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A three year longitudinal study on the seasonal Japanese encephalitis vector abundance in Thanjavur district, Tamil Nadu, India

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Abstract
Japanese encephalitis is spread by paddy breeding infected vector mosquitoes of the *Culex vishnui* subgroup and *Cx. gelidus* and amplified by infection of pigs / *Ardeidae* birds in nature. A longitudinal study of seasonal vector abundance of JE vectors was monitored from 2011-2014 in 3 villages from Thanjavur district by monitoring the abundance resting in and around cattle sheds during dusk hours which showed *Cx. tritaeniorhynchus* (83.41% to 61.7%) and *Cx. gelidus* (9.83% to 33.19%) to be the predominant species. Study revealed the seasonal abundance of *Cx. tritaeniorhynchus* and the *Cx. gelidus* the predominant vectors present in Thanjavur district, Tamil Nadu. Vector abundance clearly demarcates the time of public health threat in this area. Residents in these areas should take personal protection to reduce the number of mosquito bites, which include minimizing vector exposure at dusk and dawn, covering the body, using insect repellents and sleeping under mosquito nets.

Keywords: Japanese encephalitis virus, *Culex tritaeniorhynchus, Culex gelidus,* Thanjavur, Tamil Nadu.

1. Introduction
Japanese encephalitis (JE) is one of the major public health problems in many parts of South East Asia. Approximately 3 billion people (60 % of the world's population) live in JE endemic regions [1]. JE is endemic mainly to rural areas with rice fields domiciling the breeding zones for vectors. It is estimated that 1.9 billion people are currently living in rural JE- prone areas of the world. Among them, 220 million people live in proximity to rice irrigation schemes [2]. The prevalence of JE is higher in countries with lower socioeconomic status, when compared to more affluent neighbouring countries, indicating the importance of economic and social stability as additional risk factors that impact the transmission and prevalence of JE in non-immune populations [3]. This cycle involves pigs as the major reservoir / amplifying host, water birds as carriers and mosquitoes as vectors. The *Culex vishnui* subgroup of mosquitoes had been implicated as major vectors of JE. Japanese encephalitis is the most important cause of viral encephalitis and a leading cause of disability among the paediatric and rural folks in Asia. JE causes long term neurologic sequelae and the disease burden varies from country to country [4]. Understanding the seasonal JE vector pattern is a key step to effectively understanding JEV risks and also to preventing additional outbreaks of JE in endemic countries [5].

Several JE outbreaks with varying severity were reported from different parts of India [6]. Major outbreaks of JE had been reported predominantly in rural areas from different parts of the country. This disease had spread to 25 states/ union territories at varying degree of intensity [7, 8]. JEV is transmitted mostly by *Cx. tritaeniorhynchus* and *Cx. gelidus* in India [9].

The present state of our knowledge on the actual/potential species and bionomics of mosquitoes involved in JEV transmission is too meagre to permit evolving scientific strategies for effective control of the disease. It is imperative that seasonal vector abundance is to be made to map out areas of influence of known JE vectors. Long term observations are needed to determine the density of adults of vectors in different seasons, to study the pattern of density build up. Long term ecological studies in JE prone villages will help to gather valuable baseline data to define the mode of JE transmission in an area which will be useful to develop an early warning system to apply appropriate vector control strategies to avoid the probabilities of future outbreaks. This study was intended to understand the seasonal vector abundance of the JE vectors to undertake relevant control strategies to protect the public from this dreaded disease.

2. Materials and Methods

2.1 Study Area

Thanjavur, formerly Tanjore, is a city in the south Indian state of Tamil Nadu. Thanjavur is an important center of South Indian religion, art, and architecture. Thanjavur district lies on southern part of Tamil Nadu between 10.7825° N, 79.1313° E. This district has a total area of 36.33 km² and is inhabited by a population of 222,943 in 2011. The city is an important agricultural centre located in the Cauvery delta and is known as the "Rice bowl of Tamil Nadu". A longitudinal study of vector abundance was conducted from 2011-2014 in 3 villages of Tanjore district, namely Muthur, Keezhapattu and Kovilur which were selected with the guidance of DPH&PM Tanjore. A detailed discussion was held with Medical officers in the PHCs regarding JE cases reported from these areas. Tanjore district receives rain showers from June to September under the influence of the Southwest monsoon, and heavier rainfall from October to December from the Northeast monsoon.

