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Distribution of *Aedes aegypti* and *Aedes albopictus* in different eco zones of Thiruvananthapuram city with special reference to dengue viremia in humans

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ABSTRACT: Mosquito index study of three ecologically different ecozones of the Thiruvananthapuram district, Kerala showed sharp difference on the proportionate distribution of *Aedes aegypti* and *Aedes albopictus*. Human dengue viremia (HDV) was very high in those ecozones where *A.aegypti* density was high and HDV was low where *A.albopictus* was high. In a coastal zone of Thiruvananthapuram city, *A. aegypti* was the most abundant vector and in a hilly, arid sub urban zone, *A.albopictus* was the abundant vector. In the urban zone both species of mosquitoes showed equal distribution. Study on the circulating serotypes in the serum of HDV by Single step single tube Multiplex PCR showed all the four serotypes *viz* DENV1, DENV2, DENV3 and DENV4 in patients of Thiruvananthapuram city, which indicated the possibility of Dengue Shock Syndrome, unless there is efficient vector management. Among the four dengue serotypes, Type 1 was the most abundant virus. Abundance of microhabitats in Thiruvananthapuram city, which support *A. aegypti* may be the reason for high prevalence of dengue fever in the urban zone.

KEY WORDS: *Aedes aegypti, A. albopictus*, dengue, serotypes, microhabitat specificity

INTRODUCTION

Dengue Fever (DF) is one of the most wide spread infectious disease globally and its transmission now occurs in 128 countries (Mahalingam *et al*., 2013). Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome(DSS) are major public health problems with almost one half of the global human population may occur epidemically and endemically in any area where susceptible Aedes mosquitoes *(Aedes aegypti, A. albopictus*) breed (Farrar *et al.*, 2007). In tropics, DF occurs most frequently after rainy seasons.. After biting an infected human the mosquito becomes infective after an incubation period of 8 to 22 days with a mean duration of 11 days. In India first report of dengue was from

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Chennai in 1780 and the authentic report was available from Kolkata in 1963 (Gupta *et al*., 2012). In India severe attack of DF was reported during 1970s from various states such as Karnataka (George and Soman, 1975) , Uttar Pradesh (Chadurvedi *et al.*,1974), Maharashtra (Prasada Rao *et al.*,1981) and Rajastan (Ghosh and Sheikh, 1973) and in all these states the primary vector was *A.aegypti.*

After an epidemic in Kerala during 2003, numerous cases of DF were reported from several districts, including those in sylvian environs of Western Ghats such as Idukki and Kottayam districts (Tyagi *et al.*, 2006; Tyagi *et al.*, 2003; Tyagi and Dash, 2006). During the year 2006, a scanty distribution of DF was reported from various parts of Kerala and among the human viremia cases 65% of the total cases were reported from Thiruvananthapuram district. During 2017 a wide spread infection of DF with many DSS and accompanied deaths were reported from various districts of Kerala and in this instance also the Thiruvananthapuram district was the worst affected place in the state (Kumar *et al.*, 2013). Kerala, with an average population density of 819/sq.km provide ample survival chances for vector mosquitoes. Dengue infections are caused by four closely related virus strains named DENV1-4. The mosquito borne flavivirus is a single stranded RNA virus and are antigenically distinct from one another and have 60-80% homology. Infections result in long term protection against a particular serotype but no resistance against other serotypes (Gupta *et al.*,2014). During an outbreak of dengue fever in Delhi in 2003, serological studies of patients proved that DENV1, DENV2 and DENV3 were equally distributed but DENV4 couldn't be located (Lalith Dhar *et al.*, 2006). After a lag of three years the disease once again lashed on the population of Delhi, but that time DENV3 was the prominent serotype observed (Gupta *et al.*, 2006; Preethi *et al.*, 2008). The present study was undertaken to know the dynamics of dengue transmission in relation to micro-habitat analysis of three different sites of Thiruvananthapuram City and also on the basis of the vector density of related species of Aedes mosquitoes, *A. aegypti* and *A.albopictus*. The changing epidemiology in relation to susceptibility of vector mosquitoes to any particular serotype of DENV has to be taken into consideration of effective management of this disease. This aspect is also discussed in the present scenarios of dengue viremia in Kerala.

