospheric pressure changes in the Bay of Bengal. The northeast monsoon mainly influences the seasonal rainfall, but the area also receives a considerable amount of rain throughout the year by convectional precipitation. The eastern side of the area is surrounded partly by the Batticaloa lagoon. The houses are constructed with bricks and tiled roof. There are bare lands with fully grown grass and uncleared debris.

Aedes larval collection

Conventional black plastic ovitraps (3. 2 × 2.7 cm) were used for larval collections. A plywood paddle (4 × 0.5 cm) was placed against the upper-rim of each coded ovitrap [7] containing 100 ml of well water. Fifteen houses were randomly selected from the study site. Four ovitraps (2 indoor and 2 outdoor) were placed in each house. The outdoor ovitraps were placed 15 m apart from each other and 3 m away from the house. The indoor ovitraps were placed at one per living room in close proximity to shelves or racks or hanging clothes. Sample collections were carried out fortnightly. After the first round of collection the ovitraps were emptied and washed thoroughly and refilled with water. A new paddle was used and the process was repeated for the next round of collection. Collected eggs and larvae were brought to the Zoology Laboratory at the Eastern University and reared under laboratory conditions (28 ± 2°C, 70–80% R.H.). Larvae along with eggs in each ovitrap were reared separately in yoghurt cups (3.5 × 6.5 cm) containing

50 ml of dechlorinated drinking water. Powdered fish meal pellets were provided twice a day as larval food. The collected larvae and emerged adults were numerated and identified using standard keys [8].

Insecticide susceptibility

The standard World Health Organization bioassay kit was used for susceptibility tests [9] under laboratory conditions (temperature 28 ± 2°C and RH in the range of 70–75%). Susceptibility of 2–3 d old *Ae. aegypti* and *Ae. albopictus* was determined by exposing the adults in batches of 15–20 to the diagnostic doses of insecticide impregnated papers i.e. 4% DDT, 0.8% malathion, and 0.25% permethrin for one hour. At least three replicates of each insecticide were carried out for each population. Dead mosquitoes were counted after a recovery period of 24 hours. A control mortality of <20% was corrected using Abott's formula [10]. If the control mortalities were >20% the bioassay results were discarded.

Meteorological data and analysis of data

Rainfall data were obtained from the Meteorological Department of Batticaloa. Sample collection data were analyzed using statistical software Minitab 14.0. Student's t-test was performed to study the differential breeding preference of *Aedes* species, and Pearson correlation was used to correlate *Aedes* density with rainfall.

RESULTS

Prevalence and seasonality

During the study period a total of 10,685 *Aedes* mosquitoes were collected from the study sites. *Ae. aegypti* and *Ae. albopictus* constituted 57% and 43% of the total respectively. In both localities, outdoor collections outnumbered indoor collections, with *Ae. aegypti* as the predominant species (Table 2). A seasonal shift and variation in the density of *Ae. aegypti* and *Ae. albopictus* in indoor and outdoor collections were observed. The highest 58% was recorded during the dry season (April–July) and 73% during the wet season (August–December) (Table 3). *Aedes aegypti* was the predominant species during the pre-monsoon period (August–September) and *Ae. albopictus* during the monsoon season (October–December). In the indoor collections, *Ae. aegypti* density was high during the dry season (Fig. 2). In the outdoor collections, *Ae. aegypti* was the predominant species during the dry season and *Ae. albopictus* during the wet season (Fig. 3). Although both species preferred to breed in outdoor ovitraps (Fig. 4 & 5), *Ae. aegypti* showed a positive tendency to breed in indoor ovitraps ($p < 0.02$). As

Table 2. Total number of *Aedes* mosquitoes collected during the study period

Indoor			Outdoor			Total
Ae. aegypti	*Ae. albopictus*	Sub total	*Ae. aegypti*	*Ae. albopictus*	Sub total	
2412	1126	3538	3493	3654	7174	10685

Table 3. Recorded number of positive ovitraps during the study period

Month	Batticaloa Municipal Area					
	Number of positive ovitraps (out of 30)				% of positive ovitraps (out of 60)	Ovitraps positive for mixed breeding of *Ae. aegypti* and *Ae. albopictus* (out of 60)
	Indoor		Outdoor			
	Ovitraps positive for *Aedes* breeding	Ovitraps positive for mixed breeding of *Ae. aegypti* and *Ae. albopictus*	Ovitraps positive for *Aedes* breeding	Ovitraps positive for mixed breeding of *Ae. aegypti* and *Ae. albopictus*		
April	10	3	15	6	42	15
May	10	2	13	5	38	12
June	15	2	20	11	58	22
July	13	6	12	4	42	17
August	15	2	14	5	48	12
September	14	3	14	6	47	15
October	10	2	17	9	45	18
November	15	1	21	10	60	18
December	20	1	24	15	73	27

