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Research Paper

ENTOMOLOGICAL AND VIROLOGICAL INVESTIGATION IN A FOCUS OF DENGUE AND CHIKUNGUNYA VIRUS TRANSMISSION IN KERALA

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Abstract

Dengue and chikungunya virus infection has become a public health problem in Kerala. Since 2009, suspected cases of dengue fever were reported from Kottayam district. Although, fever outbreak occurred every year, reports on virus detection from mosquito vectors from the district are currently not available. The present study is to identifying the primary vectors involved in transmission so that appropriate public health measures could be implemented. The household entomological study was conducted in randomly-selected sites where major outbreaks of Dengue occurred during 2009 and 2012**,** Kottayam district, Kerala state during 12 months in 2012-2013. Water-holding containers were inspected for presence of Aedes mosquito larvae. Laboratory reared adult mosquitoes were analyzed by RT-PCR for the presence of DEN and CHIK virus. A total of 3921 containers were inspected in which 484 (13.21) containers were positive with *Ae. albopictus*. The Maximum Breteau and Pupae index were found to be 111.22 and 901.52 during the month of June and August. The maximum breeding of *Ae. albopictus* was found in large buckets (33.57%), plastic sheet (17.87%), and split coconut shell (14%). Mosquitoes were analyzed by RT-PCR for the presence of dengue and chikungunya virus. A total of 1689 (96 pools) adult mosquitoes were tested, and all tests were negative for dengue and chikungunya virus. Based on replicate in dry and wet seasons, the occurrence of Dengue virus in Kerala *Ae. albopictus* could be considered a potential vector in the Kottayam district for transmission to humans.

Key words: *Aedes albopictus,* Breteau index, Pupae index, dry and wet season, dengue, chikungunya.

INTRODUCTION

Dengue is considered an important public health problem all over the world. Its occurrence has been registered in more than 100 countries. More than 2.5 billion people are estimated to live in risk areas for the transmission of this disease [1, 2]. In 2013, a total of 74454 dengue and 18639 chikungunya cases were reported form the country in which 7911 dengue and 219 chikungunya cases were reported from Kerala[3, 4]. Dengue and chikungunya viral disease is endemic in Kerala. With the lack of a commercial dengue and chikungunya vaccine, prevention of dengue and chikungunya outbreaks depends on the surveillance of vectors and therefore, efforts should be directed toward its effective control[5].

Dengue virus was first detected in the state in 1997 during the epidemic of dengue like illness that occurred in Kottayam district. Since that initial outbreak, dengue has continued to cause periodic and widespread epidemics in Kottayam and other district of the state[6]. The state

had the first outbreak of chikungunya during 2006 along the coastal areas of Alleppey, Ernakulam and Trivandrum districts and again in 2007 chikungunya cases were reported from 6 districts in which Kottayam was worst affected district[7,8,9]. Mosquito borne diseases, including dengue, chikungunya, Japanese encephalitis and filariasis are major public and veterinary health and West-Nile virus cases have been reported in Kerala [10,11].

Aedes aegypti and *Aedes albopictus*, two mosquitoes belonging to the *Stegomyia* subgenus, are major vectors of dengue and chikungunya virus [12]. Detection of dengue virus in natural *Aedes* populations has shown to be a potential additional tool for early prediction systems of dengue outbreaks[13, 14, 15]. Though being aware of the presence of this virus in Kerala, monitoring of dengue and chikungunya virus activity in mosquito vectors has been performed sporadically. Therefore, in the view of endemic situation in Kottayam district an entomological and virological studies were conducted in Kottayam districts, to identify the vector responsible for the virus transmission in field populations of *Ae. aegypti* and *Ae. albopictus.*

MATERIALS AND METHODS

Study area

Kottayam district lies between North Latitude 9.48 ^{0} and 9.9 ^{0} and East Longitude 76.4 ^{0} and 77 ^{0}. **K**ottayam district has a total area of 2208 sq. km. with three ecological zones (Low land, Midland and Highland). In the study region of the district, rubber is the main crop and coconut, tapioca, pepper, banana, pineapple are also cultivated. The district experienced a long period of rain from June to October and a short dry season from November to March.

