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Determination of critical density in *Culex tritaeniorhynchus* Giles, 1901 (Diptera: Culicidae) as a deciding factor influencing the transmission of Japanese encephalitis virus in southern India

P. Philip Samuel*, V. Thenmozhi, J. Nagaraj, D. Ramesh, M. Muniaraj and N. Arunachalam

Centre for Research in Medical Entomology (ICMR),Madurai 625002, Tamil Nadu, India.
Email: philipsamuelpaulraj@gmail.com

ABSTRACT: Japanese encephalitis virus (JEV) causes a severe neurobiological health hazard, namely, Japanese encephalitis (JE) which has been rampant in Cuddalore district in the 1970s and 1980s, transmitted mainly by *Culex tritaeniorhynchus.* The authors have been working on various aspects of transmission dynamics of JE in Cuddalore district, south India, for past more than two decades and have analyzed varied data on ecology and biology of vector *Cx. tritaeniorhynchus,* to develop a model for depicting the critical density which is a primary requisite to develop control strategy against vector. Such a model of critical density for the vector will be helpful to forecast prospective outbreaks of the disease exacting heavy morbidity and mortality in many parts of India. © 2016 Association for Advancement of Entomology

KEYWORDS: Cuddalore, JE virus, *Culex tritaeniorhynchus,* Critical density

INTRODUCTION

Japanese encephalitis (JE) is widespread over South East Asia and Pacific regions where 3 billion people are at risk of infection. It is a leading cause of viral encephalitis in Asia, caused by a virus from the family Flaviviridae and mainly occurs and prevailing in rural setting, especially in rural and suburban areas where rice growing and pig farming culture coexist (Campbell *et al.,*2011; Halstead and Jacobson, 2008). It is of greater public health importance since it produces mild infection to permanent brain damage with increasing case fatality rate through causing serious inflammation of the membranes around the brain (Bhowmik *et al.,* 2012). Japanese encephalitis virus (JEV) is transmitted to humans by infective bites of female mosquitoes mainly belonging to *Culex tritaeniorhynchus*, *Culex vishnui* and *Culex pseudovishnui*. In India, *Culex vishnui group (Culex tritaeniorhynchus, Culex vishnui and Culex pseudovishnui)* are the chief vectors of JE which breeds particularly in stagnant water in the flooded rice growing fields (WHO, 2001). JEV is maintained in an enzootic cycle between mosquitoes and amplifying vertebrate hosts, primarily pigs and wading ardeid birds. Humans are incidental or dead-end hosts, because they usually do not develop a level or duration of viremia necessary to infect mosquitoes (Susan *et al.,* 2012).

JE disease was first reported in Japan in 1924, was subsequently reported in other Asian countries whereas in India the first case was reported in the state of Tamil Nadu in 1955. Till 2012, about 17 states/UTs in India have reported incidence of JE.

* Author for correspondence

Since 2008, a maximum number of JE cases were reported in the years 2011 (1214) and 2012 (745) compared to previous years. It was estimated that an average incidence of JE cases and deaths were about 719 and 121 respectively can occur annually. Eastern and central regions of India are highly affected with maximum number of cases and deaths compared to other regions.

There are a number of studies related to serological and epidemiological aspects of JE in India. But there is lack of studies on entomological investigation, especially for the estimation of number of mosquito bites required (single host (pig)) to maintain virus transmission. CRME has field station at Cuddalore district in Tamil Nadu and has been working on vector borne diseases including JE during last two decades. In this paper, there was an attempt to develop a mathematical model on the basis of MacDonald's model on malaria and Gordon Smith's model on Western Equine Encephalomyelitis (WEE) with necessary inputs and refinements in order to determine the number of mosquito bites required (single host (pig)) to maintain JE virus transmission in Cuddalore district.

MATERIALS AND METHODS

Study area:

JE has been highly endemic in Cuddalore district in Tamil Nadu. The district is located in the South East of Tamil Nadu, Southern India. The district has an area of 3564 sq.km with a population of 2.6 million. Tamil Nadu is one of the leading rice growing states in India, in which Cuddalore district alone contributes 6.65% of production to the states. The major JE outbreak had occurred in Cuddalore district during 1981 (Kabilan *et al.,* 2004) and since then it has been reported to have high morbidity rate (Gajanana *et al.,* 1995; 1997). A prospective study conducted during 1989-91 in some villages of the study area was reported a high rate of seroconversion in sentinel pigs against flaviviruses and high vector densities with high infection frequency for JEV (MIR of JEV = 10.4).

