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A longitudinal study on abundance and infection frequency of Japanese encephalitis vectors in Tirunelveli district, Tamil Nadu, India

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Abstract
Japanese encephalitis virus (JEV) is transmitted by *Culex vishnui* subgroup, primarily *Culex tritaeniorhynchus* Giles, and amplified by infection of pigs / *Ardeidae* birds in nature. A longitudinal study of vector abundance and infection frequency of JE vectors was conducted from 2011-2013 in 4 villages namely Senthimangalam, Ariyanayagipuram, Kuthalaperi and Magiladi of Tirunelveli district. A total of 13,343 adult mosquitoes were collected and processed by antigen capture ELISA. JEV was detected from ten species of mosquitoes – 28 positive pools, viz. *Cx. tritaeniorhynchus* (10), *An. subpictus* (7), *Cx. infula* (2), *Ma. annulifera* (2), *Ma. uniformis.* (2), *Cx. bitaeniorhynchus* (1), *Cx. quinquefasciatus* (1), *An. pallidus* (1) *An. barbirostris* (1) and *Ar. subalbatus* (1). Vector incrimination with JEV indicating a possible public health threat in the near future and helps programme people to develop appropriate control strategies.

Keywords: Japanese encephalitis virus, *Culex tritaeniorhynchus*, *Anopheles subpictus*, Tirunelveli, Tamil Nadu.

1. Introduction
Japanese encephalitis (JE) is one of the major public health problems in many parts of South East Asia. The disease causes 35,000 cases of encephalitis and 10,000 deaths each year, and about 30% of survivors develop serious permanent sequelae [1]. A recent estimate states that 378 million individuals are exposed to the risk of becoming infected with JEV in India [2]. JE is a viral disease caused by JE virus (JEV). 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.

JE, a disease with a very high mortality and morbidity has emerged as a major public health problem in India since 1955 from Vellore, north Arcot district [3]. Since 1973, epidemics of JE have occurred in West Bengal, Bihar, Uttar Pradesh, Assam, Andhra Pradesh, Tamil Nadu and Karnataka [4]. An extensive outbreak of encephalitis occurred in the State of Tamil Nadu, South India, mainly in Tirunelveli district [5]. Serological evidence of a probable JE etiology was obtained in 64.4% of the cases tested [6]. Tirunelveli is located on southern tip of Tamil Nadu where a JE epidemic was recorded during 1977-78 and subsequently this area remains endemic till date [6].

Under "Transfer of technology" our centre – Centre for Research in Medical Entomology and Tamil Nadu Public Health Department have established a "*JE surveillance network programme*" to monitor JEV activities whole the year in the districts of Tamil Nadu. In this programme nine zones (Cuddalore, Trichy, Vellore, Virudhunagar, Dindigul, Tirunelveli, Coimbatore, Thanjavur & Salem) were included and JEV activities has been continued to monitor since 1996 [7]. The vector infections in desiccated mosquitoes were regularly recorded from the six endemic zones viz. Cuddalore, Trichy, Tirunelveli, Virudhunagar, Dindigul & Vellore and the results of infection of the positive pools used to be intimated to concern health authorities to take necessary control measures. However, in recent past two years (since 2007 onwards) there is increase in infection rate in vector mosquitoes was recorded in Tirunelveli district. The silent JEV transmission in vectors since 2007 onwards indicates that there is some shift or alteration is taking place in mode of JEV infection / activity in this endemic zone.

The results of JE surveillance network programme showed that on average 2.8% pools of desiccated vector mosquitoes were found positive. This unique JE transmission dynamics need

to be investigated by prospective entomological study which will define the mode of transmission in this endemic area and also be helpful to develop appropriate control strategies to avoid the probabilities of future outbreaks.

2. Materials and Methods

Study Area

Tirunelveli district, lies on southern part of Tamil Nadu between 8°05' and 9°30' north latitude and 77°05' and 78°25' east longitude. This district has a total area of 6,823 square kilometers, and is inhabited by 3,072,880 people (2011) with a approximate density of 450 per Km^2 [12]. The Tamiraparani River provides consistent irrigation to a large agricultural area.