2.2 Mosquito collection

Mosquitoes were sampled from identified villages at bimonthly intervals during 2011 to 2014. Adult mosquitoes were collected resting on bushes and thatched roofs of cattle sheds during dusk hours. Mosquito samples were transported to the field laboratory and identified up to species level [10]. Mosquito (only females) abundance was calculated as number collected per man-hour (PMH).

3. Results

A three year longitudinal study on vector abundance in Thanjavur district during 2011-14 showed that Culicines formed 96.06% and the Anophelines formed 3.93%. Total collection recorded during the pre-monsoon season was 44.5% (144.17 PMH density) which increased during post monsoon season 55.5% (179.52 PMH) collections. Abundance of the Culicine mosquitoes increased from 44.2% (137.48 PMH) to 55.5% (173.48 PMH) whereas the Anophelines decreased from 4.6% (6.69 PMH) to 3.36% (6.04 PMH) during pre to post monsoon seasons.

Culex tritaeniorhynchus was found dominant (78%) in all the study villages followed by *Cx. gelidus* (15.08%). Total abundance of *Cx. tritaeniorhynchus* decreased from 88% (126.8 PMH) to 70.4% (126.33 PMH) but the PMH density did not show any variation between pre to post monsoon seasons. But *Cx. gelidus* showed remarkable change in its abundance from 4.5% (6.56 PMH) to 23.4% (42.24 PMH) from pre to post monsoon seasons. *Culex* species abundance increased during the third year 2013-14 after a steep decline during the first two years (Fig.1). Anophelines and other culicines maintained lower density throughout this period. Compared to SW monsoon NE monsoon rainfall was more. Composition of Anophelines showed that there is a decrease in the case of *An. subpictus* from 4.1% (5.9 PMH) to 0.45% (0.81 PMH) whereas there was an increase in the case of *An. peditaeniatus* species from 0.3% (0.44 PMH) to 2.06% (3.7 PMH) during pre to post monsoon periods (Fig 2).

Though there was no significant variation in the different study villages during different seasons for *Cx. tritaeniorhynchus*, *Cx. gelidus* showed significant variation only in the village Muthur, between pre and post monsoon periods (p= 0.041) (Table 1). But *Cx. tritaeniorhynchus* showed significant variation between the 3 pre-monsoon periods and also during the 3 post monsoon periods in all the villages (Table 2). But *Cx. gelidus* showed significant variation during the post monsoon period (Table 2). Similarly between different years, there was no significant variation in the vector abundance (Table 3).There was no significant variation between the years during pre and post monsoon seasons for both *Cx. tritaeniorhynchus* and *Cx. gelidus* (Table 4.). *Cx. tritaeniorhynchus* during the SW monsoon was more in density compared to NE monsoon in all the years whereas *Cx. gelidus* showed always increase in its density only during the NE monsoon period and increase in its abundance from the SW monsoon season collections (Fig.1). Thus there was a gradual build up of *Cx. gelidus* from 2011-14 whereas *Cx. infula* showed increase in the first two years and a few collections were reported in the third last year (2013-14) (Fig 3). *Neomelaniconion lineatopenne* (Ludlow, 1905) was collected in more numbers during the pre-monsoon period (4.29%) and was collected in both the seasons. Other culicines (0.64 to 1.48%) and the other Anophelines (0.2 to 1.24%) increased in the density and showed an increasing trend from pre to post monsoon periods. Among the Anophelines the predominant *Anopheles subpictus* (1.63%) recorded more during the pre-monsoon period which was replaced by *Anopheles peditaeniatus* (2.39%) abundance during the post monsoon period. Though there was a moderate positive correlation (r=0.326; *P*= 0.301) between the volume of rainfall and the density of *Cx. tritaeniorhynchus*, it was not statistically significant. Likewise, the density of *Cx. gelidus* also did not show significant relationship with rainfall (r=0.068; *P*= 0.834).