Mosquito collection and estimation of infection rate:

Mosquitoes were collected during April 2011 to December 2012 by using aspirators and compared its results with the previous studies in order to understand the features and biological characteristics of the mosquitoes. The virus infection rate at mosquitoes was expressed as minimum infection rate (MIR) per 1000 female's tested (Chiang and Reeves, 1962).

$$MIR/1000 = \frac{\text{Number of mosquito pools positive}}{\text{Total number of mosquitoes tested}} \times 1000$$

Daily survival rate (P = Proportion of mosquito survival one day) of *Cx. tritaeniorhynchus* was calculated by using a formula gc"p_i, where gc is the length of gonotrophic cycle and p_i is the parity rate (Davidson, 1954).

JE transmission modeling:

The life cycle of JEV is maintained between mosquitoes and hosts including pigs, birds and humans where humans are incidental or dead-end hosts since they do not develop required concentration for survival and replication of JE virus in their bloodstreams to infect feeding mosquitoes. In the mosquito borne diseases, the parasite transmission model was initially developed and estimated in 1957 by MacDonald for Malaria (Mac Donald, 1957). In 1970, Gordon Smith was estimated a transmission model for Western Equine Encephalomyelitis virus (WEE) (Smith, 1987) on the basis of Mac Donald's model with certain refinements since the virus transmission cycle for WEE was different from the cycle of Malaria parasite. Since the life cycle of Japanese encephalitis (JE) virus is closely related to WEE, West Nile and St. Louis encephalitis viruses, in this study, an attempt was made to estimate the model for JEV transmission on the basis of existing models (Mac Donald, 1957; Smith, 1987; Ebel and Kramer, 2009) of transmission with necessary changes. The various models estimated so far in mosquito borne disease are given in Table 1. There are factors like number of *Culex tritaeniorhynchus* bites/host,

Table 1. Comparison of inclusion/exclusion factors engendering in deriving various formulas for calibrating 'Critical Vector Density'

Sl. No	Author (Year)	Area (Species)	Models	Factors development of Model	Chronological
1	MacDonald G. (1957)	Malaria(*Anopheles*)	$R_0 = \frac{ma^2 b p^n}{-r(\log_e p)}$	**m** = the number of vectors per man **a** = the proportion of mosquito feeding on man /gonotrophic cycle **b** = probability that a human gets the infection after being bitten by an infective mosquito **p** = the proportion of vector surviving one day (Survival probability) **n** = the incubation period of parasite in the vector **r** = the daily rate each human recovers from infection	
2	Garrett-Jones (1964)	Malaria (*Anopheles gambiae)*	$C = \frac{ma^2 p^n}{-(\log_e p)}$	**m** = the number of vectors per man **a** = the proportion of mosquito feeding on man /gonotrophic cycle **p** = the proportion of vector surviving one day (Survival probability) **n** = the incubation period of parasite in the vector	Derived from Macdonald's Model but excluded certain parameters like 'b' and 'r'
3	Smith C.E.G. (1987)	Western Equine Encephalomyelitis (WEE) (*Culex tarsalis)*	$R_o = \frac{m\, b\, h\, S_m\, v\, S_v\, p^i}{-\ln p}$	**m** = the number of vectors per bird(critical vector density) **b** = no.of feeds by mosquito each day/ gonotrophic cycle **h** = proportion of blood meals taken from birds **Sm** = vector competence for WEE	Derived from Macdonald's Model with certain refinement on following parameters 1.Replaced denominator in variable 'm' (Replaced anthrpophilic into zoophilic)

Sl. No	Author (Year)	Area (Species)	Models	Factors development of Model	Chronological
			$m = \frac{-\ln p}{b\,h\,S_m\,V\,S_v\,p^i}$ (where R_0=1)	**V** = duration of infective viraemia in birds **Sv** = the proportion of birds susceptible to infection **p** = the proportion of vector surviving one day (Survival probability) **i** = the extrinsic incubation period of virus in the vector	2. **'a'** replaced with **(b). (h)** 3. '**b'** replaced as $\mathbf{S_m}$ (vector competence) 4. Added **V** = duration of infective viraemia in birds 5. Added **Sv** = birds susceptibility. 6. '**n'** replaced as '**i'**
4	Paulo *et al.* (2005)	Dengue (*Aedes albopictus*)	$C = \frac{ma^2 p^n}{(-\ln p)}$	**m** = the number of vectors per man **a** = the proportion of mosquito feeding on man /gonotrophic cycle **p** = the proportion of vector surviving one day (Survival probability) **n** = the incubation period of virus in the vector	Used similar model derived by Garrett-Jones (1964)
5	Ebel and Kramer (2009)	West Nile Virus (*Anopheles maculipennis*)	$V_C = \frac{ma^2\,b\,p^n}{(-\ln p)}$	**m** = the number of vectors per host **a** = the proportion of mosquito feeding on host /gonotrophic cycle **b** = (Vector competence) **p** = the proportion of vector surviving one day (Survival probability) **n** = the incubation period of virus in the vector	Based on existing derivation except replacement of feature of parameter 'm' (Replaced denominator in variable 'm'. Replaced anthrpophilic into zoophilic)