A longitudinal study of vector abundance and infection frequency was conducted from 2011-2013 in 4 villages of Tirunelveli district. The study villages namely Senthimangalam in Rajavallipuram PHC, Ariyanayagipuram in Mukkudal PHC, Kuthalaperi in Manur PHC, and one control village Magiladi in Thirukurankudi PHC of Tirunelveli Zone were selected with the guidance of Tamil Nadu State Health Department, Zonal Entomological Team, located at Tirunelveli (Fig. 1). Discussed with Medical officers in the PHCs regarding JE case repots. The census data of the index villages were collected from the respective villages. Numerous Little Egret birds in the paddy fields were noted. The amplifying host pigs were also recorded in the village Ariyanayagipuram.

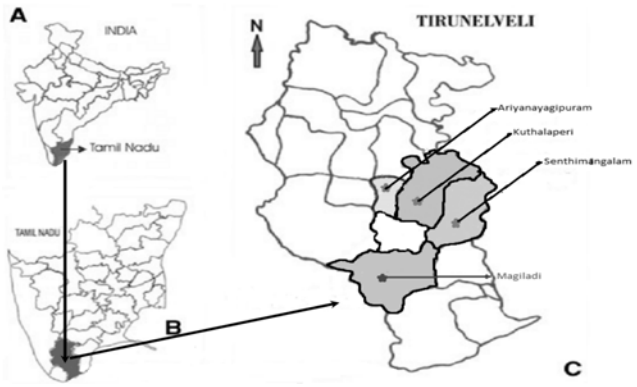


Fig 1: Study area map

Tirunelveli district receives rain showers from June to August under the influence of the southwest monsoon, and heavier rainfall from September to December from the northeast monsoon (Fig. 2).

2.1 *Mosquito collection*

Mosquitoes were sampled from identified villages at bimonthly intervals during 2011 to 2013. Adult mosquitoes were collected resting on bushes and thatched roofs of cattle sheds during dusk hours and from human dwellings (indoor resting) and outdoor resting places during day time 8-10 a.m. Mosquito samples were transported to the field laboratory, lightly anaesthetized with ether, species identified [8] and sorted on ice into pools of 1-50 specimens/pool. Unfed mosquitoes were pooled on the same day of collection, whereas engorged female mosquitoes were held for 48 h for digestion of blood meals before pooling. Mosquito (only females) abundance was calculated as number collected per man-hour (PMH).

Mosquito pools were stored at -80 °C until processed for virus detection and isolation as described [9]. Two systems were used:

2.2 *Antigen capture ELISA*

Monoclonal antibody 6B4A-10 (reactive against all viruses in JE/WN/SLE/MVE complex) was used as capture antibody and monoclonal antibody peroxidase conjugate SLE MAB 6B6C-1 (reactive against all flaviviruses) as detector antibodies (supplied by Dr. T.F. Tsai, Centers for Disease Control and Prevention, Fort Collins Co., USA). A mosquito pool was considered ELISA positive if its optical density (OD) value is $\geq$ mean + 4SD of the six normal pools.

2.3 *Insect bioassay*

Toxorhynchites splendens mosquito larvae were inoculated with ELISA positive pools intracerebrally, incubated for 7-10 days at 29 °C and then tested by the indirect immunofluorescence assay (IFA) on head squeeze preparations (Toxo-IFA). Smears were tested with JEV specific monoclonal antibody, MAB 112 (supplied by Dr. Kimura Kuroda, Tokyo Metropolitan Institute of Neurosciences, Japan), and detected by FITC conjugated anti-mouse immunoglobulin (Dakoppats, Denmark).

2.4 *Virus infection rate*

Virus infection rate in mosquitoes was expressed as minimum infection rate (MIR) per 1000 females tested [9].

MIR = No. of positive pools / Total number of mosquitoes tested X 1000.

2.5 *Meteorological data*

Besides entomological studies, data on other parameters like minimum temperature, maximum temperature and rainfall were collected month wise from meteorological department during study periods.

2.6 *Statistical analysis*

Data analysis was done with the SPSS 16.0 version statistical package. Maximum likelihood estimation (MLE) was calculated using pooled Infrate V4.2007 – software of CDC, USA. Mosquito density and MIR were correlated with climatic factors.

