Chapter 4

Objective 1:

Epidemiological profiling of Japanese Encephalitis (JE) disease and evaluation of environmental factors influencing JE vector ecology in the targeted study area

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4.1: Introduction:

*Japanese encephalitis (*JE) is a disease of public health importance because of its epidemic potential and high case fatality rate. JE in patients who survive complications may lead to lifelong sequelae[1]. Though JE is primarily a disease of rural agricultural areas, where vector mosquitoes proliferate in close association with pigs and other animal reservoirs, epidemics have also been reported in peri-urban areas where similar conditions may exist[2]. For investigation of an outbreak, the first principle is to have in place a system to receive early warning signals and confirm diagnosis. In areas of low JE endemicity every single suspect JE case needs to be investigated. However in areas where JE is endemic the term outbreak can be applied to an unusual increase in suspected JE cases compared to the normal transmission or increase beyond the normal range due to seasonal variations. This normal range will be different from place to place. The purpose of JE and ASE Surveillance is to estimate disease burden and understand the disease pattern in terms of its influence on morbidity and mortality. The incidence of JE and AES will form the basis of any future planning for prevention and control of this disease. JE and AES surveillance would thus mean generation of authentic and valid information on epidemiological, clinical, laboratory and entomological parameters on regular basis.

Accordind to Tyagi, (2015)[3], the exact number of mosquito species and subspecies in India is unknown due to the lack of a reliable compilation of data on the topic. However, 16 mosquito species have been recognized as possible JE vectors, primarily *Culex vishnui, Culex tritaeniorhynchus*, and *Culex pseudovishnui*[4]. Female mosquitoes get infected after feeding on a vertebrate host harbouring JE virus and after 9-12 days of extrinsic incubation period, they can transmit the virus to other hosts. It is reported that, *Culex vishnui* subgroup of mosquitoes are very common, widespread and breed in water with luxuriant vegetation, mainly in paddy fields and their abundance may be related to their breeding in rice fields, shallow ditches, pools, fish ponds, etc.[5]. Preference for breeding places during rainy season and irrigation channels bordering the paddy fields, support breeding during non-monsoon season[6]. In view of the breeding habitats of the vector mosquitoes, JE is usually associated with rural areas with paddy cultivation. Thus people residing near rice cultivated areas, become vulnerable to JE infection. Therefore, the vector density of most prominent vectors of JE breeding in paddy fields was studied. This study will provide platform to select the best vector management practice to prevent JE infections to those who are susceptible to infection due to involvement in outdoor activity of rice cultivation practice.

As a mosquito-borne disease, environmental factors play a significant role in JEV transmission. Weather factors, including temperature and precipitation, have been reported to drive the JE transmission by affecting the mosquito life cycle including the development time of immature mosquito stages and mosquito density[7]. Temperature and rainfall were found to be closely associated with mosquito density. The two thresholds, maximum temperature of 22–23°C for mosquito development and minimum temperature of 25–26°C for JEV transmission, play key roles in the ecology of JEV[8].

4.2 Materials and methods

4.2.1 Study site

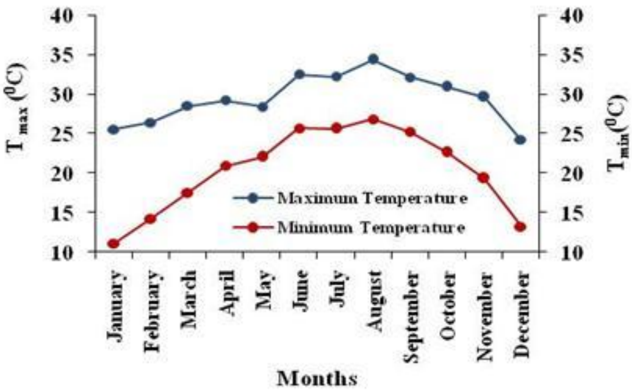
Details of study site is described in 3.1.1.1 in Chapter 3, materials and methods, page no.43