Sl. No	Author (Year)	Area (Species)	Models	Factors development of Model	Chronological
6	Ciota *et al.* (2013)	West Nile Virus (*Culex pipiens*)	$V_C = \frac{mh^2\ b\ p^n}{(-\ln p)}$	**m** = the number of vectors per host **h** = (blood feeding rate)the proportion of mosquito feeding on host / gonotrophic cycle **b** = (Vector competence) **p** = the proportion of vector surviving one day (Survival probability) **n** = the incubation period of virus in the vector	Based on existing derivation except replacement of feature of parameter '**m**' (Replaced denominator in variable 'm'. Replaced anthrpophilic into zoophilic) 1.The variable '$\mathbf{a^2}$' was replaced as '$\mathbf{h^2}$'
7	Garaza Hernandez *et al* (2013)	Dengue (*Aedes aegypti*)	$C = \frac{ma^3\ b\ p^n}{(-\ln p)}$	**m** = the number of vectors per man **a** = the proportion of mosquito feeding on man /gonotrophic cycle **b** = (Vector competence) **p** = the proportion of vector surviving one day (Survival probability) **n** = the incubation period of virus in the vector	Derived on the basis of Garrett-Jones (1964)' s Model with following modifications 1.Included the variable '**b**' 2.Modified $\mathbf{a^2}$ as $\mathbf{a^3}$ based on the proportion of mosquito feeding on man/ gonotrophic cycle
8	Current Derivation	Japanese Encephalitis (*Culex tritaeniorhynchus*)	$m = \frac{-\ln (p)}{-a\ .\ S_c\ .\ V_c\ .\ p^n}$	**a**= the proportion of mosquito feeding on pig /gonotrophic cycle $\mathbf{S_c}$=Proportion seroconversion rate of sentinel Pigs **Vc** = Vector competence **P** = Survival probability **n** = Extrinsic incubation period	Derived from Macdonald and Smith C.E.G's Model with certain refinements on following parameters 1. Replaced denominator in variable 'm' (birds replaced as pigs as source of blood feeding) 2.Replaced '**b. h**' as '**a**' 3.'**Sv**' replaced into '**Sc**' Seroconversion in pigs (Susceptibility) 4.'**Sm**' replaced into '**Vc**' (Vector competence) 5. Excluded the attribute '**V**' (duration of viraemia)6. Parameter '**i**' replaced as ' **n**'

mosquito survival rate, gonotrophic cycle, mosquito susceptibility, vector competence and incubation period for JEV in vector are influencing JEV transmission and required for its estimation. The factors responsible for influencing JEV transmission and required for estimation of transmission model was constructed by using two decadal research activities (published data) of CRME in Cuddalore district.

Critical vector (Culex tritaeniorhynchus) density:

The basic reproduction rate (R_0) is defined as "an average number of secondary infections produced when one infected individual is introduced into a host population where everyone is susceptible" and is commonly used term to predict epidemic dynamic of infectious diseases. If $R_0 > 1$, the disease is spread indefinitely; if $R_0 < 1$, the disease will die out where the value for $R_0=1$ the disease is likely to exist. The basic reproduction rate (R_0) for JEV transmission was expressed as

$$Ro = \frac{m.\ a\ .\ S_c\ .\ V_c\ .\ P^n}{-\ln(P)}$$

Where,

- m: the number of JE mosquitoes bite/pig/night (Critical density).
- *P*: the Proportion of mosquito surviving one day.
- a: the proportion blood feeing on pig (h) / Gonotrophic cycle.
- S_c: the seroconversion (JE) in sentinel pigs (proportion of pigs susceptible to infection).
- V_c: the vector competence of *Cx. tritaeniorhynchus* (proportion of Vector susceptible to infection).
- n: the incubation period of the JE virus in the vector.

