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Entomological investigations on sporadic Japanese encephalitis sero-positivity in Tamil Nadu, India

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ABSTRACT: In India, Japanese Encephalitis (JE) continues to be a public health issue in some parts of our country. JE surveillance includes early reporting of clinical cases, sentinel sero-surveys and vector surveillance in the endemic areas. In the present study, JE longitudinal vector surveillance and epidemiological investigations were carried out for the first time during two consecutive years in the endemic district of Tamil Nadu. 22,538 mosquitoes were collected, species identified and screened for JE virus by RT-PCR. Predominant was *Culex tritaeniorhynchus* (60%) and followed by it *Anopheles subpictus* (23%), *Culex quinquefasciatus* (8%) and *Culex gelidus* (3%). It suggests that *Culex tritaeniorhynchus* may act as major vector and *An. subpictus* may act as secondary vector. Monsoon and post-monsoon seasons favour breeding of *Cx. tritaeniorhynchus* leading to vector abundance. Preferential resting sites for *Cx. tritaeniorhynchus* were pig and cattle shed. Although clinical cases have been reported seasonally in the three blocks, the presence of virus among field caught mosquitoes could not be established by RT-PCR. It may be due to the low titre value of JE virus in mosquitoes. This is the first report of JE investigations in the endemic district of Tamil Nadu and it helps to formulate the effective control strategies for JE virus transmission.

KEYWORDS: Flavi virus transmission, vector surveillance, *Culex tritaeniorhynchus*, *Anopheles subpictus*, *Culex gelidus*

INTRODUCTION

Globally more than three fourth of the population is exposed to vector borne diseases and among these, mosquito transmitted diseases that are more prevalent in tropical and sub-tropical countries contribute the major burden. Mosquito-borne diseases, especially malaria, dengue, filariasis and Japanese encephalitis, remain endemic in many tropical countries (Poopathi *et al.,* 2014; Franklinos *et al.,* 2019). Japanese encephalitis (JE) caused by Flavivirus is a major public health concern in rural as well as suburban areas of Asian countries and sporadic spread occurs in northern parts of

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Australia and some parts of Western Pacific. Twenty-four JE endemic nations in Western Pacific and South East Asian areas continue to have Japanese Encephalitis Virus (JEV) transmissions, exposing more than three million populations to the risk of infection with an estimated 68,000 clinical cases every year (WHO, 2019). The first JE case in India was reported at Vellore, Tamil Nadu in 1955 (Webb *et al.,* 1956) and JEV was isolated from human brain tissue in 1958 (Carey *et al.,* 1968). Subsequently, many JE epidemics were reported in 1973, 1978, 2005, 2006 and 2007 from all Southern states and some Eastern and North-Eastern states. Widespread epidemics were also reported from large states like Uttar Pradesh, Madhya Pradesh and Maharashtra (Banerjee *et al.,* 1979; Dhanda and Kaul. 1980; Kabilan *et al.,* 2004; Tiwari *et al.,* 2008; Kumari and Joshi, 2012). In the state of Kerala, JE is endemic in the state of Alappuzha, Kottayam, Trivandrum and Thrissur (Tyagi *et al.,* 2014). In 2009, JEV genotype I was first reported in Gorakhpur region (Fulmali *et al.,* 2011). In India, among symptomatic cases, case fatality was reported to be 20 to 30%, and during the last 10 years, it has been drastically reduced due to better case management. However, permanent neurologic or psychiatric sequelae are not reported in India. JE virus is transmitted through zoonotic cycle among mosquitoes (vectors) and vertebrate – amplifying host primarily pigs and birds (carriers). Infection in the human population is incidental and due to poor viral multiplication in human tissues, there is no transmission from human to mosquitoes. *Culex vishnui* groups consisting of *Cx. tritaeniorhynchus* Gilles, *Cx. vishnui* Theobald and *Cx. pseudovishnui* Colless have been associated as principal vectors for JE. Nevertheless, JE virus has been detected from 16 mosquito species belonging to the genera *Culex* (10), *Anopheles* (3) and *Mansonia* (3) (Kanojia *et al.,* 2003).

In India, twenty four states still are considered as endemic zones. In 2007, a health education programme was conducted to improve the hygiene of population at risk in India. In Tamil Nadu state, an extensive epidemic was reported in 1981 in Cuddalore district, since then, the disease were reduced until 2013 with no death. Latest JE case death was reported during the year 2014 in Tamil Nadu and since then JE occurrence was increasing (NVBDCP, India). Though the Department of Public Health and Preventive Medicine, Tamil Nadu introduced SA 14-14-2 type of JE vaccine more than 10 years back for the children aged one to fifteen years, 12 districts continued to be listed as endemic in the State of Tamil Nadu (NVBDCP, Tamil Nadu). One among the state is the Thiruvallur district where entomological and epidemiological investigations are reported in the present study. Concurrently vector control activities are also being implemented in three JE control units Perambalur, Villupuram, and Cuddalore under the supervision of the monitoring unit at Chennai. However, sero-positive cases of JE are being reported almost every year in these districts indicating JE virus circulation in Tamil Nadu. There has been a change in the epidemiological trend in JE during last decade. JE cases in adult were reported from several districts where JE vaccination programme has been in operational (TAG, 2017). The Government of India introduced E adult vaccination in three states viz: Assam, Uttar Pradesh and West Bengal (Vipin and Ramachandran, 2015). Changing epidemiological trends warranted vector surveillance at several parts of the country. Considering the above seriousness on JE incidence entomological and epidemiological investigation were carried out in the JE endemic district of Tami Nadu to find out the seasonal abundance, adult density, vector infection rate which was not reported earlier to suggest effective strategies for vector control.

