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Japanese encephalitis vectors in Thanjavur district, Tamil Nadu, India

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Abstract
Japanese encephalitis virus (JEV) is a mosquito-borne zoonotic pathogen. JEV is transmitted by paddy field breeding mosquitoes of the *Culex vishnui* subgroup, primarily by *Culex tritaeniorhynchus* vectors and amplified by pigs / Ardeidae birds in nature. Mosquitoes were sampled from identified villages during different season intervals from 2011 to 2014. Adult mosquitoes were collected which were resting on bushes and thatched roofs of cattle sheds during dusk hours from 2011-2014 in 3 villages namely Muthur, Keezhapattu and Kovilur of Thanjavur district. A total of 17,479 adult mosquitoes were collected, belonging to eighteen species of mosquitoes of six genera: *Anopheles* (5 species), *Armigeres* (1 species), *Culex* (7 species), *Aedes* (2 species), *Mansonia* (2 species) and *Mimomyia* (1 species). The predominant JE vector *Cx. tritaeniorhynchus,* (PMH 126.57) was collected followed by *Cx. gelidus* (PMH 24) from the study villages. As compared with *An. subpictus* (PMH 3.39) and *An. peditaeniatus* (2.07) the other JE vector species namely *Cx. vishnui* were very few in number. Study reveals the abundance and prevalence of the major vector *Cx. tritaeniorhynchus* in Thanjavur district Tamil Nadu. JE vector abundance recorded is a measure of the maximum number of vector mosquitoes prevalent in this area and this will enable people to develop appropriate vector control strategies.

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

1. Introduction
Japanese encephalitis (JE) is a vector-borne viral disease that is endemic in most parts of Asia and is one of the major public health problems in many parts of South East Asia. Worldwide, it is estimated that around 68,000 cases occur annually, 40,000 in the Western Pacific Region alone [1]. Approximately 3 billion people i.e. 60% of the world's population live in JE-endemic regions and 15,000 deaths per annum are notified from wide geographical range. A recent estimate states that 378 million individuals are exposed to the risk of becoming infected with JEV in India [2]. Approximately 20–30% of JE cases are fatal and 30–50% of survivors have significant neurologic sequelae [3]. JEV is maintained in a zoonotic cycle, which can be both enzootic and epizootic. This cycle involves pigs as the major reservoir / amplifying host, water birds as carriers and mosquitoes as vectors. The *Culex vishnui* subgroup of mosquitoes consisting of *Culex tritaeniorhynchus* Giles, *Culex vishnui* Theobald and *Culex pseudovishnui* Colless have been implicated as major vectors of JE [4]. Since 1973, epidemics of JE have occurred in West Bengal, Bihar, Uttar Pradesh, Assam, Andhra Pradesh, Tamil Nadu and Karnataka [5]. Government of India had launched vaccination campaign in the highly endemic states of Assam, Karnataka, West Bengal, Uttar Pradesh, Andhra Pradesh, Bihar, Haryana, Maharashtra and Tamil Nadu which has resulted in reduced incidence of JE in these states [6].
Thanjavur district, with extensive rice cultivation, has not fortunately witnessed JE epidemics and the case incidence is low as compared to the relatively dry neighboring Trichy and Cuddalore districts, which have suffered severe outbreaks in the past. Between 1986-91, the annual case incidence (ACI) per 100,000 human population in Thanjavur district was only 0.4 whereas the ACI in Trichy and Cuddalore districts were 2.2 and 4.7 respectively (source: DPH&PM, Tamil Nadu).
In view of the above, entomological studies carried out in Thanjavur district, the silent JE virus transmission in vectors since 2007 indicates that there is some shift or alteration in the mode of JEV infection / activity in this endemic zone. This unique JE transmission was investigated by prospective entomological study which recorded the abundance and the presence of different JE vectors in this district which in turn was helpful to develop appropriate vector control strategies to the probability of future outbreaks.