3. Results

A three year prospective study on vector abundance and vector infection in Tirunelveli district during 2011-13 showed that *Cx. tritaeniorhynchus* was found dominant in all the study villages followed by *An. subpictus*. A total of 13,343 adult mosquitoes were collected, belonging to nineteen species of mosquitoes of four genera: *Anopheles* (7 species), *Armigeres* (1 species), *Culex* (9 species) and *Mansonia* (2 species) from four villages namely Magiladi, Senthimangalam, Kuthalaperi and Ariyanayagipuram in Tirunelveli district. Greater numbers of JE vector *Cx. tritaeniorhynchus*, (9,937) were collected from the study villages, as compared with *An. subpictus* (1432) and *Cx. gelidus* (992). The other JE vector species namely *Cx. vishnui* was very few in number (337). There was only one *Cx. pseudovishnui* collected from the study villages.

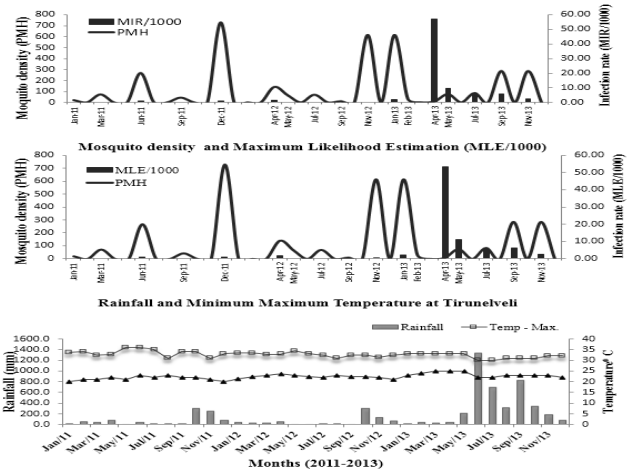
All the 527 pools were processed for JE virus detection by antigen capture ELISA and 28 pools were found positive.JE virus detected from ten species of mosquitoes – 28 positive pools, viz. *Cx. tritaeniorhynchus* (10), *An. subpictus* (7), *Cx. infula* (2), *Ma. annulifera* (2), *Ma. uniformis.* (2), *Cx. bitaeniorhynchus* (1), *Cx. quinquefasciatus* (1), *An. pallidus* (1) *An. barbirostris* (1) and *Ar. subalbatus* (1). JEV infection was high in Ariyanayagipuram (13), followed by Senthimangalam (8), Kuthalaperi (4) and Magiladi (3) (Table.

1). Peak JEV infection was noticed in the month of September (7) followed by April (6), January (5), November (4), December (3), July (2) and June (1).

Table 1: Japanese encephalitis virus infection in mosquitoes in Tirunelveli district (2011-2013)

Village															
Species	**Senthimangalam**			**V.Ariyanayagipuram**			**Kuthalaperi**			**Magiladi**			**Total**		
	No. of mosquitoes	No. pools	No. pools +ve	No. of mosquitoes	No. pools	No. pools +ve	No. of mosquitoes	No. pools	No. pools +ve	No. of mosquitoes	No. pools	No. pools +ve	No. of mosquitoes	No. pools	**No. pools +ve**
Cx.bitaeniorhynchus	23	2	0	2	2	**1**	15	3	0	4	2	0	44	9	**1**
Cx.fuscanus	1	1	0	0	0	0	1	1	0	2	1	0	4	3	**0**
Cx.fuscocephala	3	2	0	0	0	0	15	3	0	27	3	0	45	8	**0**
Cx.gelidus	690	26	0	183	12	0	3	3	0	116	6	0	992	47	**0**
Cx.infula	19	5	1	82	7	**1**	16	3	0	2	1	0	119	16	**2**
Cx.pseudovishnui	1	1	0	0	0	0	0	0	0	0	0	0	1	1	**0**
Cx.tritaeniorhynchus	3772	89	**3**	3167	76	**3**	1368	34	**2**	1630	46	**2**	9937	245	**10**
Cx.vishnui	79	9	0	38	10	0	117	5	0	103	8	0	337	32	**0**
Cx.quinquefasciatus	38	6	0	18	4	**1**	0	0	0	3	3	0	59	13	**1**
Ma.annulifera	32	8	**1**	15	9	**1**	0	0	0	0	0	0	47	17	**2**
Ma.uniformis	51	9	**2**	11	4	0	0	0	0	0	0	0	62	13	**2**
An.barbirostris	16	5	0	6	3	**1**	0	0	0	0	0	0	22	8	**1**
An.culicifacies	3	2	0	0	0	0	0	0	0	0	0	0	3	2	**0**
An.nigirimus	0	0	0	6	1	0	0	0	0	0	0	0	6	1	**0**
An.pallidus	7	4	0	12	4	0	2	1	0	7	3	**1**	28	12	**1**
An.peditaeniatus	28	4	0	30	5	0	34	1	0	0	0	0	92	10	**0**
An.subpictus	177	13	0	620	23	**5**	312	12	**2**	323	19	0	1432	67	**7**
An.tesselatus	2	1	0	1	1	0	0	0	0	0	0	0	3	2	**0**
Ar.subalbatus	65	10	1	2	1	0	6	3	0	37	7	0	110	21	**1**
Grand total	**5007**	**197**	**8**	**4193**	**162**	**13**	**1889**	**69**	**4**	**2254**	**99**	**3**	**13343**	**527**	**28**