ENTOMOLOGICAL SURVEILLANCE

House-to-house surveys were performed during dry and wet seasons from March – 2012 to February- 2013 in randomly-selected sites where major outbreaks of Dengue occurred. Briefly, each survey consists of a brief questionnaire, inspection of household for water-holding containers. The searches were carried out in domestic and peridomestic habitats, viz. coconut shells, metal containers, tire, plastic containers, cement tanks, glass bottles, mud pots, plastic sheets, etc. A total of 889 houses were sampled in the district. Live larvae and pupae collected in positive receptacles were samples and reared into adults to identify the species. Containers positive for *Aedes* larvae were recorded. Laboratory emerging adult mosquitoes were separated by species and combined into pools with each pool containing a maximum of 50 mosquitoes. Mosquitoes were stored at –80°C till used for virus detection. Larva habitats were characterised according to their setting, type and capacity (Small:<2 litres, Medium: 2 to 25 litres, large >50) (Table 2). Three larval indices and one pupae indices i.e. The House Index (HI), Container Index (CI), Breteau index (BI) and Pupae index (PI) were calculated by the standard procedure (WHO 1999).

VIRUS SCREENING

The newly laboratory emerged adult mosquitoes (both males and females) tissue extracts were prepared by homogenizing in 1.5 milliliters of phosphate buffer saline (PBS pH 7) containing 4% bovine serum albumin (BSA) and added 3 glass beads, size of 3.0 to 3.5 millimeter (mm). The mosquitoes were homogenized in bead beater homogenizer for 60 sec (Biospec. Products Inc. USA) then it was centrifuged at 800 x g for 10 min at 4°C. The supernatant was transferred to a fresh tube. Two hundred micro liter aliquot was used for RNA isolation using Trizol reagent (Invitrogen) according to the manufacturer's instructions and remaining supernatant was stored in -80 ^{0}C until use. Dengue virus was detected from the supernatant using reverse transcriptase PCR (RT-PCR) techniques as described earlier [16]. Similarly the Chikungunya virus was detected as described by Yergolkar et al. 2006[17].

RESULT

The results of the present study showed that presence of only *Ae. albopictus.* None were positive with *Ae. aegypti* during household survey. It was observed that the Breteau index of *Ae. albopictus* began to increasing in April (66.15) with onset of summer rain and declined in

November (32.2) end of the raining season (Fig. 1). The Maximum Breteau index and Pupae index were found to be 111.22 and 901.52during the month of June and August which is corresponds to the rainy season. Several indices were calculated to estimate the *Ae. albopictus* population density including the house index, container index, Breteau index and pupae index (Table 1).

Table 1. House index, Container index, Breteau index, total pupae collected and pupae per container in both survey of Kottayam district.

Season	House index	Container Index	Breteau Index	Pupae Index
Dry season	23.4	10.97	39.63	124.87
Wet season	39	15.08	74.95	435.50

A total of 3921 containers were inspected in the localities surveyed during the study. It was found that 484 (13.21) containers were positive with *Ae. albopictus*. The most abundant containers available at the localities were large buckets (33.57%) as the most preferred breeding place among domestic containers, plastic sheet (17.87%) among discarded containers filled by rain and split coconut shell (14%) during rainy season (table 2). According to the number of pupae collected, in both the season large buckets were more important pupae producers (38.68%), followed by plastic sheet (16.75%) and discarded waste water containers (11.76%). A complete listing of containers and infestation rates is presented in Table 3.

Table 2. Seasonal distribution of larvae habitats identified according to habitat type

Container types	Dry season		Container index	Wed season		Container index
	Container searched (%)	Container positive (%)		Container searched (%)	Container positive (%)	
Containers filled by human action						
Syntex/Drum/Cement tanks storage	300 (16.15)	27 (15.08)	9	201 (9.73)	17 (5.57)	8.45
Animal drinking pans	20 (1.07)	2 (1.11)	10	5 (0.14)	3 (1.63)	166.6
Buckets/Utensils	680 (36.61)	60 (33.51)	8.82	412 (19.95)	73 (23.93)	17.71
Containers filled by rain						
Small tin/ Plastic waste/ Jars/Pots	349 (18.79)	22 (12.29)	6.30	363 (17.57)	25 (8.19)	6.88
Rubber tire	58 (3.12)	14 (7.82)	24.1	58 (2.80)	25 (8.19)	43.10
Plastic sheet/Roof gutter	142 (7.64)	32 (17.87)	22.53	270 (13.07)	62 (20.32)	22.96
Rock and mud pools	37 (1.99)	0 (0)	0	57 (2.76)	2 (0.65)	3.50
Natural containers filled by rain						
Coconut/Coco shell	124 (6.67)	15 (8.37)	12.0	249 (12.05)	55 (18.03)	22.08
Bamboo stumps/Tree holes	147 (7.91)	7 (3.91)	4.76	452 (21.88)	41 (13.44)	9.0
Total	1857	179		2068	303	