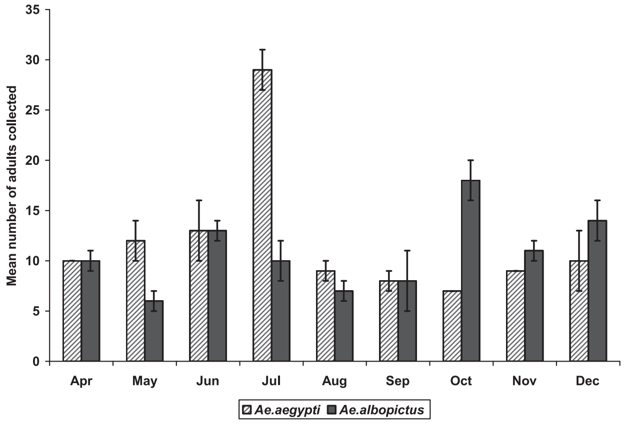

Fig. 2. Monthly variation in the mean number of *Aedes* adults collected from indoor ovitraps

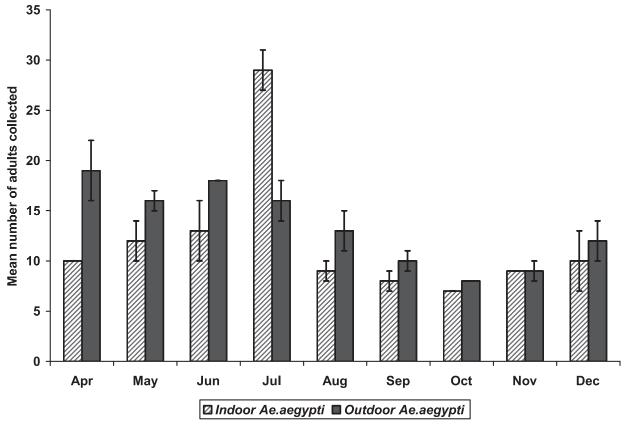

Fig. 4. Monthly variation in the mean number of *Ae. aegypti* collected from indoor and outdoor ovitraps

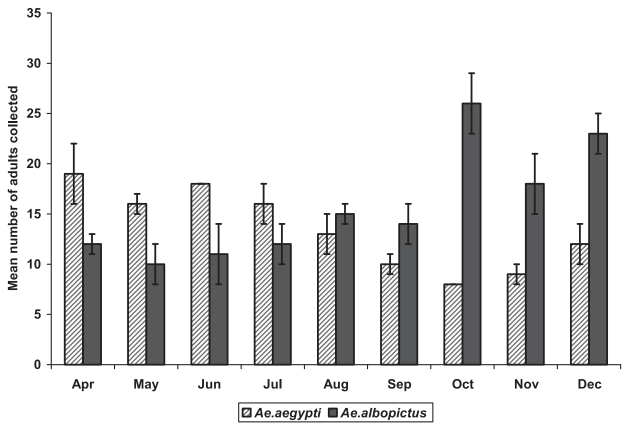

Fig. 3. Monthly variation in the mean number of *Aedes* adults collected from outdoor ovitraps

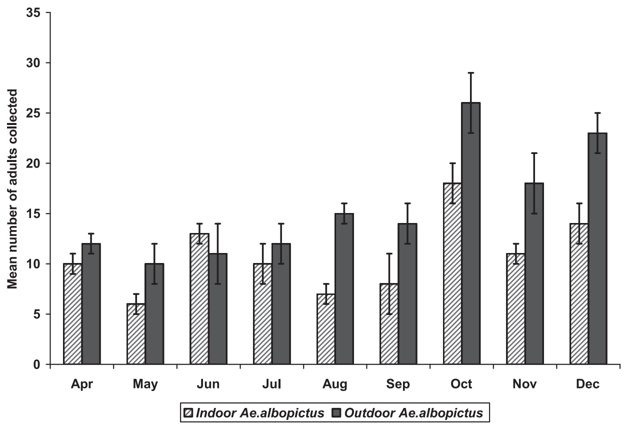

Fig. 5. Monthly variation in the mean number of *Ae. albopictus* collected from indoor and outdoor ovitraps

expected, a positive association was observed between *Aedes* density and rainfall (Fig. 6).

Susceptibility to common insecticides

Both *Ae. aegytpi* and *Ae. albopictus* populations were highly resistant to 4% DDT and highly susceptible to 0.25% permethrin (Table 4). Both species showed susceptibility to 0.8% malathion but the difference was not statistically significant ($\chi^2 = 1.409$, df = 1, p = 0.235).