The basic reproduction rate allows ($R_0 = 1$, endemic area) the calculation of the critical density (m) threshold of hosts necessary to maintain virus transmission. Thus the critical density (*Cx. tritaeniorhynchus*) for JEV transmission can be expressed as:

$$m = \frac{-\ln(P)}{a\ .\ S_c\ .\ V_c\ .\ P^n}$$

Data analysis:

Mathematical and statistical analysis of data for derivation of JE transmission model and its graphical presentation were done by using Microsoft Excel 2007 version and SPSS version 16.0. We have conducted online searches of published literatures on mathematical models related to vector density for JEV transmission through various database without restriction to languages or geography and finally ensures that the model for estimating critical density in *Culex tritaeniorhynchus* for transmission of JEV has yet to be derived. After initial search restricted to published data was done, a model was designed and derived on the basis of MacDonald, 1957 and Smith, 1987 models by including/excluding the parameters required for this estimation.

RESULTS

Mosquito abundance:

The mosquito vectors of JEV were longitudinally monitored for its abundance and virus infection in the villages of Cuddalore district. The *Culex vishnui* group, comprising *Cx. tritaeniorhynchus, Cx. vishnui* and *Cx. pseudovishnui* are proven vectors of JE in Southern India) (Reuben et *al.,* 1988). A three year longitudinal study was conducted during 1991-1994 with the objectives of monitoring vector abundance and JEV infection frequency in mosquitoes in the villages covered under the Nallur PHC, an area endemic for JE, in Cuddalore district. In which, a total of 422,621 female mosquitoes were collected and found that *Cx. tritaeniorhynchus* (62.6%) was the predominant species (Gajanana *et al.,* 1997). During April' 2011 to December' 2012, a total of 15,941 female mosquitoes representing 24 culicine species were collected. About 90.5% of total catch was contributed by the JE vectors, in which, *Cx.*

gelidus was 48.6%, *Cx. tritaeniorhynchus* was 40.7% and remaining 1.8% by *Cx. Vishnui*. A two decade research activities and the comparative analysis of the various studies related to vector abundance monitoring has revealed that *Cx. tritaeniorhynchus* was the principal vector. A maximum per man hour (PMH) density was observed (661.50) in October 2002 during the last ten years with reporting peak level during the months of October and November (Figure 1). A critical vector density was determined for *Cx. tritaeniorhynchus* since this particular species was found and reported as a predominant vector compared to other species in influencing the JEV transmission in Cuddalore district.

Blood feeding habit (h):

Assessment of blood feeding habits of *Cx. Vishnui* complex was done during the period of December 1988 and December 1990 and found out that a relatively high proportion of recognized vectors of JE virus were *Cx. tritaeniorhynchus* and *Cx. vishnui*, had fed mainly on cattle in addition to humans and pigs. Pig feedings (h) accounted for 4.4 – 5.4%, cattle feedings for 84.6 – 88% of the total feeding and human feedings for 2.4 – 6.2%, but reported that there were no ardeid - positive feeding (Reuben *et al.,* 1992). Though the ardeid was as one of host for JEV transmission, it was excluded while estimation of this model since there was no evidence of blood fed on ardeid in the study area.

Gonotrophic cycle (Gc):

The frequencies of blood meals taken and the survival rates of vector mosquitoes are important parameters influencing transmitting capacity of pathogens (Nat *et al.,* 1998). Mosquito gonotrophic cycle (Blood-feeding - egg maturation - oviposition) is repeated in several times throughout adult female mosquito's life cycle (Paaijmans and Mathew Thomas 2011). Mori (1983) and Somboon *et al.* (1989) estimated daily survival rate of JE vectors by using Davidson's method (Davidson, 1954) and found that the gonotrophic cycle duration was around 3-4 days. In Cuddalore district, JE cases mainly were occurred during the months of September – November. During these months, the gonotrophic cycle in females of the *Cx. vishnui* subgroup reached 3 days. Female mosquitoes infected after taking a viraemic blood meal and would become infective 9 day later after completing 3 gonotrophic cycles (Gajanana *et al.,* 1997).