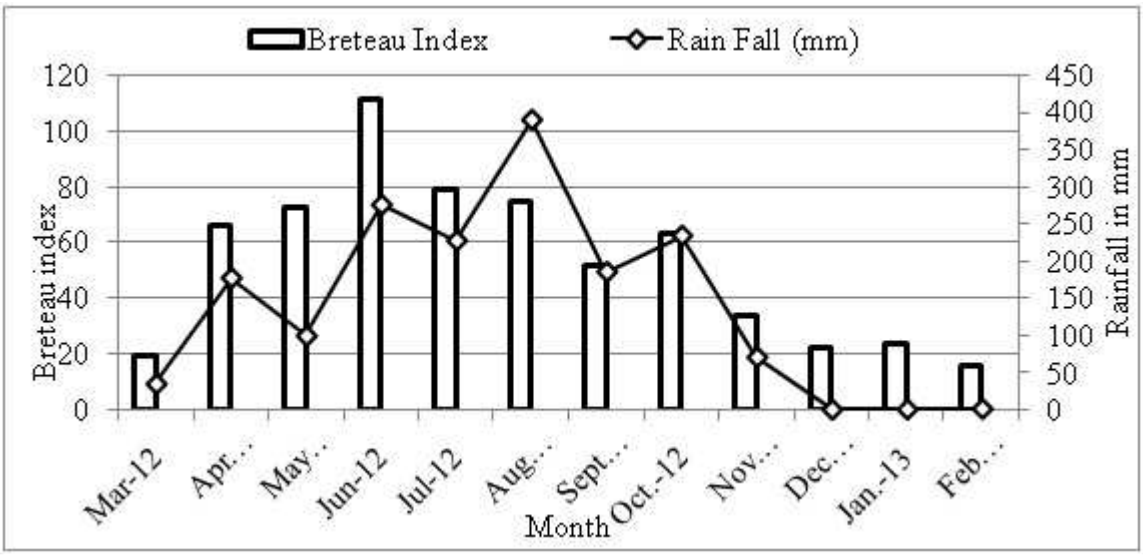

Fig 1. The relationship of increasing larval population (BI) with rainfall (in mm)

Table 3. Seasonal distribution of Pupae habitats identified according to habitat type

Types of Containers	**Dry season**	**Wet season**	Pupae Productivity
	Pupae collected (%)	Pupae collected (%)	
Syntax/Cement tanks storage	61 (10.8)	1144 (68.25)	53.86
Bowl (ant trap, pet dish etc)	13 (2.31)	5 (0.002)	0.80
Small tin/ Plastic waste/ Jars/Pots	66 (11.76)	104 (6.20)	7.59
Buckets/Utensils	217 (38.68)	**151** (9.00)	16.45
Rubber tire	36 (6.41)	23 (1.37)	2.63
Plastic sheet/Roof gutter	94 (16.75)	103 (6.14)	8.80
Rock and mud pools	12 (2.13)	**22** (1.31)	1.51
Coconut/Coco shell	50 (8.91)	81 (4.83)	5.85
Bamboo stumps/Tree holes/Leaf axils	12 (2.13)	43 (2.56)	2.45
Total	561	1676	

2.3. Container volume

The first group with a <2 liter capacity a total of 2385 container was composed of coconut shell, discarded plastic container, bottles, jars and earthen pots that 252 (10.56%) container positive. The second group with a 2-25 liter capacity 1035 containers was composed of small and large buckets, part of plastic sheet and tires were 97 (9.37%) container positive. The third group was composed of plastic drum, barrel and syntax 350 were 64 (18.28%) container positive. Containers were frequently found unlidded (90.18%) and rest of the containers was found half lidded. Containers found outside, at low sun exposure, filled with rain water, not fully lidded and with polluted water (fallen leaf and tree bark) (83%), were more likely to be infested.

Table 4. Percentage of water holding containers of each type and of pupae collected container index, and pupae per container for every variable measured and every type of container.