MATERIALS AND METHODS

Study Area: The Indian state Kerala has a total area of 38863 km ^{2} and a population of 36.6 million, with 31.16% lives in urban areas. Thiruvananthapuram district of Kerala is fairly humid and warm throughout the year with relative humidity ranging from 70-90% and temperature ranging between 22-35.5^{0}C respectively. The annual precipitation is high, reaching upto 300cm/ year (Meteorological Department, Meteorological Centre, Thiruvananthapuram). The larval surveys were undertaken in all months. March, April and May (summer), June, July and August (south west monsoon), September, Ocober and November (northeast monsoon) and December, January and February(pre-summer). In this four seasons temperature and humidity exhibits sharp difference which determines the mosquito biology and their distribution. The monthly entomological data of different seasons were pooled together for analysis. In Thiruvananthapuram district incidence of dengue was Ã2 / one hudred thousand population during the year 2006 and about 65% of dengue cases in Kerala were reported from Thiruvananthapuram district (Thangaradham *et al.*, 2006). Since Thiruvananthapuram forms the epicentre of this disease, sites such as two lanes of Kunnukuzhy (Urban site), Sreekaryam (Sub-urban) and Kannanthura (Coastal site) were selected for entomological and clinical study.100 houses were selected from each site for the study. Kunnukuzhy is within the heart of the city and poulation density is highest among the three study sites. The study site in Sreekaryam is moderately an elevated and airid zone.

Entomological survey: In each of these representative sites, 100 houses were thoroughly checked for the breeding of Aedes mosquitoes. The survey was carried out on outdoors, indoors and

also at premises of houses. The breeding sites such as cisterns, cement tanks, metal containers, plastic drums, grinding stones, mud pots, plastic bottles, flowerpots, flower vases, polythene sheets and natural breeding sites such as coconut shells, tree holes, fallen spates or bracts were observed from these localities. Among the above breeding sites mud pots were found to be possessing highest number of larval and pupal density.

Small containers (< 20 litter) were drained through strainer in to white larval sampler (25x20x4 cm) to collect the immature stages of mosquitoes. Large breeding places like ground level cement tanks; fountains etc were sampled using a 250ml larval dipper. Five dips were taken from the surface water of each breeding place. The collected larvae were separately brought to the laboratory and identified to species level using standard mosquito and larval identification key (Tyagi *et al* 2015 and DHS Kerala 2011) (CITE). The duration of study was three years from January 2014 to December 2016.

The details of survey were recorded in a format specially designed for this purpose. From the entomological data the following indices were calculated as described in standard methods, House Index (HI), Container Index (CI) and Breteau Index (BI) (WHO, 2009).

Formula for calculating the Mosquito larval indices:

House Index (HI)

= number of houses positive for *Aedes* breeding/ houses checked X 100

Container Index (CI)

= number of positive containers/ total containers checked X 100

Breteau Index (BI)

= number of positive containers/ total number of houses searched X 100

Blood Sample collection: The three sites on which the entomological study was conducted during the last three years were selected for blood sample collection. As the investigators are familiar with the local people of this area they have co-operated for blood sampling after getting a signed affidavit/ declaration from them. Institutional ethical clearance for the above sampling was already obtained (Univ.Coll.IEC/Dept.Zool/001/Vect.Borne Dis.dt.28/05/2014). Blood samples were collected from patients who are suspected for dengue infection. A total of 150 samples were collected during this period. 50 patient samples with high dengue fever was selected from the total samples and were subjeted for the study.

Single step Single tube Multiplex PCR (SSMPCR): The study was done at Laboratory Medicine and Molecular Diagnostics, a division of Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. Reverse Transcription Polymerase Chain Reaction (RT PCR) was used as state of the art technique for the detection of serotype. Indigenously developed Single step Single tube Multiplex PCR (SSMPCR) was used as the technique for the detection of specific serotype (Dayakar *et al.*, 2015).

Viral Nucleic Acid (RNA) Isolation: Viral nucleic acid (RNA) was isolated from the patient serum was carried out using QIAGEN's QIAamp Viral RNA Extraction Kit®(QIAGEN, Germany), following the manufacturers protocol.

One-step one-tube Multiplex PCR amplification with serotype specific primers: A direct one-step, single tube multiplex PCR amplification was performed for differentiation of dengue virus serotypes. This method involves the usage of the isolated viral RNA directly with D1 consensus primer and serotype specific primers TS1, TS2.TS3,TS4 in a single tube reaction. The isolated viral RNA was directly used as the template. 0.5 µL each of the serotype specific primers DEN1,TS2, TS3, TS4 as reverse primers. The primers used were taken from previous publication. Primers were added along with 0.5 µL D1 forward primer along with TaKaRa's PrimeScript One Step RT-PCR Master Mix (TaKaRa ® Japan) consisting of 12.5 µL of 2X One Step RT buffer, 0.5 µL of Taq Polymerase (5 units/µL), 0.5 µL of 5X Reverse Transcriptase Enzyme and 3.0 µL of RNase Free

dH_2O. A total reaction volume of 25µL was subjected to PCR for 40 cycles, with an initial cDNA synthesis step at 42^0 C for 5 min, initial denaturation at 94^0 C for 10 sec, denaturation at 94^0 C for 30 secs, annealing at 55^0 C for 60 secs, and extension at 72^0 C for 60 sec, and a final extension at 72^0 C for 60 sec. The amplicons were then detected using 1.5% agarose gel electrophoresis. The PCR product size as estimated using 100bp ladder (Takara™).