2. Materials and Methods

2.1 Study site

Thanjavur district, lies in the southern part of Tamil Nadu between 10.7825° N, 79.1313° E. This district has a total area of 3411 square kilometer and is inhabited by 2,405,890 people (2011) with an approximate density of 705 per Km^2. Thanjavur has 4.5 lakh ha under irrigation. A longitudinal study of vector abundance was conducted from 2011-2014 in 3 villages of Thanjavur district. The study villages namely Muthur, Keezhapattu and Kovilur were selected in consultation with the DPH&PM, Tamil Nadu. Thanjavur experiences a tropical climate characterized by moderate summers and mild winters whereby there is not much temperature variations in different seasons. The summer season of Thanjavur begins from the month of March and lasts till the middle of June; temperature goes up to about 40 ºC. Thanjavur experiences rain during June, July and August. The average rainfall is about 111.37 cm. Winter season which starts from the middle of October or beginning of November and ends in February is very pleasant, the temperature goes down to 20 ºC. The northeast monsoon winds bring intermittent rainfall in Thanjavur during winter.

2.2 Methodology

Mosquitoes were sampled from identified villages at seasonal intervals during 2011 to 2014. Adult mosquitoes were collected which were resting on bushes and thatched roofs of cattle sheds during dusk hours. Mosquito samples were transported to the field laboratory, lightly anaesthetized with ether, species identification was carried out [7] and the vector abundance was calculated as number collected per man-hour (PMH).

3. Results and Discussion

A three year prospective study on vector abundance in Thanjavur district during 2011-14 showed that *Cx. tritaeniorhynchus* was found dominant in all the study villages followed by *An. subpictus*. A total of 17,479 adult mosquitoes were collected, belonging to eighteen species of mosquitoes of six genera: *Anopheles* (5 species), *Armigeres* (1 species), *Culex* (7 species), *Aedes* (2 species), *Mansonia* (2 species) and *Mimomyia* (1 species) from three villages namely Muthur, Keezhapattu and Kovilur of Thanjavur district. Greater numbers of JE vector *Culex tritaeniorhynchus*, (13,670) were collected from the study villages, as compared with *Anopheles subpictus* (366) and *Cx. gelidus* (2635). The other JE vector species namely *Cx. vishnui* was very few in number (32). There was only one *Cx. pseudovishnui* collected from this study. There were 5 species from *Anopheles* - *An. subpictus* 2.09%, *An. peditaeniatus* 1.28%, *An. barbirostris* 0.27%, *An. pallidus* 0.09% and *An. tessellatus* 0.19%, one species of *Armigeres* - *Ar. subalbatus* 0.19%; 7 species of *Culex* - *Cx. tritaeniorhynchus*78.21%, *Cx. vishnui* 0.18%, *Cx. pseudovishnui* 0.01%, *Cx. infula* 0.50%, *Cx. gelidus* 15.08%, *Cx. fuscocephala* 0.01% and *Cx. quinquefasciatus* 0.10%; 2 species of *Aedes* - *Ae. lineatopennis* 1.24% and *Ae. vexans & vexans* 0.38%; 2 species of *Mansonia* - *Ma. uniformis* 0.16%, *Ma. annulifera* 0.01% and one species from *Mimomyia* - *Mi. luzonensis* 0.01% recorded from this study (Fig.1).