MATERIALS AND METHODS

Study area: Thiruvallur district, Tamil Nadu is geographically located between the Latitude of 13°08'37.54" N and Longitude of 79°54'32.00" E. The elevation of the area ranges from 183 m Above Mean Sea Level (AMSL). Being at the North end of the Tamil Nadu State, it is bordered by the Southern end of Andhra Pradesh in North, Kancheepuram district in the South, Bay of Bengal in East and Vellore district in West. The most common occupation of the population is agriculture and the district has more than 131.17 thousand hectares of cropped areas. Seven seasonal rivers

are the major sources of water for cultivation through North-East monsoon (52%) as well as South-West monsoon rain (41%) respectively. The annual average rainfall through both monsoons is 1104 mm. The incidence of JE cases was obtained from the collaborating agency of Department of Public Health and Preventive Medicine and King Institute of Preventive Medicine and Research, Chennai. Based on the JE clinical cases reported in the Thiruvallur district from 2011 to 2016, the villages of Ellapuram, Sholavaram and Thiruvelangadu were selected for this study. It is important to emphasize that, so far entomological surveillance was not carried out in these villages, even though these villages were reported with larger number of JE cases for the past several years.

Mosquito collection: Mosquito collections were carried out indoor and outdoor resting sites during throughout the study period in dusk hours (between 18.00 and 19.30 hours) by using manual and mechanical aspirator. Each selected village was sampled fortnightly for entomological study from January 2017 to December 2018. With the aid of the torch, mosquitoes were collected by manual and mechanical aspirator from walls, ceiling, under furniture, hangings, and curtains. Search for mosquitoes was carried out systematically starting from the main door and moving clockwise inside the house room by room. While collecting mosquitoes from indoor, attention was given to the preferential resting locations for mosquitoes. Resting places were recorded in the standard proforma to determine the preferential indoor resting sites. Collections were carried out for at least 30 minutes depending upon the size of the house and crowding of the domestic items and utensils. New standard CDC miniature light trap was fixed near the pigsties, bushes and cattle sheds, two meters above ground level set before sunset and collected after sunrise in the next morning. The mosquitoes caught during dusk hours and light traps in the field were stored in liquid Nitrogen and transported in labelled containers to the laboratory for species identification.

Species identification and storage: Standard taxonomic key was adopted for mosquito species identification (Reuben *et al.,* 1994). The wild caught mosquitoes were segregated species wise and pooled (25 mosquitoes per pool) for virological assay.

Seasonal abundance: Information in terms of seasonal abundance was collected in four seasons *viz*: winter - cool and dry (December to February), summer - hot and dry (March to June), monsoon - cool and wet (July to September) and finally post-monsoon cool and wet (October to December) seasons. The abundance of JE vector was calculated for different seasons. Vector density was recorded as the number of female mosquitoes per man hour (PMH) spent during collection. The relative density of female mosquito was estimated as the number of females collected PMH and it was denoted as per man hour density (PMD).

Virus detection tests: Virus detection in mosquitoes was carried out by standard Reverse Transcriptase PCR assay. First, wild caught mosquito pools (25 mosquitoes in each pool) were homogenized in a Remi motar (Remi Elektrotechnik Ltd, India) by using separate pestle for each pool. Following centrifugation at 12,000 rpm for 30 minutes, the supernatants were separated and utilized for extraction of viral RNA by Trizol method. The Reverse Transcriptase-PCR reaction was accomplished by Superscript-III one step RT-PCR with Platinum Taq High Fidelity (Invitrogen, Life Technologies, California, USA). Primer pairs JEV-Ef (5'-TGYTGGTCGCTTCCACAYCTC -3') and JEV-Er 5'-AAGATGCCACTTCCACAYCTC-3' were used to amplify the JEV. Following parameters were applied to carry out RT-PCR: 55ÚC for 30 minutes; 94ÚC for 2 minutes; 40 cycles of 94ÚC for 15 seconds, 57ÚC for 30 seconds, and 68ÚC for 1 minute; and a prolonged elongation at 68ÚC for 5 minutes. Purification of RT-PCR products was done following the manufacturer's instructions using the quick Gel Extraction kit (QIAGEN). JEV was confirmed in 1.5% gel electrophoresis along with positive control which was received from NIV, Pune, India. Mosquito virus infection rate (MIR) was expressed using the formula:

$$MIR = \frac{\text{No. of positive pools}}{\text{Total no. of mosquito mosquitoes tested}} \times 1000$$

Data analysis: All field collected information was entered in Microsoft Office 2013 Excel, cross-checked independently. Wherever required, the data were checked with original field data sheets and appropriate corrections were done in digital data before analysis. Data from Excel was transported to statistical software SPSS for analysis. Descriptive statistics mean, standard deviation and proportion were calculated on version 16.0 IBM SPSS statistics for windows.