Correlation between climatic variable and minimum infection rate of *Cx. tritaeniorhynchus* and *An. subpictus* was analysed. Maximum likelihood estimation (MLE) [10] was calculated (Fig. 2). *An. subpictus* showed strong correlation significance with minimum temperature (P< 0.005) whereas *Cx. tritaeniorhynchus* showed moderate/slight correlation significance with minimum temperature (P =0.05). MIR of both *Cx. tritaeniorhynchus* and *An. subpictus* does not correlate with rainfall and maximum temperature (P>0.05).



Rainfall & Temperature data source:

1) India meteorological department (2011-2012); www.accuweather.com/en/in/tirunelveli (2013); www.indiaagrimet.gov.in
2) MLE/1000 statistical analysis source: PooledInfrateV4.2007-software of CDC, USA

Fig 2: Correlation of Mosquito Density and Vector Infection Rate with Meteorological Data in Tirunelveli District (2011-2013)

4. Discussion

Tirunelveli district had experienced severe drought conditions in 1975 and 1976. This was followed by heavy rains in 1977 which resulted in increased acreage under paddy cultivation, flooding of low lying areas and formation of rain water pools and consequently a tremendous increase in the mosquitogenic potential. During the study period, chikungunya [11] and dengue [12] outbreak was reported in Tirunelveli district but not JE.

In India, JEV has been isolated from 16 species of mosquitoes; 10 species of *Culex* and three species each of *Anopheles* and *Mansonioides*. In the genus *Anopheles*, 3 species that carry JEV are *Anopheles peditaeniatus* Leicester, *An. barbirostris* van der Walpand *An. subpictus* Grassi [13]. JEV has been isolated from *An. subpictus* in Karnataka [14], Kerala [15] and in Tamil Nadu [16]. In the present study also, 7 out of 28 positives (25%) were from *An. subpictus* and also next to the JE primary vector *Cx. tritaeniorhynchus* (10/28, 36%). With the isolation of JEV from *An. subpictus* in this study, it can be demonstrated that this species is feasible to acquire the infection in nature, and may transmit the infection. Thus, *An. subpictus* may act as a secondary or bridge vector in JEV transmission in Tirunelveli as they prevailed in high density.

In Vellore district, *An. subpictus* was the most dominant species after *Cx. vishnui* group and was collected throughout the year [17]. *An. subpictus* has quite often been suspected to be involved in the epidemiology of JE transmission as predicted in Gorakhpur district, Uttar Pradesh in North India [18]. Indoor resting collections the predominant species was the *Cx. vishnui* sub-group comprising 42.6% of the total collection, followed by *An. subpictus* (40.4%) [19, 20] suspected both *An. subpictus* and *An. hyrcanus* as 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 [21].

5. Conclusion

The silent JEV transmission in this study indicates that there is some shift or alteration is taking place in mode of JEV infection / activity in this area. This longitudinal study reveals vector incrimination with JEV, indicating a possible public health threat in the near future and helps programme people to develop appropriate control strategies to avoid the probabilities of future outbreaks. As of now, no JE surveillance studies have been carried out in this region, and this study is to make an attempt to understand the disease scenario and vector dynamics in relation with weather variables. In Tirunelveli district, the role of *An. subpictus* has to be studied along with other JE vectors. A longitudinal study is needed to understand the role of *An. subpictus*.

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