Table 1: Comparison of mean density of mosquito species during pre and post monsoon season in the study villages of Thanjavur district (2011-2014)

Villages	Species	Mean PMH (± SD)		*P* value
		Pre monsoon (July-August)	Post monsoon (December-January)	
Muthur	*Cx. tritaeniorhynchus*	52.06 ± 37.78	144.28 ± 105.46	0.132
Keezhapattu		221.61 ± 82.99	170.22 ± 109.11	0.31
Kovilur		106.78 ± 48.43	64.50 ± 60.45	0.132
Muthur	*Cx. gelidus*	19.17± 25.76	123.67 ± 140.35	0.041*
Keezhapattu		0.39 ±0.49	1.00 ± 1.108	0.279
Kovilur		0.11± 0.272	2.06 ± 4.08	0.152

*Significant at 5%

Table 2: Comparison of mean density of predominant vector species between pre and post monsoon season from different study villages in Thanjavur district (2011-14)

Species	Season	Mean PMH (± SD)			*P*value
		Muthur	Keezhapattu	Kovilur	
Cx. tritaeniorhynchus	Pre monsoon	52.06 ± 37.78	221.61 ± 82.99	106.78 ± 48.43	0.001*
	Post monsoon	144.28 ± 105.46	170.22 ± 109.11	64.50 ± 60.45	0.000**
Cx. gelidus	Pre monsoon	19.17± 25.76	0.39 ±0.49	0.11± 0.272	0.190
	Post monsoon	123.67 ± 140.35	1.00 ± 1.108	2.06 ± 4.08	0.000**

*Significant at 0.5%**Significant at 0.1%

Table 3: Comparison of mean density of vector species during pre and post monsoon season in Thanjavur district (2011-14)

Year	Species	Mean PMH (± SD)		*P* value
		Pre monsoon (July-August)	Post monsoon (December-January)	
2011-2012	*Cx. tritaeniorhynchus*	153.44 ± 108.28	122.61 ± 94.10	0.623
2012-2013		104.89 ±61.89	66.78 ± 49.21	0.18
2013-2014		122.21 ± 107.81	189.61 ± 116.68	0.31
2011-2012	*Cx. gelidus*	2.500 ±5.50	9.94 ± 13.16	0.156
2012-2013		2.78±3.46	14.78 ± 28.10	0.665
2013-2014		14.89 ± 28.24	102.00 ± 155.71	0.149

Table 4: Comparison of mean density of vector species during pre and post monsoon season observed during different years in Thanjavur district

Species	Season	Mean PMH (± SD)			*P* value
		2011-2012	2012-2013	2013-2014	
Cx. tritaeniorhynchus	Pre monsoon	153.44 ± 108.28	104.89 ±61.89	122.21 ± 107.81	0.671
	Post monsoon	122.61 ± 94.10	66.78 ± 49.21	189.61 ± 116.68	0.136
Cx. gelidus	Pre monsoon	2.500 ±5.50	2.78±3.46	14.89 ± 28.24	0.564
	Post monsoon	9.94 ± 13.16	14.78 ± 28.10	102.00 ± 155.71	0.386