RESULTS

Preferential resting sites: It was observed that pig and cattle sheds were found to be the most preferential resting sites for the JE vectors such as *Cx. tritaeniorhynchus* and *An. subpictus* respectively. Whereas, the preferential resting site for *Cx. gelidus* was found in vegetative bushes around the domestic houses (Table1). As reported earlier *Cx. tritaeniorhynchus* prefers more on pig sheds whereas *An. subpictus* prefers cattle sheds.

Vector abundance: The seasonal abundance of JE vectors in the endemic district of Tamil Nadu is presented in table 2. It was observed that only the JE vectors of *Cx. tritaeniorhynchus, An. subpictus, Cx. quinquefasciatus* and *Cx. gelidus* were found spreading throughout the four seasons. Comparative density analysis for the high, moderate and less densities indicates the pattern *Cx. tritaeniorhynchus* > *An. subpictus* > *Cx. quinquefasciatus* > *Cx. gelidus*. *Ar. subalbatus* a rare species on the JE transmission was also collected from these study sites (0.2 to 5.6%) (Fig. 8).

The vector species composition in the entire study period revealed fourteen mosquito species from four genera namely *Culex, Anopheles, Aedes* and *Armigeres* were collected from the study blocks of the Thiruvallur district and these comprised of *Culex* (6 species), *Anopheles* (5 species), *Aedes* (2 species) and *Armigeres* (1 species). Though *Cx. tritaeniorhynchus* (60%) and *An. subpictus* (23%) continue to be the major and secondary JE vectors respectively, the collection of *Cx. quinquefasciatus* (8%) *Cx. gelidus* (3%) *Ar. subalbatus* (2%), *An. barbirostris* (*1%)* were also high in these areas and other eight JE vectors such as, *Cx. fuscocephala* (0.4%), *An. vagus* (0.3%), *Cx. bitaeniorhynchus* (0.3%), *An. culicifacies* (0.06%), *Ae. linneatopennis* (0.013%), *Ae. vexans* (0.09%), *Cx. infula* (0.004%) and *An. minimus* (0.004%) were also recorded (Fig. 2). The mosquitoes reported from Ellapuram block includes *Cx. tritaeniorhynchus* (53%), *An. subpictus* (29%), *Cx. quinquefasciatus* (10%), *Cx. gelidus* (3%), *Ar. subalbatus* (2%) *An. barbirostris* (1%) and *An. vagus* (1%) (Fig. 3). In Sholavaram block, *Cx. tritaeniorhynchus* (69%), *Cx. quinquefasciatus* (12%), *Cx. gelidus* (8%), *An. subpictus* (6%), *Ar. subalbatus* (2%), *Cx. bitaeniorhynchus* (1%), *An. barbirostris* (1%) and *Cx. fuscocephala* (1%) were recorded (Fig. 4). Mosquitoes reported in Thiruvelangadu block include *Cx. tritaeniorhynchus* (65%), *An. subpictus* (30%), *Cx. quinquefasciatus* (2%), *An. barbirostris* (2%) and *Cx. fuscocephala* (1%) (Fig. 5).

Consolidated analysis on seasonal variation with respect to vector density showed that the PMD increased during March - June and steadily increased upto post monsoon season of October – December. Thereafter the density declined in the winter season (December – February) (Fig. 6). There was increase in vector abundance during monsoon and post-monsoon for all the four JE vectors though abundance was much higher for *Cx. tritaeniorhynchus* and *An. subpictus* vectors in Ellapuram and Tiruvelangadu blocks. In addition, there was a perceptible increase in vector abundance during summer months and that may be responsible for maintenance of the virus in the environment (Fig. 7 a,b,c). RT-PCR assay results on the detection of JE virus infection in the mosquito pools collected from these three blocks. It was observed that JE positives were not traceable though 902 pools with 22538 mosquitoes were examined (Table 3).

DISCUSSION

Several studies have established that *Cx. tritaeniorhynchus* is the primary vector of Japanese Encephalitis in different locations of India