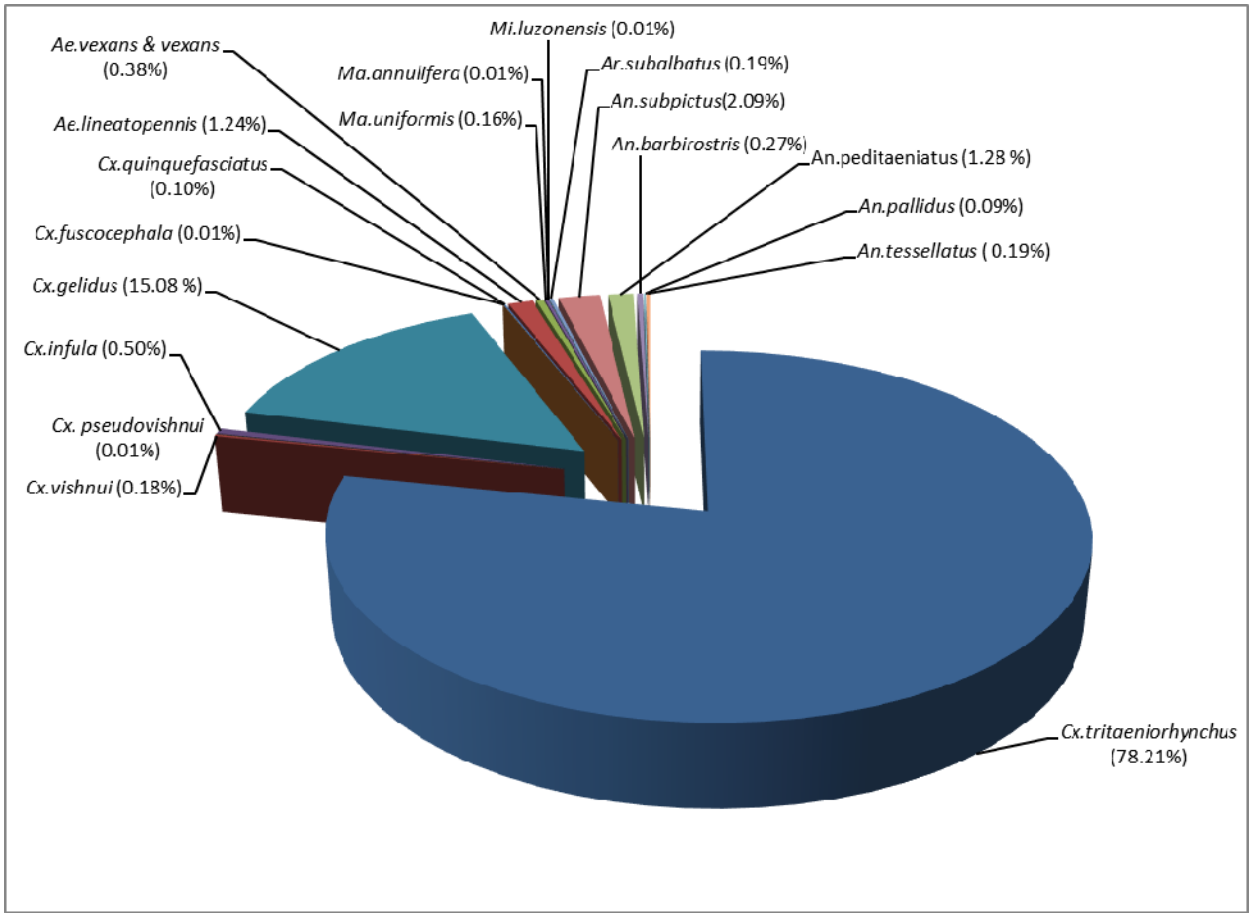

Fig 1: Mosquito species composition among the study villages in Thanjavur district (2011-2014)