DISCUSSION

Ovitrap based studies are reported to be an economical, reliable and rapid method to assess *Aedes* control measures and to detect low infestation [11]. The present study revealed a high prevalence of potential dengue and chikungunya vectors in the Batticaloa district and indicated that their presence might have contributed to local transmission of dengue in the area. *Aedes aegypti* is generally regarded as highly anthopophagic and *Ae. albopictus* as less domesticated [4, 5]. Comparatively greater abundance of *Ae. albopictus* in rural areas and breeding in outdoor sources has been reported [12]. However, the present study revealed that *Ae. albopictus*, like *Ae. aegypti*, is highly domesticated and able to breed in both indoor and outdoor ovitraps.

A noticeable fact is the seasonal shift in the density of the two species. *Ae. aegytpi* tends to predominate during the pre-monsoon season and *Ae. albopictus* during the monsoon season. Since the district receives monsoon rain from October to December, *Aedes* density increases during this period and a positive association between rainfall and *Aedes* density is observed. The high density of *Aedes* mosquitoes recorded in June is attributed to the unexpected rain experienced in this month.

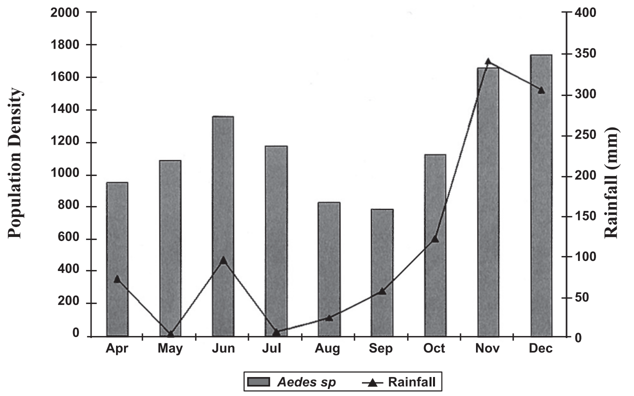

Fig. 6. Association between rainfall and *Aedes* density during the study period

Table 4. Insecticide susceptibility to common insecticides

Common insecticides	No. of resistant individuals (No. of exposed individuals)	
	Ae. aegypti	*Ae. albopictus*
4% DDT	96 (100)	96 (100)
0.8% malathion	60 (100)	45 (100)
0.25% permethrin	7 (100)	8 (100)

The continuous presence of both species throughout the study period is a major concern to health authorities because it shows that local transmission is possible even during the dry season. A similar seasonal shift was also reported from Jaffna district located in the northern dry zone of the country [13]. The seasonal shift may indicate a possible interspecies competition that is yet to be established. Mixed infested (infested with both species) ovitraps were collected during the study period at a low percentage (1–6%) indoors and high percentage (4–15%) outdoors. The noticeable seasonal shift and sharing of the same breeding sources by these two species warrants a detailed study on the ecology and niche preference of the two vectors. In addition, the indoor breeding nature of the two vector species is a concern for health officers and because these vectors are reported to be daytime biters and infants and women who stay mainly indoors during the day may thus be at risk since. Therefore the groups at risk should be protected by adopting personal protective measures.

The dengue vector control programme of the district consists mainly of source reduction and thermal fogging. As a dengue vector control measure, fogging is employed by the health authorities during disease transmission periods. Malathion in liquid form was used until 2008 and was replaced by pesguardd® (a synthetic pyrethroid) in 2009 (personal communication, Office of the Regional Director for Health Services, Batticaloa). The present study reveals that while both species are highly resistant to 4% DDT and susceptible to 0.25% permerthrin, they are moderately resistant to 0.8% malathion. The organochloride (DDT) and organophosphate (malathion) resistance pressure can be attributed to extensive use of these chemicals for the control of malaria in the district. The development of resistance to malathion might have prompted the health authorities to use pyrethroid in place of malathion. A study on the resistance mechanism of these two populations in the district is needed in order to propose suitable chemical control measures in the district.

Conducive environmental conditions coupled with human activities are the major contributing factors in the propagation of *Aedes* mosquitoes. The fact that all the sampled houses had positive ovitraps is an indication of human involvement in providing suitable breeding grounds for *Aedes* mosquitoes. In Sri Lanka, intuitions such as hospitals, transport bus depots, factories and schools are also cited as potential breeding sources for dengue vectors [14]. Discarded containers and water storage receptacles are important breeding sites for *Aedes* mosquitoes in the country [15, 16]. Control of dengue vectors is achieved largely through source reduction, health education and adulticiding with thermal fogging in the country. Considering the nature of breeding of dengue vectors in the district public awareness and health education are essential to control the transmission of dengue. It is recommended that health authorities continue vector surveillance throughout the dry and wet seasons to avoid any future outbreaks in the district.

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