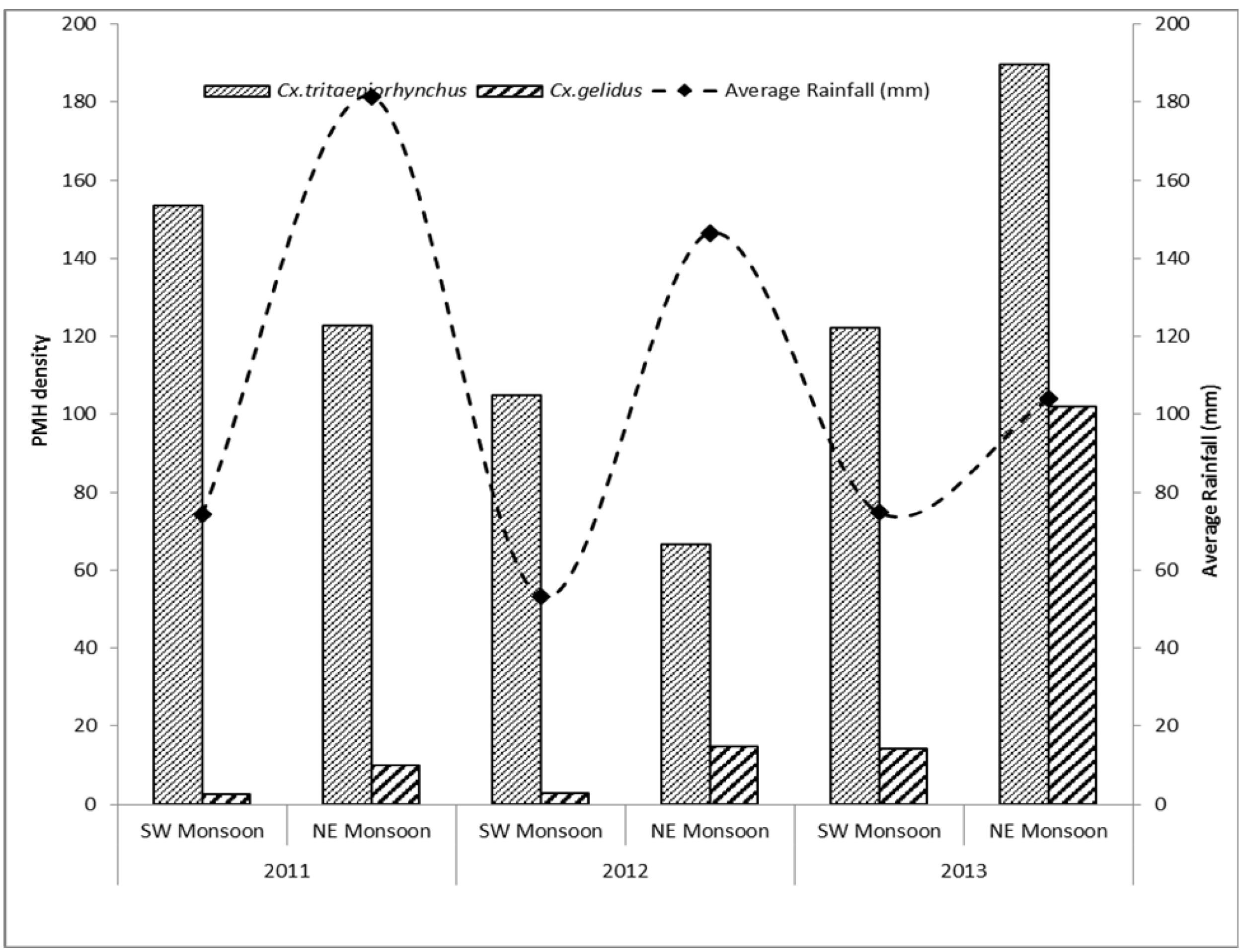

Fig 1: Season abundance of major *Culex* species correlated with rainfall in Thanjavur district, Tamil Nadu