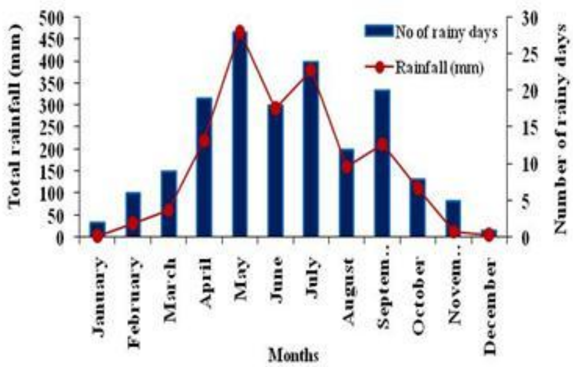
4.2.2 Meteorological data

Meteorological data was collected from Indian Meteorological Department that revealed that the study site has a moderate climate with high humidity throughout the year. Temporal pattern of mean maximum and mean minimum monthly temperature showed that maximum mean monthly temperature ranged from 24.2° to 34.3°C and mean minimum temperatures ranged from 11.1° to 26.8°C. Mean maximum temperature during the study period was 29.48°C and mean minimum temperature was 20.34°C with

January and December being the coldest and August the hottest months of the season with mean minimum temperature ranging from 11.1° to 13.2°C [Figure 4.1(a)].

Total rainfall recorded during the experimental period was 1961.5 mm and total rainy days were 152 days. Temporal variability in the rainfall and number of rainy days per months are detailed in the Figure 4.1. Maximum rainfall was received June at the advent of monsoon, meanwhile heavy showers from June to September contributed to maximum rainfall accounting to 37.05% of the total rainfall received. November and December received only 0.4 and 0.2 cm of rainfall. However, January was the driest period of all receiving only 0.1 cm of rainfall. Overall, there were 152 rainy days during the study period with maximum number of rainy days occurring from May to September (28-12 rainy days/months) and minimum during November-December (5-1 rainy days per months) [Figure 4.1(b)].



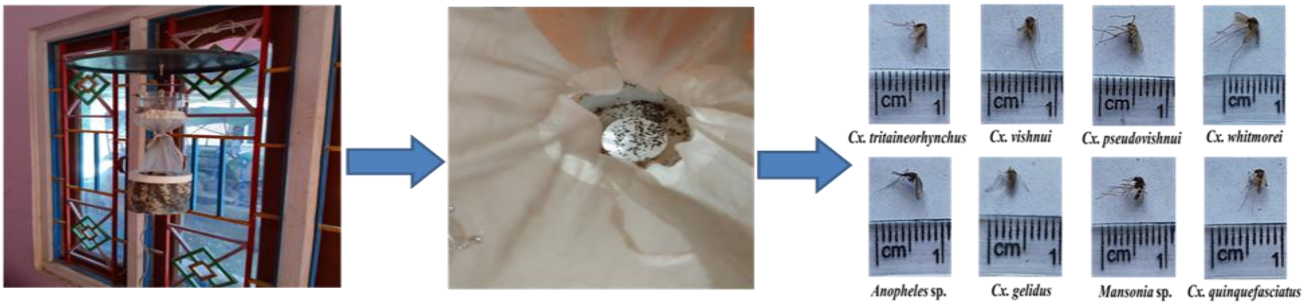


(a) ***(b)***

Figure 4.1. (a) Maximum and minimum temperature distribution (b) vents and number of rainy days

4.2.3. Case Fatality Rate (CFR) and Sample positivity rate (SPR): Details of calculation of SPR and CFR is described in section 3.1.1.5 of Chapter 3 (materials and methods) page no 47.

4.2.4. Mosquito sampling: Figure 4.2 shows collection and identification of mosquitoes. Details of collection of mosquito samples are described in section 3.1.2.1 Chapter 3, materials and methods Page no. 48.



Collection of adult mosquitoes using CDC miniature light trap

Killed mosquitoes using chloroform inside the trap

Identification of different species

Figure 4.2: Pictures showing collection and identification of adult mosquitoes

4.2.5. Relative density of vector mosquitoes: Compilation of mosquito sampling data and its calculation for relative density is described in section 3.1.2.2 of Chapter 3, page no.49

4.2.6. Statistical analysis

The number of significant JE vectors of *Culex* species which are known to breed in agricultural fields were collected and pooled and standardized to numbers per trap per night (12 dark hours) density. SPSS (IBM) and Microsoft excel were used to find mean, SD, ANOVA and correlation analysis.