Statistical analysis: Descriptive statistics of mean and confidence interval was used to calculate the larval indices. Significance of larval indices in different areas was compared by using ANOVA. Paired sample t test was used to compare significance of indoor and outdoor positive habitats. ANOVA, correlation and t test were carried out using the SPSS version of16.0 software.

RESULTS

Three year study on the distribution of different species of mosquitoes in the selected sites such as urban, semi urban and coastal zones of Thiruvananthapuram City proved that *A.aegypti* and *A.albopictus* are the dominant mosquito species. Sporadic occurrence of *Culex quinquefasciatus*, *Anophelis stephensi* and *Armingerus subalbatus* were also located in the study sites. Occurrence of *Culex quinquefascitus* was observed in foul smelling water collection such as leakages of drainage vessels and septic tanks possessing rich sources of putrefied animal wastes. Larvae of *Ar.subalbatus* were observed in accumulated water associated with cattle sheds. Larvae of *A. aegypti* and *A. albopictus* were observed in comparatively less polluted water with no foul smell. Even indoor collection of water for drinking purposes, stored in closed containers possessing very little space between the lid and rim of containers were the breeding sites of both species of Aedes. The three eco zones of Thiruvananthapuram city showed marked variations on the occurrence of *A. aegypti* and *A. albopictus*.

The semi urban zone of Thiruvananthapuram city (Sreekaryam) is a dry and elevated site from sea level, where almost 90% of the mosquito larvae observed were *A. albopictus* and the remaining was shared by *A.aegypti* and no other species of mosquito was observed (Table 1). In coastal zone, almost 80% of the mosquito larvae were that of *A.aegypti* and remaining 20% of the larvae were *A.albopictus* (Table1). Coastal zone possessed a scanty distribution of *Anopheles stephenci* larvae, which were observed in water collection with less pollution or less stench. In the city core area (Kunnukuzhy ward) both *A.aegypti* and *A.albopictus* were observed almost equal proportion. At some sites of this zone where one or two cattle were reared, the accompanying area showed sporadic occurrence of *Ar. subalbatus*. During the whole study period of three years the proportionate distribution *A.aegypti* and *A.albopictus* in three different study sites did not show any change. The vector density of the three eco zones were different. The larval indices such as HI, CI and BI were high in sub urban and coastal zones but of urban zone, it was very low, compared to other two zones. During the study period of 2014-16 the larval indices of each zone did not exhibit year wise variation (Table 1), which indicated that ecologically each microhabitat possessed a comparatively stable vector density.

Another observation made during the study period was the difference on the egg laying behavior of *A.aegypti* and *A.albopictus* in different seasons. During summer months, the containers of the outdoor(house premises) were dry and no mosquito larvae were located in outdoor area and the indoor containers such as stored water for domestic purposes, drainage tray of refrigerator, water of bath rooms and latrines were with enough larvae. During rainy season there were plenty of water accumulations in every outdoor site and almost all were with larvae but the indoor containers were free of larvae.

Analysis of the epidemiology of dengue viremia in Kerala during the period of six years, from 2012 to 2017, proved that, during the whole six year period dengue viremia was very high in Thiruvananthapuram district (Table 2). In the all six years, dengue viremia in Thiruvananthapuram

Table 1. Larval indices of three eco-zones of Thiruvananthapuram city and the distribution of *A. aegypti* and *A. albopictus*

Study sites	Range	Larval Indices of three years		
Urban zone	Range	2014	2015	2016
HI	(6-18)	10.5	11.5	11.5
CI	(11-86)	43	39.5	23.5
BI	(10-27)	14.5	17.5	15.5
Proportion of *A. aegypti & A. albopictus*		1:1	1:1	1:1
Semi urban zone				
HI	(24-46)	31	32	33
CI	(48-65)	55	59	58
BI	(27-46)	34	37	38
Proportion of *A. aegypti & A. albopictus*		1:9	1:9	1:9
Coastal zone				
Hi	(24-46)	31	32	32
CI	(35-70)	48	56	58
BI	(18-46)	29	37	35
Proportion of *A. aegypti & A. albopictus*		10:3	10:3	10:3

district was 30 to 50% of the total number DF of the state. Simultaneous study on Dengue viremia in humans and on the distribution of vector mosquitoes in three different study sites proved that Dengue fever was negligibly low in semi urban area, where the dominant mosquito was *A.albopictus*. During the three years study (2014-2016) only two DENV cases were detected from this site. Urban and coastal zones showed prevalence of Dengue fever and high prevalence was observed in the coastal zone, in which *A.aegypti* was abundantly present.