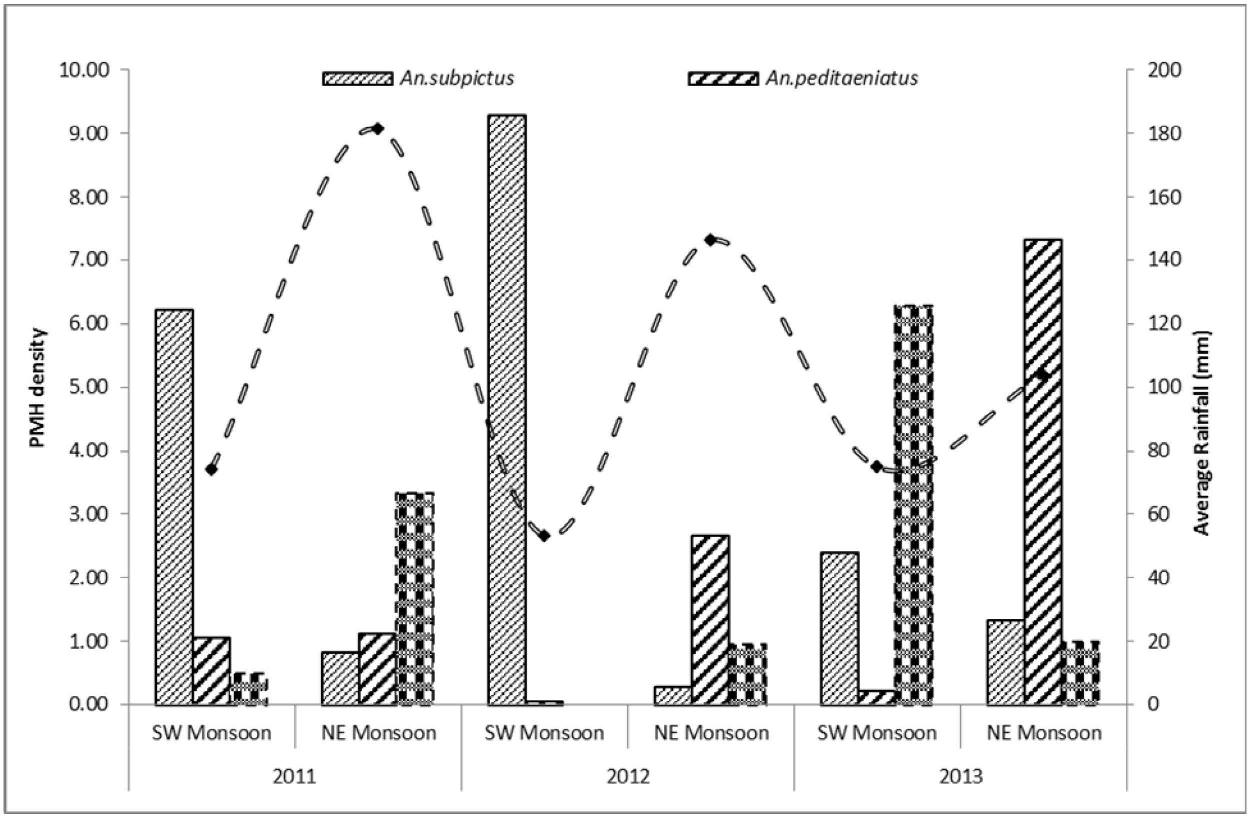

Fig 2: Seasonal abundance of major Anopheles mosquito species correlated with rainfall level in Thanjavur district (2011-2014)

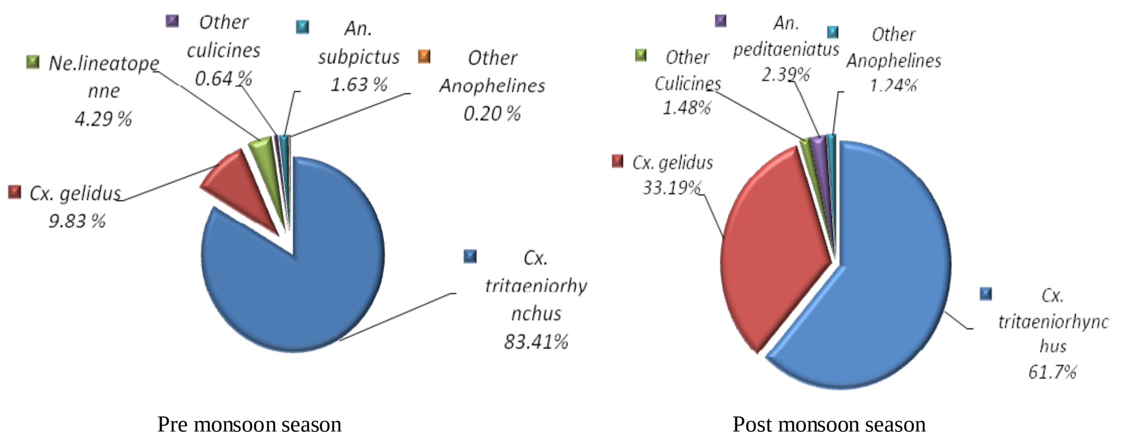

Fig 3: Species composition of mosquitoes collected from pre & post -monsoon seasons in Thanjvur district, Tamil Nadu (2011-14)

4. Discussion

A detailed longitudinal study of the JE vectors of Thanjavur district was not undertaken so far. The present study gave a clear picture on the composition, relative abundance and seasonal population fluctuation of JE vectors from these study areas. Present study demonstrated the seasonality of mosquitoes. The combined mosquito population gradually build up during pre-monsoon, reached its maximum during post-monsoon season. Percentage of these mosquitoes collected during pre-monsoon and post-monsoon were 44.5% & 55.5% respectively. Many of the species showed increase in the population from pre to post monsoon periods. Similar trend was observed in Kottayam, Kerala where *Cx. tritaeniorhynchus* total composition decreased from 83.41% to 61.7% and an increase in its abundance recorded from 9.83% to 33.19% from the pre to post monsoon season was noticed [11].