4.3 Results and discussion

4.3.1 Case Fatality Rate (CFR) and Sample positivity rate (SPR)

A trend of occurrence of JE during 2013 to 2019 revealed that Bihaguri sub center witnessed at least 1case (2017) or up to 7 JE cases (2019) in the past 7 years (Table 4.3). However, in 2013 highest JE cases were reported in other regions such as North Jamuguri, Balipara in 2014, Dhekiajuli in 2015 and 2016, North Jamuguri in 2018 and Gohpur in 2019. Maximum JE cases were reported in the study area i.e., Bihaguri and corresponds to the relative density, CFR and SPR determined in the current investigation (Table 4.3). Maximum average SPR in these 7 years (2013-2019) was observed in 2019 (32.28 %), which is higher than already reported JE statistics in Sonitpur, Assam i.e., 18.43% by Borah et al., 2018[9] and 11.51% by Baruah et al., (2018)[10] for Kamrup district in 2018 and lower than reported by Baruah et al. (2018)

in i.e., 74.49% in Sivasagar district[10]. CFR peaked in 2018 and declined in 2016 and 2019 (Table 4.1).

In 2019, notable positivity rate of JE out of total AES registered was 31.25% in Sonitpur which was found to be much higher than reported by Kumar, (1990)[11] (23% in India). JE incidences were highest in the month of July and agreed to the relative densities, CFR and SPR determined in the study area (Table 4.1). Of the total recorded 40 cases at Sonitpur, 7 cases representing 17.50% were from Bihaguri, (Table 4.1). Almost an equal sex distribution was observed in case of JE infestation in entire Sonitpur district in the North Bank Plain Agroclimatic Zone. Most of the JE cases belonged to less than 6 years age group, mostly males except in the year 2016 [Table 4.2(a), 4.2(b)]. The introduction of vaccinations in Assam is the reason behind the decline in AES and JE in children under the age of six[21]. Nonetheless, rising adult JE incidence suggests that surveillance improvements for monitoring adult JE and AES cases must continue in order to assess whether adult JE immunization is necessary JE cases were also frequent among male population of a higher age group. Similar findings are reported in Nepal by Akiba, 2001[12] in study conducted on JE infestation in children belonging to age group less than 6; however, higher age group infestation was low in Nepal. In the current investigation, a greater number of males were infected than female, i.e., 15.66% more infections in male population in the study area. The reason behind it is more exposure of males than females in outdoor activities (most likely during cropping season) which makes them vulnerable to mosquito bites. The average number of JE cases was 4.57 per year in the period 2013 – 2019 in the North Bank Plain Agroclimatic Zone, Bihaguri.

Table 4.1: A report on AES and JE cases, and case fatality rates (CFR) and sample positivity rates (SPR)

Year	AES/JE cases	JE positive cases	JE death	CFR (%)	SPR (%)
2013	96	14	6	42.86	14.58
2014	231	44	9	20.45	19.05
2015	170	30	7	23.33	17.65
2016	105	21	3	14.29	20.00
2017	124	12	2	16.67	9.68
2018	75	18	7	38.89	24.00
2019	127	41	6	14.63	32.28

Table 4.2(a). Age distribution of AES and JE affected population

Age group	2013	2014	2015	2016	2017	2018	2019
0-6	4	4	4	2	4	5	8
>6	4	39	21	19	8	11	33

Table 4.2(b). Gender distribution of AES and JE

Gender	2013	2014	2015	2016	2017	2018	2019
Male	5	26	17	8	8	7	25
Female	3	17	8	13	4	9	16

4.3.2 Characterization and quantification of JE vector mosquitoes in the study area

4.3.3 Spatiotemporal distribution of AES and JE

AES cum JE distrubtion was noticeable in the mid year humid periods i.e., May and July during rainy days, Figure 4 showed dominance of JE in hot and wet period and distinctly higher in study site named Bihaguri than all other subcenters (Table 4.3). The JE peak was intense in the month of July that relates to a similar study in Nepal reporting increase in cases after the rainy season, JE cases started appearing in the month of April and May, reached maximum in late August to early September and finally decline towards October (Figure 4.3).

Table 4.3: Record of JE cases in different Block Public Health Center in Sonitpur in the North Bank Plain Agro Climatic Zone of Assam

BPHC	2013	2014	2015	2016	2017	2018	2019	Average
Balipara	1	12	3	2	2	1	7	4.00
Gohpur	2	6	4	1	1	1	10	3.57
Biswanath Chariali	2	3	2	3	0	2	2	2.00
North Jamuguri	4	4	5	4	2	5	2	3.71
Rangapara	0	5	2	0	1	2	1	1.57
Dhekiajuli	2	4	6	5	5	2	4	4.00
Bihaguri	3	6	5	6	1	4	7	4.57
Behali	0	4	3	0	0	1	8	2.29