In India, JEV has been isolated from 16 species of mosquitoes; 10 species of *Culex* and three species each of *Anopheles* and *Mansonioides*. Among the genus *Anopheles*, 3 species that carry JEV are *Anopheles peditaeniatus*, *An barbirostris* and *An. subpictus* [8]. *Anopheles subpictus* breeds profusely in water collections and fallow rice fields [9]. In southern India the larval incidence was high throughout the year. In Vellore district, *An. subpictus* was the most dominant species after *Cx. vishnui* group and was collected throughout the year [4]. JEV has been isolated from *An. subpictus* in Karnataka [10], Kerala [11] and Tamil Nadu [12]. A two year study in the Cuddalore district of Tamil Nadu showed that the abundance of *An. subpictus* was the dominant species among *Anopheles* in the rice ecosystem was much lower than that of *Cx. tritaeniorhynchus*. The adult density was higher in the hot months than in the cool months. *An. subpictus* is strongly zoophilic feeding mostly on bovines (83%) and poorly on pigs and human. *An. subpictus* has quite often been suspected to be involved in the epidemiology of JE transmission as observed in Gorakhpur district, Uttar Pradesh in North India [13, 14]. In North Arcot district, indoor resting collections showed the predominant species as *Cx. vishnui* sub-group comprising 42.6% of the total collection, followed by *An. subpictus* (40.4%) [15, 16]. It was suspected that both *An. subpictus* and *An. hyrcanus* were secondary vectors for JE as they prevailed in high density. Substantial densities of *An. subpictus* and *An. peditaeniatus* during JE season suggest the supportive role of these species [17]. These species appear to play the role of secondary vectors mainly in zoonotic transmission. *An. subpictus*, *An. peditaeniatus* and *An barbirostris* were recorded in Gorakhpur [13, 14]. The role of *An. pallidus* and *An. tessellatus* which were already recorded earlier in West Bengal [18] and Gorakhpur [13] in the JEV transmission is yet to be established.

Culex members of the *Cx. vishnui* subgroup comprising of the *Cx. tritaeniorhynchus*, *Cx. vishnui* and *Cx. pseudovishnui* are extremely common and widespread which mainly breed in paddy fields. Blood meal analysis showed that these mosquitoes were principally cattle feeders though human and pig feeding was also recorded in villages near Madurai and Cuddalore districts of Tamil Nadu [19] and Dibrugarh district in Assam [20]. The maximum isolation of JEV was from the *Cx. vishnui* subgroup [6, 8, 10, 11, 21-24] mosquitoes which were shown to be capable of transmitting the virus in the laboratory [25-27] and in this collection *Cx. vishnui* subgroup constituted 78%. *Cx. vishnui* subgroup has been recognized for many years as the major vector and it plays an important role in the epidemiology of JE in India. The predominant vector mosquito was *Cx. tritaeniorhynchus* (78.21%) followed by few numbers of *Cx. vishnui* (0.18%) and *Cx. pseudovishnui* (0.01%).

Of the total mosquitoes collected in Madurai district, *Cx. infula* comprised 13.4% in dusk collection and 34.5% in man-biting collections. *Cx. infula* mainly feeds on cattle, human and birds [19]. During the rainy season immature stages of *Cx. infula* were collected from the paddy fields in Madurai and Cuddalore districts of Tamil Nadu. One isolation of JEV from *Cx. infula* in Madurai and five JEV antigen detections from Cuddalore district have been reported. Vector competence study showed that the infection and transmission rates are 60% and 47% respectively [28]. In this study, *Cx. infula* contributed to 0.5% of the total collection. This species is expected to play a role in JE transmission because of its seasonal abundance and man biting habits during JE season besides its competence to transmit JEV.

Culex gelidus species which prefers marshy depressions containing abundant aquatic vegetations [16] was abundantly available during the north-east monsoon season in Vellore district [4]. Adults were found closely associated with man and domestic animals and most of them feed on bovids [15] and pigs [19]. JEV has been isolated three times in Mandya district of Karnataka [21] and 5 times in Cuddalore district of Tamil Nadu [23]. *Culex gelidus* was the second dominant species (15.08%) recorded in this survey. Though this mosquito is only seasonal due to its feeding habit and infection rate, they may have an important role in the zoonotic cycle.

Culex fuscocephala species mainly breeds in ground pools and the adults were collected inside cattle sheds, pig baited traps and in light traps in Bankura [29]. In Vellore district, adults were collected mainly on cattle or buffaloes. Blood meal analysis showed that they mainly feed on bovids, and rarely on pigs [15]. JEV has been isolated from *Cx. fuscocephala* once in Mandya district [21] and six times in Cuddalore district [23]. In this particular collection the density was very meagre (0.01%). Similar to *Cx. gelidus* this species may have an important role in the maintenance of zoonotic cycle.