Molecular diagnosis and serotyping of clinically proven dengue viremia of 50 cases were performed (Fig.1). The results showed that DENV1 was the most abundant (more than 60%) and the share of all the other three different types of virus were below 40% and it is clear from Fig.2. There were 32 cases of DENV1 alone among the total of 50 patients studied. There were co-infection of three DENV1 with DENV4, two DENV1 with DENV3 and two DENV1 with DENV2. No other types of co-infection were observed, which clearly indicated that DENV1 has adaptive advantage over the other

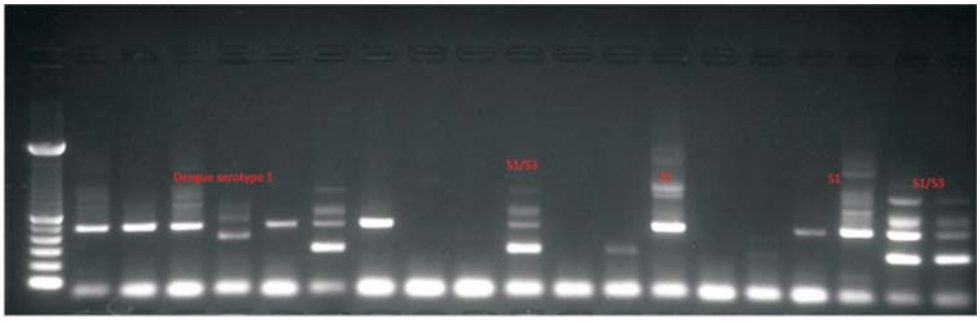

Fig. 1 : Agarose gel electrophoresis of human serum showing different dengue serotypes along with co-infection

Table 2. Incidence of dengue fever in Kerala State and in respect of Thiruvananthapuram district .(Directorate of Health Services, Kerala)

Year	Kerala state		Thiruvananthapuram District	
	DF	Death	DF	Death
2012	4056	16	2447	4
2013	7729	24	4072	5
2014	2548	13	1250	3
2015	4114	29	991	9
2016	7218	21	2158	7
2017	19994	37	8502	13

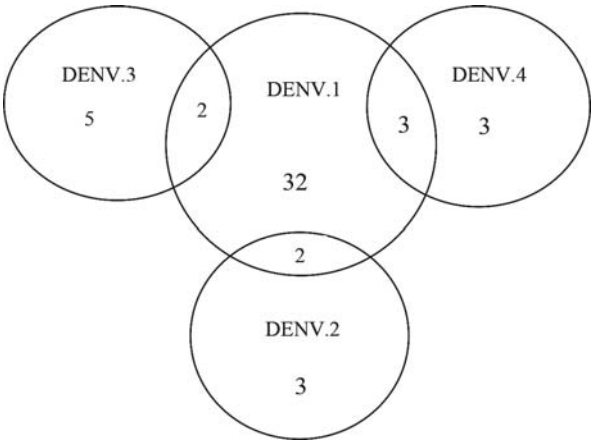

Fig. 2: Distribution of four different types of dengue viruses in Thiruvananthapuram city

serotypes in causing symptomatic disorders in humans (Tyagi *et al.*, 2003).

DISCUSSION

The present study clearly showed that the species diversity of mosquitoes in three eco-zones of Thiruvananthapuram city exhibited contrasting difference especially on the distribution of *A. aegypti* and *A. albopictus*. In the coastal zone *A.aegypti* was the dominant species and in the hilly suburban zone *A. albopictus* was the dominant species. In the city core area (urban) almost equal distribution of both species of Aedes mosquitoes were observed. Similar observations were reported from Rajastan in which different ecozones such as desert area, forest and river area and semi arid area exhibited sharp difference on the distribution of *A.aegypti* and different strains of DENV (Bennet and Joshi, 2009). During the last three years (2014-2016) there was no significant change on the proportionate distribution of *A.aegypti* and *A.albopictus* was observed. This clearly indicated that both species of mosquitoes are highly precise on their preference in niche selection. Previous reports also supports the present investigation that, in Trissur district of Kerala, the rubber plantations possessing coconut shells used for collecting rubber latex showed the larvae of *A. albopictus* only during rainy season and no *A. aegypti* larvae in the whole plantation area (Sumodan, 2003).