Vector competence (Vc):

The virus transmitting capacity of a mosquito was influenced by various factors such as the ability of an ingested virus to survive and its development in the mosquito tissues and penetrates into the salivary glands in order to transmit into a new host. Vector competence is estimated as the proportion of mosquitoes with a disseminated infection to the total number of exposed mosquitoes, often expressed as dissemination rates within a vector population (Christofferson and Christopher, 2011). Philip Samuel *et al.,* 1998 have developed a system for assessing vector competence of mosquitoes and done experiments in three different areas (Cuddalore, Madurai and Alleppey) and reported that the estimated vector competence of *Cx. tritaeniorhynchus* (i.e. transmission rate) was ranged from 32 -74% ,in which, the range was about 32 % in Cuddalore.

Host (pig) susceptibility (Sc):

Gajanana *et al.,* 1995 have done a cohort prospective serological study (1989-1991) among primary school children and also monitored the prevailing status of JE virus infection among sentinel pigs in the study area. In this study, about 124 pigs were examined and 66 (53%) animals was found to be infected with JEV and seroconverted during the transmission season of JEV.

Survival probability (P):

The parous rate (Rate of parous mosquitoes) is one of the useful parameters to describe the age structure and net reproductive rate of the mosquito population. It is not only used to ascertain the daily survival rate of adult mosquitoes but also used to determine the recruitment rate of adults, the adult longevity and the length of a gonotrophic cycle, therefore, any changes in the parous rate will reflect and bring changes in the population (Yoshio *et al.,* 1991). In Cuddalore district, human cases were mainly affected and reported during the months of September to November every year. It was estimated that the parity rate (PR= the proportion of parous from the total number of ovaries dissected Ndoen *et al.,* 2012) was 0.33 in September, 0.42 in October and 0.32 in the month of November and the probability of the vector surviving one day (survival probability (*P*) was 0.69, 0.75 and 0.69 respectively (Table 2) and an the average survival rate of *Cx. tritaeniorhynchus* (*P*) was 0.8 during this transmission season (Reuben, 1963). The survival probability of *Cx. tritaeniorhynchus* ranged between 0.64 – 0.76 during April 2011 to December 2012.

Extrinsic incubation period (EIP) in vector (n):

The prolonged development period of mosquito larval and the longer extrinsic incubation period of JE virus at cooler temperature will reduce the virus transmission rate (Solomon *et al.,* 2000). Due to prolonged viraemia, mosquitoes get the opportunity to pick up infection from pigs easily (NVBDCP). After an extrinsic incubation period of 9-12 days, infected female mosquito transmits the virus to other vertebrate hosts (NVBDCP, 2006). It was estimated that extrinsic incubation period of JEV in *Cx. tritaeniorhynchus* was 9 - 10 days (n = 9-10 days) in Cuddalore district. Female infective mosquitoes taking a viremic blood meal and would become infective 9d later after completing 3 gonotrophic cycles. The proportion of infective female mosquitoes among those infected would be about $P^n = 0.13$ ($P = 0.8$, n = 9, $P^n = 0.13$) (Gajanana *et al.,* 1997). The proportion of vector surviving in the incubation period (P^n, n = 9-12 days) was ascertained after 9 days during the years 2011 and 2012 and was given in Table 3.

Mosquito life expectancy: There are two factors such as gonotrophic cycle (gc) and parity rate (PR) were required and used here to estimate the life

Table 2. Parity Rate (PR) and survival probability (*P*) of *Cx. tritaeniorhynchus* in Cuddalore district (Transmission seasons)

Year	Month	Dissected	Parous	PR	*P*	Age (days)
2011	September	106	39	0.37	0.72	3.03
	October	102	40	0.39	0.73	3.24
	November	88	23	0.26	0.64	2.26
2012	September	27	9	0.33	0.69	2.73
	October	90	29	0.42	0.75	3.49
	November	50	21	0.32	0.69	2.66

Table 3. The proportion of *Cx. tritaeniorhynchus* surviving the virus - during in the transmission seasons (2011 and 2012)

Year	Month	P	P^9	P^{10}	P^{11}	P^{12}
2011	September	0.72	0.051	0.037	0.027	0.019
	October	0.73	0.062	0.046	0.033	0.025
	November	0.64	0.019	0.012	0.008	0.005
2012	September	0.69	0.037	0.026	0.018	0.012
	October	0.75	0.076	0.057	0.043	0.032
	November	0.69	0.034	0.023	0.016	0.011

expectancy and infective life of mosquito. The PR, proposed by Davidson, 1954 was used to arrive this derivation. The gonotrophic cycle (gc) value was found to be 3 days. The life expectancy (age) of *Cx. tritaeniorhynchus* was in the range value of 2.26 - 3.03 in 2011 and 2.66 - 3.49 in 2012 during the transmission season (Table 4) which likely to be change based on the survival probability (P = 0.60 - 0.80) of the mosquito. Therefore, based on the survival probability, the life expectancy (age) and the expected infective life (days) of *Cx. tritaeniorhynchus* was estimated and given in Table 5 (Figures 2 and 3).