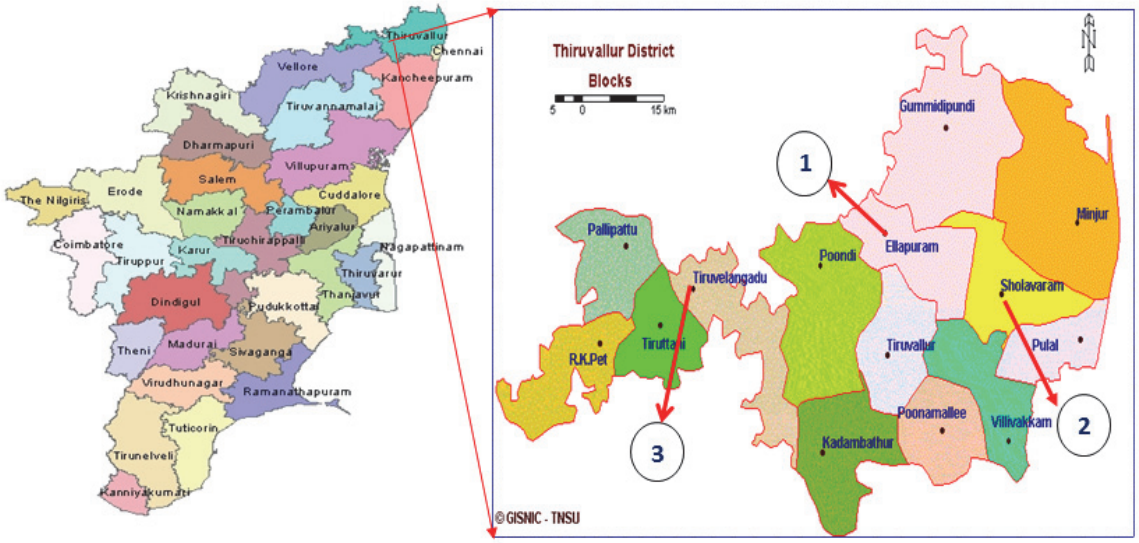

Fig. 1 Study area – Thiruvallur district, Tamil Nadu, India

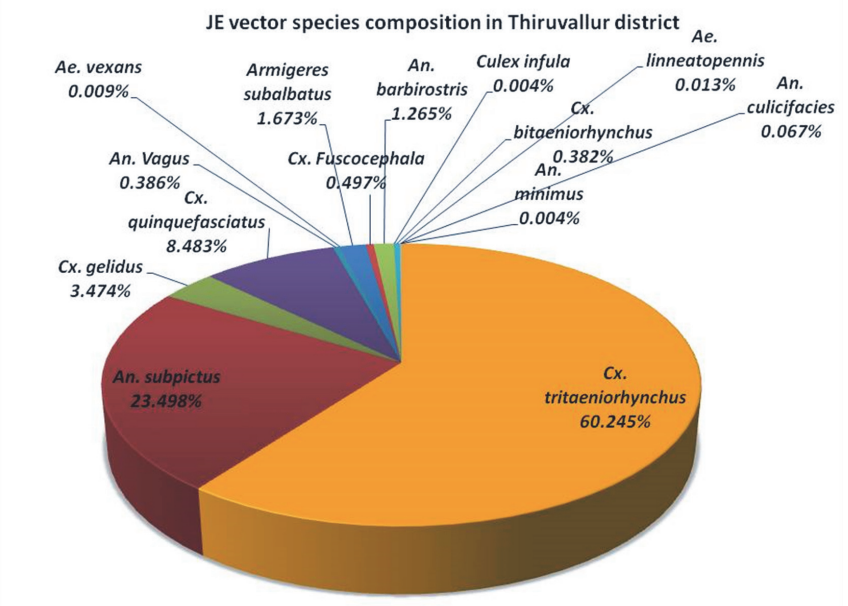

Fig. 2 Mosquito fauna of Thiruvallur district, Tamil Nadu, India

(Dhanda *et al.,* 1997; Burke and Monath 2001; Murthy *et al.,* 2002; Kanojia, 2007; Arunachalam *et al.,* 2009; Ramesh *et al.,* 2015; Samuel *et al.,* 2018). In the present study, although JE confirmed cases were reported in the study areas, so far no JE-vector surveillance was carried out. Thiruvallur district is an adjacent location and has a close proximity the metropolitan areas of Chennai and transmission potential to urban areas is also to be considered. Therefore, this report has significant public health importance for the district programme officer (Thiruvallur district, Tamil Nadu). Further, in Kerala, Karnataka, Andhra Pradesh, Utter Pradesh and West Bengal, various secondary

vectors have been identified, which include, *Ma. indiana, Cx. gelidus, Cx. whitmore, Cx. pseudovishnui, Cx. epidesmus, An. peditaeniatus, An. subpictus* and *Ma. uniformis* (Kelly Hope *et al.,* 2004; Pani *et al.,* 2004; Khan *et al.,* 1996; Arunachalam *et al.,* 2009). In Tamil Nadu also (Cuddalore, Villupuram and Tanjore), *Cx. gelidus* was reported as the secondary vector (Ramesh *et al.,* 2015; Samuel et al., 2015, 2016a,b, 2018). In the present study, *An. subpictus* was reported for