Culex tritaeniorhynchus

The pattern of rainfall and agriculture may affect the larval habitats and vector population size [12, 13]. A high density of *Cx. tritaeniorhynchus* was recorded in paddy cultivated areas of Cuddalore (erstwhile South Arcot) and Madurai districts of Tamil Nadu and Mandya district of Karnataka [14-16]. The abundance of vector species is the most important factor for the outbreak of disease [17]. *Cx.tritaeniorhynchus* was the most abundant species and showed two density peaks, first in pre-monsoon and second in post monsoon. This species has also been incriminated as a major vector of JE in India as well as in many countries of Southeast Asia [6, 16, 18-20]. *Cx. tritaeniorhynchus* yielded maximum number of JE isolates in India [16, 21]. *Cx. tritaeniorhynchus* was identified as the primary JE vector in Kerala and Tamil Nadu [13, 16, 22]. This species is known to breed in rice fields and its population dynamics is closely associated with paddy cultivation [13]. *Cx. tritaeniorhynchus* showed increase in abundance during pre-monsoon and post monsoon period corresponding to rice crop. The occurrence of JE cases was found to be associated with high density of *Cx. tritaeniorhynchus* [13]. The major proportionate contribution of this species was found to be a significant feature in several JE affected areas in India [16, 23, 24]. *Culex tritaeniorhynchus* is the major vector of JE in Thanjavur district. Continuous monitoring and control measures are essential for the prevention of JE in this district which is maintained in the silent transmission mode. JE transmission is mainly facilitated by two important factors namely, global climate change and the modulation of agriculture [25]. Within the past 40 years, rice agriculture in JEV endemic countries has increased by 20%, thereby expanding *Cx. tritaeniorhynchus* habitat and increasing human risk of exposure to vector populations [2]. The larval habitat of *Cx. tritaeniorhynchus* is primarily low lying flooded areas containing grasses and flooded rice paddies, but this species can also be found in urban environments in close proximity to human populations [26]. An increase in the amount of flooded rice field habitat has shown to be positively correlated with increases in adult populations of *Cx. tritaeniorhynchus* in Korea. Locations of human cases of JE generally fell within the higher probability areas of *Cx. tritaeniorhynchus* [5]. Rapid response and prevention are the immediate requirements. When there is a precise probability on the key element-'vector abundance', predictability will be stronger for taking precautionary action to avert the possible disease outbreaks [27]. *Culex tritaeniorhynchus* is largely responsible for transmission of JE in Kurnool district of Andhra Pradesh, India, although other species such as *Cx. gelidus* have been identified in urban areas of the study area. The mosquitoes *Cx. tritaeniorhynchus* and *Cx. gelidus* vector abundance increased after the monsoon period and lowest in dry season [28].

Culex gelidus

This species of mosquito was reported from Maharashtra, Goa [29], Rajasthan [30], Karnataka, Kerala [31], Tamil Nadu [16], Andhra Pradesh [32, 33], Uttar Pradesh [24], West Bengal [34] and Assam [31, 35]. Extensive studies carried out in Mysore district of Karnataka state showed only a negligible percentage (0.02%), compared to the total mosquito population [36, 37]. In Mysore, breeding of these species was seen only in ground pools and not in paddy fields. In Mandya and Kolar districts, JEV endemic areas in Karnataka, *Cx. gelidus* population was found to be <10% of the total mosquitoes collected during 1983–88. Recently a spurt in the population of *Cx. gelidus* was observed in many southern states of India, *viz*. Andhra Pradesh, Tamil Nadu and Kerala. *Cx. gelidus* population was approximately 50% of the total mosquito population in peri-urban areas of Kurnool district of Andhra Pradesh during a four year study [32]. This has been substantiated by subsequent studies carried out by Murthy *et al.* 2010 [9] as they observed predominantly high percentage (68.05%) of *Cx. gelidus* in the urban areas of Kurnool district. In rural areas, higher prevalence of *Cx. tritaeniorhynchus* (57.51%) was observed. Cuddalore district of Tamil Nadu reported high density of *Cx. gelidus* as *Cx. tritaeniorhynchus, Cx. vishnui, Cx. gelidus* and *Cx. fuscocephala* constituted 93.6% of the total mosquito population during 1991–94 [16]. A tremendous increase in the population of *Cx. gelidus* was observed in the last few years. In a year-long study conducted during 2012–13, 57.9% of the total mosquito collection was constituted by *Cx. gelidus* whereas during 2009, it was recorded only 17% of the total population in the same area [38]. *Cx. tritaeniorhynchus* population of Alappuzha has shown Mansoniods during the inter-epidemic period to maintain the JE virus [13, 39, 40]. South India points a gradual replacement of *Cx. tritaeniorhynchus* mosquitoes by *Cx. gelidus* in the urban and semi-urban areas. The availability of ample breeding habitats, *i.e.* rice fields and water bodies such as ponds, ditches and canals have attributed for the sudden increase in *Cx. gelidus* populations. Changes in environment and pollution of water bodies must have given an advantage to *Cx. gelidus* over *Cx. tritaeniorhynchus* as the former is known to breed mostly in muddy pools with high concentration of organic matter [41]. The accumulated manures and stagnant water left over in the paddy field converted into sugarcane field facilitate *Cx. gelidus* breeding.