Critical vector density (m):

Critical density is used to estimate the number of mosquitoes required to bite a single host (pig) in order to maintain virus transmission in an area .The values required for estimation of critical vector density (m) were collected from the results of various studies conducted on JE during the two decades in Cuddalore district (Tyagi *et al.,* 2011). The critical vector density for the study area was estimated to be around 595 which mean a minimum of 595 mosquito's bite/pig/night is necessary in order to maintain virus transmission in the particular area (Table 6).

DISCUSSION

Culex tritaeniorhynchus is the most abundant mosquito, accounting for 41 to 63 % of the total mosquitoes species in Cuddalore district since this district is a major rice growing area in Tamil Nadu where pig rearing culture also coexist. This species usually bite at night; preferably on cattle, pigs and humans in the study area. The blood feeding pattern of the mosquitoes were assessed and found that cattle feeding was predominant and accounted for around 88%, followed by human blood feeding (6%) and pig feeding (5%). Though 88% of blood feeding pattern of mosquito was depend on the cattle, it will not play an active role in influence the virus

Table 4. Life expectancy and expectation of infective life (days) of *Cx. tritaeniorhynchus* in Cuddalore district during the transmission seasons (2011 and 2012)

Year	Month	P	Expectation Life	Expectation of infective life (days)			
				n = 9	n = 10	n = 11	n = 12
2011	September	0.72	3.03	0.156	0.112	0.080	0.058
	October	0.73	3.24	0.201	0.147	0.108	0.079
	November	0.64	2.26	0.042	0.027	0.017	0.011
2012	September	0.69	2.73	0.102	0.070	0.049	0.034
	October	0.75	3.49	0.266	0.199	0.150	0.113
	November	0.69	2.66	0.090	0.062	0.043	0.029

P = probbility of *Cx. tritaeniorhynchus* survival through one day, n = Extrinsic Incubation period (JE virus in the vector). Life expectation is expressed as (1/-ln(p)) and expectation of infective life as (p^n/ -ln(p).

Table 5. Expectancy of life or age and infective life of *Cx. tritaeniorhynchus* in Cuddalore district

Survival probability (P)	Life Expectation (1/-ln(p))	Expectation of infective life (days)			
		n = 9	n = 10	n = 11	n = 12
0.60	1.96	0.020	0.012	0.007	0.004
0.65	2.32	0.048	0.031	0.020	0.013
0.70	2.80	0.113	0.079	0.055	0.039
0.75	3.48	0.261	0.196	0.147	0.110
0.80	4.48	0.601	0.481	0.385	0.308

Table 6. Computation of the critical density (m) of *Cx. tritaeniorhynchus* in Cuddalore district

Proportion of host (pig) blood feeding Index (h)	0.05
Gonotrophic cycle (gc) days	3
a = Proportion blood feeing on pig/ Gonotrophic cycle(h/gc)	0.017
Proportion of host (pig) Susceptibility (S_C)	0.53
Proportion of Vector Competence (V_C)	0.32
Survival probability (*P*)	0.8
Incubation Period (n) (days)	9
P^n = The proportion of *Cx. tritaeniorhynchus* surviving the virus	0.13
Critical Density (m)	595.3

transmission cycle (dead end host). Though the ardeid birds are one of the main hosts (reservoir) in influencing the JEV transmission cycle but in the study area, the assessment of blood feeding pattern of mosquitoes was revealed that the ardeid host was accounted for zero percentage (Reuben *et al.,* 1992). Therefore, ardeid was not considered as one of the parameter in influencing the virus transmission and excluded from the estimation of critical vector density (m). If ardeid would found be one of host, then this factor also need to be included and considered along with pigs for formulae derivation. Pigs are important amplifying host, in which naive pigs are highly susceptible to infection and reported with high mortality (CFSPH, 2007). The number of pigs in a region can affect and influence the incidence of JE cases. However, we could reduce the risk of infection through changing the type of husbandry practices and modern pig farming.