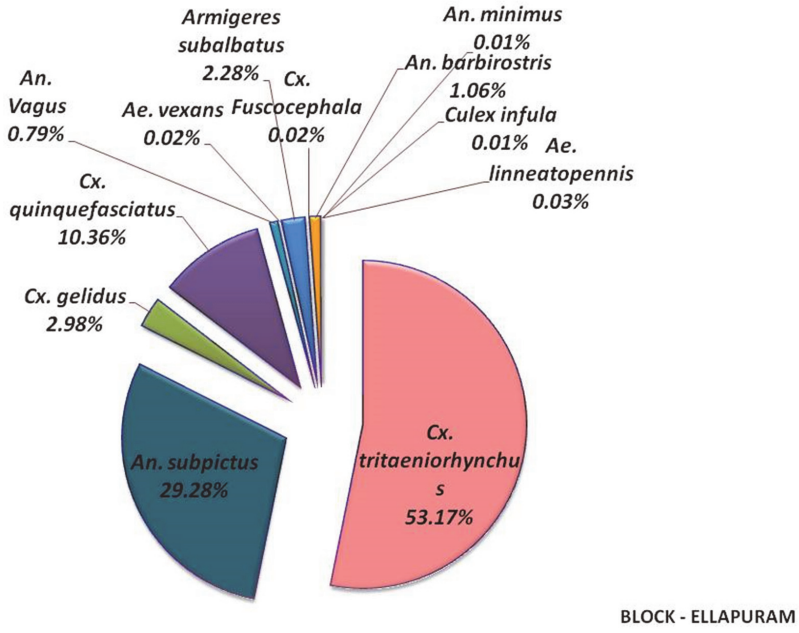

Fig. 3 JE vector abundance in Ellapuram - Thiruvallur district, Tamil Nadu, India

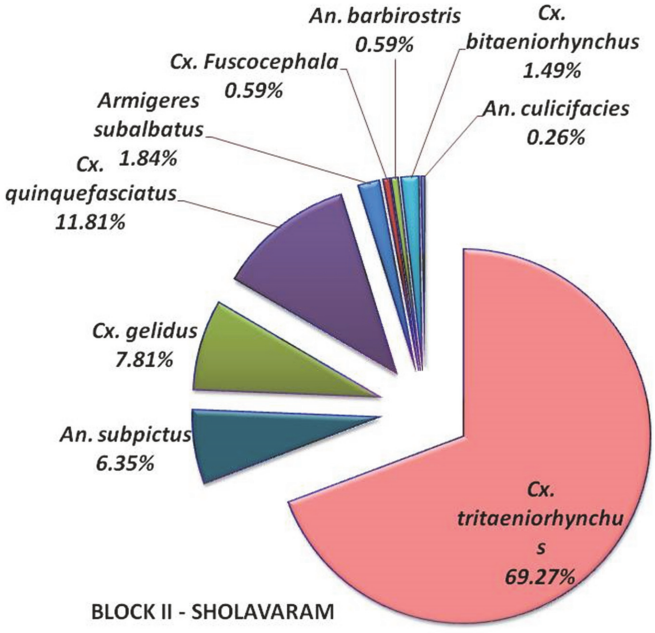

Fig. 4 JE vector abundance in Sholavaram - Thiruvallur district, Tamil Nadu, India

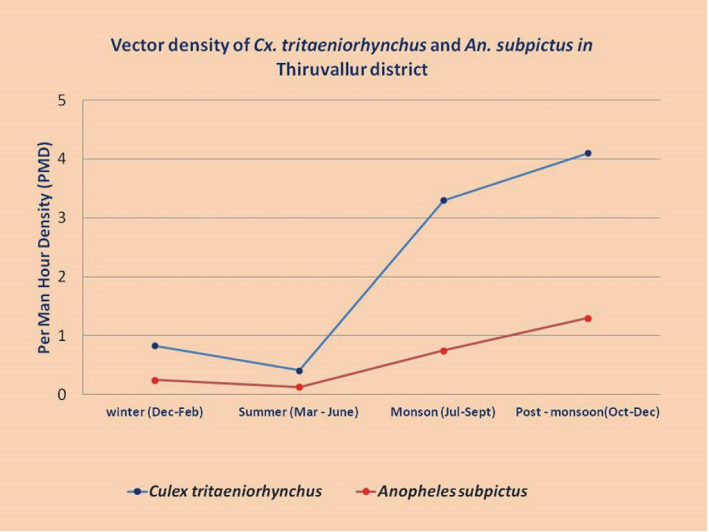

Fig. 5 JE vector abundance in Tiruvelangadu – Thiruvallur district, Tamil Nadu, India

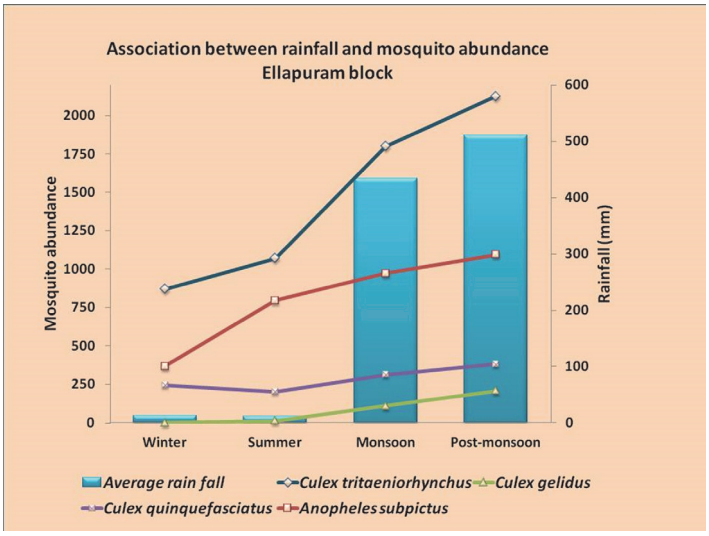

Fig. 6 Vector density of JE vector in Thiruvallur district, Tamil Nadu, India

the first time in the JE endemic district (Thiruvallur) as secondary species. Our findings corroborate with previous workers who have reported the *An. subpictus* as secondary vectors in JE prone areas such as Kuddapah (AP), Tirunelveli and Virudhu Nagar (TN) (Murthy *et al.,* 2002; Thenmozhi *et al.,* 2015; Anandh and Sevarkodiyon, 2017).