Increase in the population of *Cx. gelidus* pose a major threat to public health but the population is negligible and the major JEV vectors are *Cx. vishnui* group, mainly *Cx. tritaeniorhynchus*. However, in Assam, it has been observed that *Cx. gelidus* is widely distributed in large numbers [35]. *Cx. gelidus* occupied the second position in its abundance during this study which occupied third or fifth position in the previous studies [16]. Many isolations of JEV have been made from this species in India [16, 42]. It is suggested that more systematic studies are required to understand the decrease in *Cx. tritaeniorhynchus* population compared to *Cx. gelidus* as both share nearby breeding habitats.

Cx. infula was prevalent during and subsequent to rainy season[43]. Thus there was a gradual build up of *Cx. infula* from 2011-14 and showed an increase in the first two years and a few collections were only reported in the third last year (2013-14). Similar trend was observed in Kerala [11]. Breeding habitats and feeding habits of larval *Cx. infula* are similar in nature to those of 2 closely associated species *Cx. bitaeniorhynchus* and *Cx. epidesmus* [24].

Anophelines

The other known vectors of JE in India viz., *An. subpictus* and *An. peditaeniatus* were also caught in good numbers during the JE transmission season [21, 24, 43]. Seasonal patterns of *An. subpictus* were unimodal-monsoon season in Pakistan [44]. *An. subpictus* was a major post-irrigation vector in Sri Lanka [45] but in this area it was observed in the pre monsoon season. This suggests that all these species may be playing some role in the epidemiology of JE in the area.

5. Conclusion

Human JE cases were categorized based on the estimated probability of vector presence at the reported location5. Since several species of vector mosquitoes are involved in transmission of JE in the same area, choice of control measure will have to take into account varying bionomics of individual species. Observations in JE affected villages in Thanjavur Tamil Nadu have shown that *Culex tritaeniorhynchus* are found in high densities, followed by *Cx. gelidus*. Sporadic occurrence of few cases in widely scattered villages makes vector control difficult. It could be seen that on an average only 1.3 cases occur per village and to carry out residual spray in all the houses in the affected and JE prone villages poses problems of logistics. Even within the villages distribution of cases is seen to be widely scattered. The peak incidence of JE is seen from October to December which is the post monsoon period in the area of study. The re-emergence of JEV remains possible due to multiple factors. Increases in the pig farming industry, modification and expansion of arable lands for wetland rice farming, and a fraction of the population unvaccinated/non-immune, in combination with optimal climatic conditions, contribute to the potential for periodic outbreaks of JE as seen in Korea [46]. Vector control measures and the JEV vaccine reduced the number of cases in many regions [4]. This longitudinal study reveals the seasonal abundance of JE vectors indicating a possible public health threat. This study was carried out in this region and to make an attempt to understand the vector dynamics. In the background of the higher densities of the mosquito in the JEV endemic areas, a surveillance mechanism needs to be implemented to check the vector population. Thus residents from these areas should take personal protection to reduce the exposure to mosquito bites, which include minimizing vector exposure at dusk and dawn by covering the body, using insect repellents and sleeping under mosquito nets.

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