The two decadal studies of JE in Cuddalore district found that the *Cx. tritaeniorhynchus* was the predominant species during eighties and nineties, but during 2011 and 2012 *Cx. gelidus* was found to be the predominant species, perhaps shift in agricultural practices from paddy to sugarcane which has a direct correlation with this *Cx. vishnui* and *Cx. tritaeniorhynchus* in density of the two vector species. The assessment of biting behavior of *Cx. tritaeniorhynchus* was found that it bites throughout the year irrespective of dry and cool-wet (C-W) seasons and the mosquito abundance which influences the transmission of virus cycle and was found to be happening around the clock. The EIP in mosquito was found to be a minimum of 9 days. The duration of the gonotrophic cycle of *Cx. tritaeniorhynchus* was found as 3 days and completes its 3 gonotrophic cycles after 9 days (Gajanana *et al.,* 1997). The high parous rates imply that the probability of daily survival of the vectors for efficient transmission of infection is high (Ree and Hwang, 2000). High parity values would be evidence for high egg development and high blood intake. The interaction between the survival probability (*P*) and EIP decides and influences the infective life days. It was found that infective life was gradually increased when the survival probability (*P*) was increasing and decreasing in EIP and vice versa (Figure 3).

The Basic reproduction model was developed by MacDonald (1957) in 1957 for transmission of malaria by the *anopheline* mosquitoes (Smith, 1987) presented a modification of the Malaria reproduction rate formula of MacDonald, 1957 by replacing men by birds in order to estimate the reproductive rate of arboviruses (R) for WEE where birds are active host in disease transmission . However, this estimation was based on so many untested assumptions. In this study, we have tried and estimated the critical density for JEV transmission on the basis model of Smith *et al.,* 1987 by including and excluding the factors which required for estimation for JEV. This model was estimated that a minimum of 595 mosquito bites/

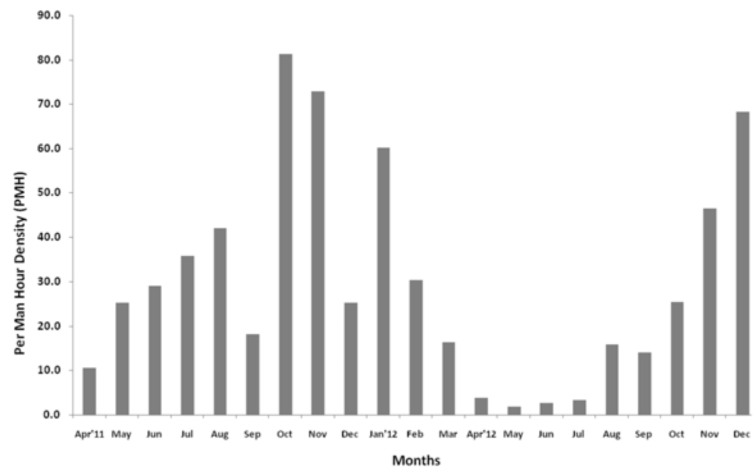

Figure 1. Cx. tritaeniorhynchus Per Man Hour density in Cuddalore district during

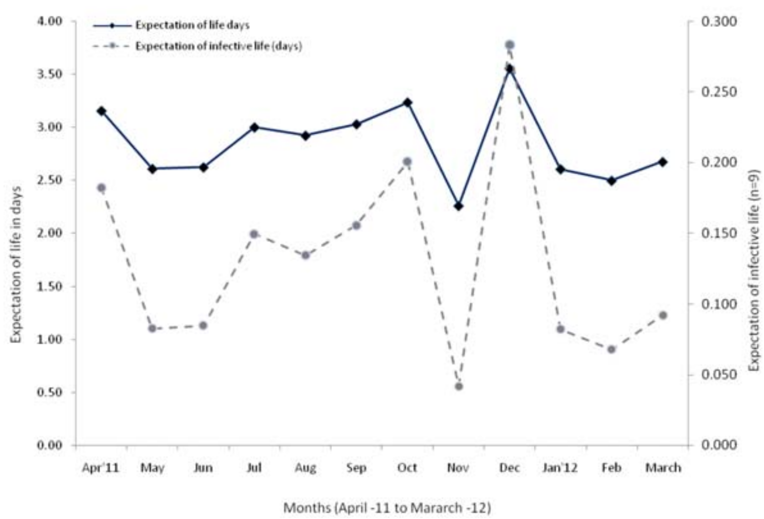

Figure 2. Expectancy of life days (age) and infective life (days) of *Cx. tritaeniorhynchus* in Cuddalore district during April 2011 to March 2012