The population dynamics of JE vectors largely depend upon rice cultivation, water bodies, temperature and humidity in the rural areas. In these study sites, agricultural farming is the primary source of income for inhabitants, therefore agricultural farming favours prolific breeding of JE vectors especially *Cx. tritaeniorhynchus* followed by *An. subpictus*. Seasonal abundance of these mosquito species showed that *Cx. tritaeniorhynchus* and *An. subpictus* were very high in cool - dry (December to February) and cool - wet (July to September) seasons. This observation is in agreement with earlier findings (Ramesh *et al.,* 2015).

The per man hour density (PMD) ranged from 0.8 – 4.1 for *Cx. tritaeniorhynchus* and 0.25-1.3 for *An. subpictus* in the study period of January to December 2017 and similar trend was also observed in the subsequent year of field studies (2018). This entomological observation revealed a significant increase in vector abundance during monsoon season as reported inother areas like Andhra Pradesh and Tamil Nadu (Murthy *et al.,* 2002; Ramesh *et al.,* 2015; Malar *et al.,* 2015; Samuel *et al.,* 2016). JE vector abundance was high in rainy season (June to December). However, when vector density was plotted site-wise, there were two peaks, first during the summer and then during monsoon in Sholavaram blocks due to increase in density of the *Cx. tritaeniorhynchus*. A similar observation was reportedwith peak density of *Cx. quinquefasciatus* followed by *Cx. tritaeniorhynchus* in Bareilly district of Uttar Pradesh (Pantawane *et al.,* 2017). In the present study, the wild-caught mosquitoes of 902 pools collected from Thiruvallur district showed no positivity for JE virus. Similar results were reported by Changbunjong *et al.* (2013) in Thailand. During our study period, only ten sero-positive JE cases were reported by Department of Public Health and Preventive Medicine, Chennai. Therefore it is understood that JE positive cases recurrently occurring in these study areas (Ellapuram, Sholavaram and Tiruvelangadu blocks) but the titre values of JE virus transmission may be low and it is presumed that this may be due to the non-appearance of JE-virus positivity in mosquito pools during our study period.

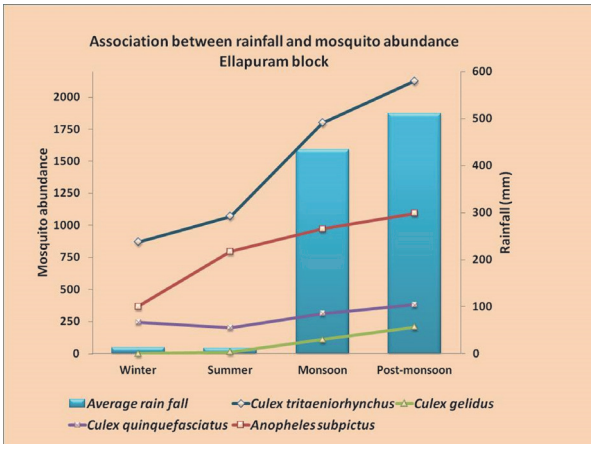

Fig. 7a Association between rainfall and mosquito abundance – Ellapuram block.

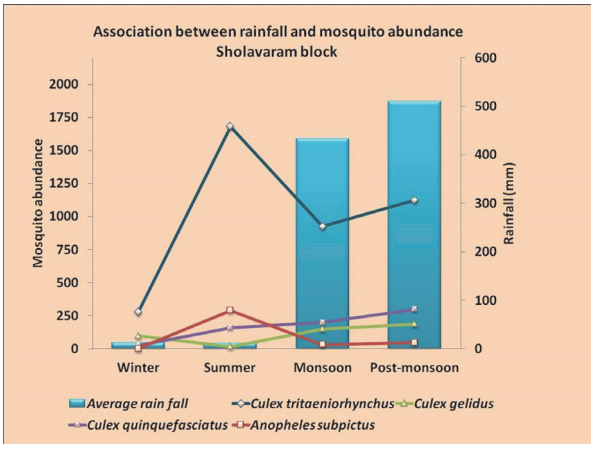

Fig. 7b Association between rainfall and mosquito abundance - Sholavaram block

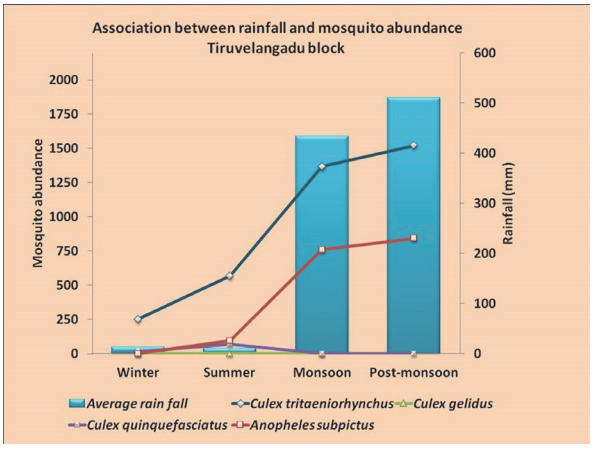

Fig. 7c Association between rainfall and mosquito abundance - Thiruvelangadu block