Culex quinquefasciatus is the most common domestic species in urban, semiurban and rural areas. It is strongly anthropophilic (53.2-62.7%); 7-14.7% cattle feeding and 1.5% feeding on pigs were also observed [19]. A single isolation of JEV was made from *Cx. quinquefasciatus* in Kolar district in 1986 [21] but the total composition from this area is very less (0.1%). *Cx. quinquefasciatus* has been shown to be capable of transmitting the JEV in the laboratory [30] but, the epidemiology of JE does not fit into the known information regarding the ecology and behaviour of *Cx. quinquefasciatus*.

Aedes lineatopennis mosquitoes (217 nos.) were collected from West Bengal and Gorakhpur UP [13, 14, 18]. Two strains of JEV were isolated from 772 (23 pools) *Ae. lineatopennis* in Yunnan Province, China [31]. *Ae. vexans & vexans* (66 nos.) mosquitoes were also collected from as in West Bengal and Gorakhpur UP [13, 14, 18]. Two strains of JEV were also isolated from 1605 (50 pools) *Ae. vexans* in Yunnan Province, China [31]. Thus *Aedes lineatopennis* and *Ae. vexans & vexans* constituted 1.24 and 0.38% respectively in this study.

Mansonia species which are the vectors of brugian filariasis breed in water with floating vegetation like *Pistia*, *Salvinia* and *Eichhornia* [32]. JEV was isolated from *Ma. annulifera* in Dibrugarh, Assam [33]. In a recent outbreak of JE in Kerala, three isolations were made from *Ma. uniformis* and one from *Ma. Indiana* [11]. JE antigen was detected in *Ma. uniformis* from Mandya district [21]. Isolations of the JEV from these species indicate that these species have access to viraemic hosts. In this collection, *Ma. uniformis* and *Ma. annulifera* showed very less in number (0.16% & 0.01%).

Mimomyia (Etorleptiomyia) luzonensis (Ludlow) is recorded from Bahour Union Territory of Puducherry [34]. This species is collected from ground pools with emergent vegetation and it is associated with species like *Cx. rubithoracis*, *Cx. gelidus* and *Ur. edwardsi*. Distribution of this species is recorded from Assam, Himachal Pradesh, Karnataka, Madhya Pradesh, Maharashtra, Manipur, Orissa, Sikkim, Tamil Nadu, Uttar Pradesh, and West Bengal. *Mi. luzonensis* species of mosquitoes were already reported from Gorakhpur [13] and also recorded in the Disease vector ecology profile, Thailand 1993 Armed Forces Pest Management Board; Defence Pest Management Information Analysis Centre; Forest Glen section, WRAMC, Washington [35]. Only one specimen was collected in this study. *Ar. subalbatus* was also collected in

small number from these areas and also reported from other areas like West Bengal and Gorakhpur, UP [13, 14, 18]. *Ar. subalbatus* is a common vector of filariasis [36]. JEV was isolated in *Ar. subalbatus* in Taiwan where there is no rice cultivation [37].

4. Conclusion

Thanjavur zone which is the rice bowl of Tamil Nadu with extensive paddy cultivation round the year has high vector abundance fortunately no cases were recorded while carrying out longitudinal studies during 2011 to 2014, though encircled by endemic neighbouring districts like Cuddalore on Northern & North-Western sides. The main reason for this paradox was that in Thanjavur zone the ratio between pig and cattle was found to be much higher (1:400) when compared to the neighbouring endemic district Cuddalore (1:4) [38]. Hence the higher population of JEV dumping host (Cattle) indirectly inhibits the process of spill over of the JE virus to humans. Although there are no JE cases reported until 2006, the silent JEV transmission in vectors since 2003 onwards indicate that there is some shift or alteration taking place in the mode of JEV infection / activity in this non endemic zone. This unique investigation by prospective entomological means defined the abundance and prevalence of many species of JE vectors in this non-endemic area and enabled to develop appropriate vector control strategies to avoid the probability of future outbreaks. Effective surveillance systems are urgently required to identify the vector mosquitoes from the different parts of India. This longitudinal study reveals a possible public health threat which can be combated by the available appropriate vector control strategies.

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