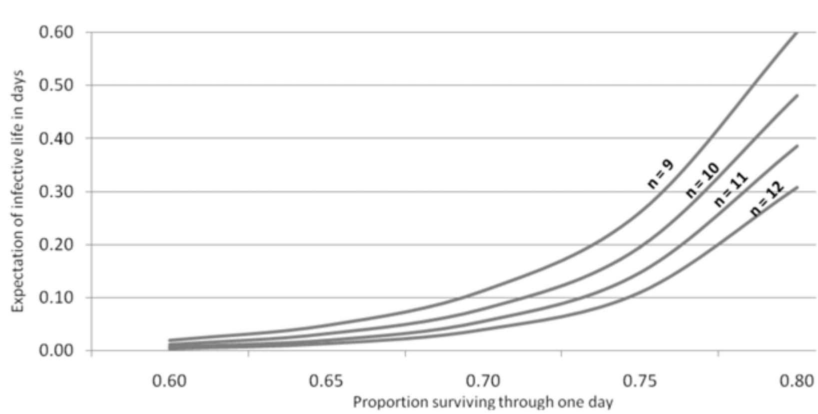

Figure 3. Survival probability and expectancy of infective life (days) of *Cx. traitaeniorhynchus* in Cuddalore district

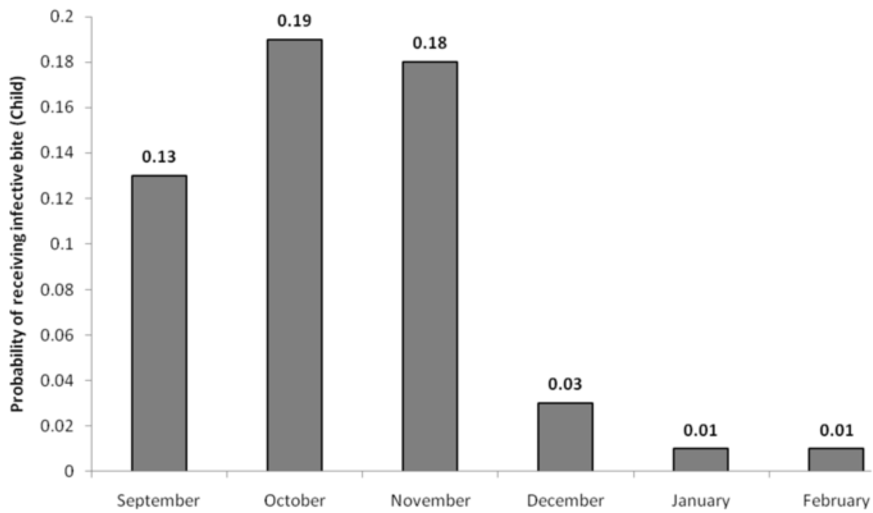

Figure 4. Monthly probability of receiving an infective bite in villages of Cuddalore district

pig/night are necessary to maintain JEV transmission.

Risk to human” Human is a dead end host in JE transmission cycle due to low and short-lived viraemia. Mosquitoes do not get infection from JE infected humans because humans usually do not develop a level or duration of viraemia sufficient to infect mosquitoes (Fischer *et al.,* 2010). Risk to humans depends on Infection rate in mosquito which relates to intensity of transmission between pigs and probability that a person is bitten by an infective mosquito. Majority of the cases were reported soon after monsoon, i.e. during August and September months. Upsurge of cases are reported during the rainy season (monsoon) (Gunasekaran *et al.,* 2012). JE cases started reporting during the months of April - May and reaching peak during late August to early September and subsequently decline from October (Ree and Hwang, 2000). The duration of the illness ranged from 1 to 14 days. The mean and median age were 5.7 +10.1 (S.D) and 7.5 years (range = 3 months – 15 years), respectively (Kabilan *et al.,* 2004). *The* distribution of cases was equal among both the sexes. In the study area, the probability of child receiving an infective bite during September and December was 0.53 which is reasonably close to the estimate of 0.50 – 0.75 was obtained from seroconversion rates in children in the same area (Figure 4) (Gajanana *et al.,* 1997). Minimum infection rate at mosquitoes (per 1000) in April 2011 to December 2012 was 0.24 (95 pools, 1 +ve pool) and average PMH was 26.8 during its peak season with 25.4 (October), 81.1(November) and 68.2(December) of the year (Figure 1). Susceptibility in humans may also depend on many other factors like age, immune status of the individual and other unknown factors. Hence, risk of the disease to humans will be evaluated with critical density of mosquitoes required to maintain the disease in pig population itself.

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