		A	B	C	D	E	F	G	H	I	Total
No of Containers	Small	2.39	43.47	6.04	86.79	53.44	64.07	86.17	99.19	99.66	2080
	Medium	3.59	56.5	56.95	12.64	46.55	35.92	12.76	0.80	0.33	962
	Large	94.01	00	36.99	0.56	00	00	1.06	00	00	880
Larval density	<10	6.81	42.8	4.51	72.34	48.7	11.70	100	37.14	60.4	133
	10-50	00	00	48.12	14.89	51.28	42.55	00	54.28	25	181
	>50	93.18	57.14	47.36	12.76	00	45.74	00	8.57	14.58	170
Under vegetation	Full	82.03	86.04	86.91	73.07	81.89	80.82	91.48	85.5	95.65	3253
	No	17.96	13.95	13.08	26.92	18.10	19.17	8.51	14.44	4.34	668
Shade	Full	92.2	81.3	40.22	91.75	94.80	93.93	92.55	92.91	97.99	3284
	No	7.78	18.60	59.77	8.24	5.17	6.06	7.44	7.08	2.00	637
Cover	Full	55.68	2.32	5.06	4.12	00	4.61	5.31	00	00	385
	No	44.31	97.67	94.93	95.87	100	95.38	94.68	100	100	3536

A. Syntax/ Drum/ Cement tanks storage / Trough, B. Bowl (ant trap, pet dish), C. Buckets/ Utensils, D. Small Tin/ plastic wastes/ Broken bottles/jars/pots, E. Rubber tire, F. Plastic sheet/ Roof gutter, G. Rock & Mud Pools, H. Bamboo stumps/ Tree-holes/ Leaf axils, I. Coconut/coco shell.

RT-PCR Test for Dengue and Chikungunya virus detection

A total of 96 pools were processed by RT-PCR during the study period. The collection consists of *Ae. aegypti* (2), *Ae. albopictus* (94), processed for dengue and chikungunya virus detection in which none of the pools were found positive for dengue and chikungunya virus.

Table 5. Detection of dengue and Chikungunya virus by RT-PCR

Sr. No	Mosquito species	Mosquitoes	Pools tested	Virus positive				
				Dengue Sero type				CHIK
				I	II	III	IV	
1	*Aedes albopictus***	1684	94	0	0	0	0	0
2	*Aedes aegypti* *	5	2	0	0	0	0	0
	Total	1689	96	0	0	0	0	0

** *Ae. albopictus* collected from house hold survey
* *Ae. aegypti* collected from tire shop

DISCUSSION

Vector borne diseases are on the rise in last few years with epidemics of dengue and chikungunya reported in many parts of the state. In the present study, all the vector indices in the district were very high and conducive for the transmission of the disease. *Ae. albopictus* was found to be the predominant species and the primary vector *Ae. aegypti* was not found during the house hold survey. *Ae. albopictus* was more frequently found to develop in artificial larval habitats. This result similar with previous studies from other district of Kerala where this species is mentioned in a wide range of container types from natural to artificial with a preference for natural larval habitats like bamboo stumps and tree holes (Rao et al., 2010), Eapen et al., 2010). Among natural larval habitats sampled, coconut shells were the most common natural larval habitats encountered as coconut constitutes a staple food for local population. Coconut husks are usually kept outdoors near houses for long periods of time and are usually infested by *Aedes* spp. especially during the rainy season. They were also considered to be one of the highly productive larval habitats for *Ae. albopictus* in Kerala (Kumar et al., 2009). In many studies, used tires and barrels were also predominant containers with observed pupal presence [18], which is similar to our findings.

In the present study, dengue and chikungunya virus was not detected in *Ae. albopictus* by RT-PCR methods. However, in Lakshadweep, La Reunion island and Kerala *Ae. albopictus* is the Chikungunya and dengue vector in the absence of *Ae. aegypti* [19, 20, 21]. However, several workers have also reported the transovarial transmission of the dengue virus in *Ae. albopictus* experimentally and from field collected mosquito larvae[22]. This species is a competent vector for dengue and chikungunya virus transmission; it bites humans. Our results showed that *Ae. albopictus* was the predominant species in all localities studied, which could have possibly played the role as the main vector responsible for the dengue transmission. Based on replicate surveys in dry and wet seasons, *Ae. albopictus* could be considered a potential vector in Kottayam district for dengue virus transmission to humans.

The smaller, common household containers and discarded plastic sheet yielded significant numbers of larvae. Objects filled by rain water in natural habitats such as coconut shell, leaf axils, tree hole and cut bamboo stumps gain in importance during the wet season. The control of *Ae. albopictus* is most difficult as the species occupies both natural habitats as well as manmade domestic/peridomestic receptacles which can hold rainwater. The control strategy would require source reduction/larvicidal application in domestic/peridomestic habitats. Rubber tapping shells used to collect latex in the rubber plantation should be flipped over when not in use. Novel strategies to eliminate breeding of *Ae. albopictus* need to be developed to control future dengue outbreaks.

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