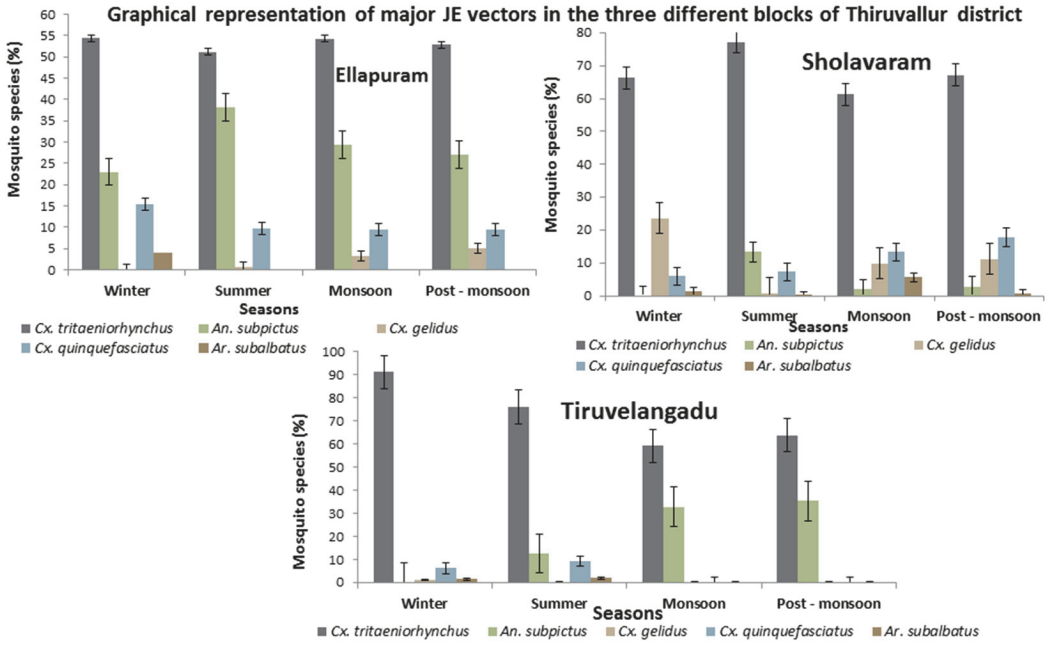

Fig. 8 Graphical representation of major JE vectors in the three different blocks of Thiruvallur district

In the present study it has been established that all three study sites continue to harbour mosquitoes responsible for JE transmission and *Cx. tritaeniorhynchus* continues to be the predominant JE vector in this region. Paddy cultivation and the close proximity of Pulicat bird sanctuary are the potential risk factors for JE transmission. Reported cases from the DDPH indicated that there has been a shift in JE incidence to higher age groups. Studies from different parts of India have established age-shift in the occurrence of JEV infection (Borathur *et al.,* 2013; Gunasekaran *et al.,* 2012). Vector surveillance along with serological studies in amplifier animals such as pigs and reservoir animals such as egret is a preferable approach which can be used as an early warning system (Baruah and Hazarika 2018).Pulicat bird sanctuary may lead the JEV transmission through migratory birds from

Table 1. Preferential resting places for Japanese encephalitis vectors

Mosquito resting places	Mosquito species Density			
	Cx. tritaeniorhynchus	*An. subpictus*	*Cx. gelidus*	*Cx. quinquefasciatus*
Thatched roof	+	++	+	+++
Sleeping mat	+	+	+	+++
Wooden shelf	+	+	+	+++
Clothes in rope	++	+	+	+++
Cattle shed	+++	++++	+	+
Pig shed	++++	++	++	++
Bushes	++	++	++++	+

+ Low density ++ Moderate density +++ High density ++++ Very high density

Table 2. Seasonal abundance of JE vector in Thiruvallur district, Tamil Nadu, India