A very remarkable observation made in the present study is the relationship between dengue viremia in the study sites and the distribution of mosquito population. In sub urban site, *A. albopictus* was the dominant mosquito, in which Dengue infection was very low (only two cases among 50 cases during three years), but in the other study site, the coastal zone, there was high prevalence of Dengue infection, in which the dominant mosquito vector was *A. aegypti* (WHO, 2003). This clearly indicates that *A. aegypti* is the only true vector of DF and the role of *A. albopictus* is insignificant.

Clinical data from the Department of Health, Govt. of Kerala, clearly showed that, Thiruvanathapuram district of Kerala carry the major share of DF, which is almost 40- 50 % of the total cases reported in Kerala for the last six years. This clearly indicated either ecological factors or genetic factors of vector mosquitoes is favoring Thirvananthapuram district to be the most favorable zone in Kerala for maintaining dengue virus. Similar type of observations are reported from Australia in which socio demographic and ecological features play a

significant role on the distribution of *A.aegypti*. People of high economic group possessing rain water harvest tank above the houses provide ample chances to this mosquito to breed in this tanks, but the people living in small houses, where the disease is uncommon because the abundant vector in such places is *A.albopictus* (Rokeya *et al.*, 2017). Compared to Thiruvananthapuram species level difference on the distribution of DENV, it is not a rare phenomenone. During 2003 dengue viremia in people of Delhi Metropolitan city showed co-circulation of DENV 1, DENV2 and DENV3 with equal efficiency and no DENV4 was located. (Chakravarti *et al.*, 2008; Lalith Dar *et al.*, 2003), but in 2006 there was a shift in favour of DENV3, which became prevalent in Delhi with negligibly low distribution other two serotypes(Gupta *et al.*, 2006). Study of dengue viremia, specifically in Kerala showed that all the four serotypes are distributed in Kerala which increases the chances of DSS in future years (Pradeepkumar *et al.*, 2015). and another study specifically in Ernakulam district of Kerala by Anoop *et al. (*2010) showed that combined infections such as DENV2 & DENV3, DENV1& DENV2 and DENV1 & DENV3 are common. Anoop *et al.* (2013) have also suggested that there is hyper endemicity of dengue viremia in Ernakulam district and suggested the possibility of local gene evolution of dengue virus and the team have also reported that there is a lineage shift of DENV3 in Kerala, probably due to exotic introduction of dengue virus from other Nations (Anoop *et al.*, 2013). In South America similar type of DENV serotypes were demonstrated in human blood and in *A.aegypti* mosquitoes and the investigators experimentaly proved that DENV1 is the most efficient among the four as a causative agent for this disease (Rosen *et al.*, 1983).

The niche selection of *A. aegypti* and *A. albopictus* exhibits microhabitat specificity, which plays an important role in the distribution of mosquitoes at species level in different sites of a broad area. The coastal zone of Thiruvananthapuram city was the prefered site for *A. aegypti* but a hilly site barely 5 km away from the coastal zone was the prefered site for *A. albopictus*, the perticular study site is arid and 40 meters above sea level. The site preference in relation to egg laying behaviour of *A. aegypti* changed in accordance with season. During summer, the prefered egg laying sites for Aedes mosquitoes were indoor collections of water, but after summer rains and and during rainy seasons the prefered sites were outdoor collection of water in discarded containers. Dengue infection in three study sites during 2014-2016 showed a co-relation with species level distribution of *A. aegypti* and *A.albopictus*. Dengue infection in areas with abundance of *A. aegypti* was higher than that of the areas with abundance of *A. albopictus.* The present study proved that *A. aegypti* and *A. albopictus* exhibits microhabitat specificity on their distribution and this has major influence on the transmission of DENV on each microhabitat. In Jodhpur of Rajastan, a desert area, people store water in houses which form effective breeding sites of *A.aegypti* where dengue infection is very common and DENV3 is the most abundant virus (Bennet and Joshi, 2009). Eventhough all the four antigenically distinct dengue virus were located in Thiruvananthapuram city, DENV1 was the most abundant serotype, which was identified through PCR technique on human blood samples. Further epidemiological analysis on the distribution of dengue serotypes and their molecular analysis on evolutionary distance is warrrented for a much complete picture.

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