Study site	Season	Mosquito species													
		Cx. tritaenior hynchus	*An. subpictus*	*Cx. gelidus*	*Cx. quinque fasciatus*	*An. Vagus*	*Ae. vexans*	*Armigeres subalbatus*	*Cx. Fuscoce phala*	*An. barbiro stris*	*Culex infula*	*Cx. bitaenior hynchus*	*Ae. linneato pennis*	*An. culicifa cies*	*An. minimus*
Ellapuram	winter	54.4	23.0	0.0	15.3	5.4	0.1	1.3	0.1	0.0	0.1	0.0	0.2	0.0	0.0
	summer	51.2	38.1	0.6	9.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	monsoon	54.3	29.3	3.3	9.4	0.0	0.0	2.1	0.0	1.6	0.0	0.0	0.0	0.0	0.0
	post - monsoon	52.8	27.1	5.1	9.5	0.0	0.0	3.9	0.0	1.6	0.0	0.0	0.0	0.0	0.0
Sholavaram	winter	66.3	0.0	23.6	6.0	0.0	0.0	1.4	0.7	0.0	0.0	0.7	0.0	1.2	0.0
	summer	77.1	13.3	0.7	7.3	0.0	0.0	0.1	1.4	0.0	0.0	0.0	0.0	0.0	0.0
	monsoon	61.3	2.0	9.9	13.3	0.0	0.0	5.6	0.0	2.3	0.0	5.0	0.0	0.7	0.0
	post - monsoon	67.1	2.8	11.2	17.8	0.0	0.0	0.7	0.0	0.0	0.0	0.4	0.0	0.0	0.0
Thiruvelangadu	winter	91.2	0.0	1.1	6.2	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	summer	76.0	12.7	0.0	9.3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	monsoon	59.1	32.8	0.0	0.0	0.0	0.0	0.0	3.1	5.0	0.0	0.0	0.0	0.0	0.0
	post - monsoon	63.8	35.3	0.0	0.0	0.0	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0

Table 3. Japanese encephalitis virus infection in field caught mosquitoes in Thiruvallur District, Tamil Nadu, India

Species	BLOCK I - ELLAPURAM			BLOCK II - SHOLAVARAM			BLOCK III – THIRUVELANGADU			TOTAL		
	Number of Mosquitoes	Number of Pools	Number of pools Positive	Number of Mosquitoes	Number of Pools	Number of pools Positive	Number of Mosquitoes	Number of Pools	Number of pools Positive	Number of Mosquitoes	Number of Pools	Number of pools Positive
Culex tritaeniorhynchus	5867	235	0	4001	160	0	3710	148	0	13578	543	0
Anopheles subpictus	3231	129	0	367	15	0	1698	68	0	5296	212	0
Culex gelidus	329	13	0	451	18	0	3	0	0	783	31	0
Culex quinquefasciatus	1143	46	0	682	27	0	87	3	0	1912	76	0
Anopheles Vagus	87	3	0	0	0	0	0	0	0	87	3	0
Aedes vexans	2	0	0	0	0	0	0	0	0	2	0	0
Armigeres subalbatus	252	10	0	106	4	0	19	1	0	377	15	0
Culex fuscocephala	2	0	0	34	1	0	76	3	0	112	4	0
Anopheles barbirostris	117	5	0	34	1	0	134	5	0	285	11	0
Culex infula	1	0	0	0	0	0	0	0	0	1	0	0
Culex bitaeniorhynchus	0	0	0	86	3	0	0	0	0	86	3	0
Aedes linneatopennis	3	0	0	0	0	0	0	0	0	3	0	0
Anopheles culicifacies	0	0	0	15	1	0	0	0	0	15	1	0
Anopheles minimus	1	0	0	0	0	0	0	0	0	1	0	0
Grand Total	11035	441	0	5776	231	0	5727	229	0	22538	902	0

another country. The study revealed that the potential primary vector and secondary vector in Thiruvallur district as *Cx. tritaeniorhynchus* and *An. subpictus* respectively and seasonal abundance of JE vectors were high during July- December which is a monsoon season in Tamil Nadu. However, in Sholavaram block there were two peaks during the year indicating that increase in mosquito density was due to high density of *Cx. tritaeniorhynchus*. In this block, first peak was observed during summer which indicates that the sporadic rain might be responsible for the vector abundance and also emphasizes fortnightly vector surveillance.

Public Health Department of Tamil Nadu reported five, six and four JE sero-positive cases from the entire district of about 370 thousand population from 12 revenue blocks during the year 2016, 2017 and 2018 respectively. Only one and 2 sero-positive cases were reported from the study areas in 2016 and 2017 respectively. These areas are also covered under JE vaccination programme. These factors indicate that JE do not occur in epidemic proportion like the State of Uttar Pradesh, India. For such a low level of sporadic transmission, the viral load in vector mosquitoes is likely to be low and therefore, we were not able to detect JE positivity in RT-PCR assays. However, reporting of the JE sero-positivity in sporadic locations cannot be ignored. A close entomological and epidemiological investigation is suggested to monitor at regular intervals. The areas are covered under this study has the Pulicat bird sanctuary of about 480 sq. km shared with the adjacent State Andhra Pradesh. The Pulicat bird sanctuary harbour thousands of greater flamingos and it is the major feeding and breeding ground many migratory birds like storks and pelicans. Locations from the study areas are in close proximity to the bird sanctuary as well as the areas of Chennai Metropolitan city. If the transmission is allowed to set in the urban metropolitan areas in Chennai, it will lead to a major public health emergency. Therefore, further extensive vector surveillance activities to provide the lead for epidemiological intelligence and thereby the prevention of urban JE epidemics. The public health authorities need to create awareness among the communities on mosquito abundance, seasonal variations, vaccination and vector control strategies to prevent JE transmission. More sensitive PCR assays to pick up low level JE infection must be developed for a robust surveillance system in India.

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