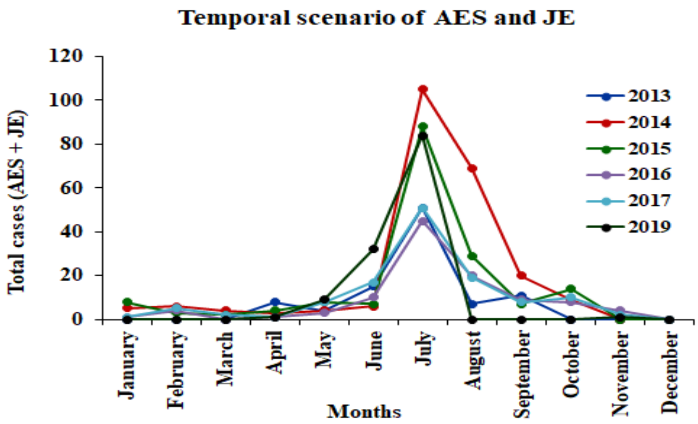


Figure 4.3. Temporal distribution of AES and JE in Sonitpur

Similar JE cases were reported a study in Chennai, majority of incidences were recorded after monsoon, gradually increased during August and September[13] and In this study, AES cases started in late April (hot and dry period) and showed up to November, but JE csaes initiated from June, obtined a maximum peak value in July (hot and wet period) and gradually declined in October (cool and wet period) (Table 4.4). However, in the month of January to February both JE and AES cases were minimum, these findings positively corresponds to the relative densities calcluated from night trap of *Culex* sp.described in Table 4.5. This investigation helped in tracing the rate and trend of emergence of JE epidemics in the North Bank Plain Agroclimatic Zone,Assam.

Table 4.4: Month wise distribution of AES and JE cases in study site (2019)

Months	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
	Cool and dry			Hot and dry			Hot and wet			Cool and wet			
AES	0	0	0	1	10	28	88	0	0	0	1	0	128
JE	0	0	0	0	0	8	31	0	0	0	1	0	40

Table 4.5. Relative density/relative abundance of mosquito species recorded in CDC light traps

Month	Jan	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec
	cool and dry			hot and dry			hot and wet			cool and wet		
***Culex tritaeniorhynchus*/trap/nt**	7.67	7	6.33	14	9.33	11.67	18.33	8.33	6	8.33	28.67*	11
Relative density	(43.4)	(24.42)	(14.07)	(16.6)	(11.2)	(6.1)	(8.79)	(17.61)	(22.50)	(67.57)	(65.65)	(70.21)
***Culex pseudovishnui*/trap/nt**	7.67	7.33	7	18	6.67	16.67*	13.33	6	9	3.67	14	2.67
Relative density	(43.4)	(25.58)	(15.56)	(21.34)	(8)	(8.71)	(6.39)	(12.68)	(33.75)	(29.73)	(32.06)	(17.02)
***Culex vishnui* /trap/nt**	2.33	14.33	32.33	52.33	67.33	158	178.33*	33	11	3.33	4	2
Relative density	(13.21)	(50)	(71.85)	(62.06)	(80.80)	(82.58)	(85.46)	(69.72)	(41.25)	(27.03)	(9.16)	(11.00)

*, p value less than 0.05; nt, night

4.3.4 Seasonal variation and JE vector occurrence

The annual rainfall was recorded as 1961.5 cm in the study sites. Maximum rainfall was received in May (i.e., 468.1 cm with 28 rainy days) at the advent of monsoon, meanwhile heavy showers from June to September contributed to maximum rainfall accounting for 38.42% of the total rainfall received. November and December received only 5.8 and 2.8 cm of average rainfall. However, January was the driest period of all, receiving only 2.8 cm of rainfall. Overall, there were 152 rainy days during the study period with maximum number of rainy days occurring from May to September (12-28 rainy days/months) and minimum during November-December (1-5 rainy days per month) (Figure 4.1). Sampling of JEV vector mosquitoes though night trapping method produced 5 genera–*Anopheles, Culex, Aedes, Armigeres* and *Mansonia*, among these, *Culex* sp. showed highest count. In the study area, *Culex* species are the potential JEV vectors, mainly *Culex tritaineorhynchus*, *Culex vishnui* and *Culex pseudovishnui*.

Culex vishnui preferred hot and dry as well as hot and wet periods i.e., days with less rainfall, humid days and also rainy days with an average of 52.33-178.33 counts in the months April to July, and gradually recede towards the end of July (Table 1, Table 5). *Culex pseudovishnui* was abundant throughout the year, occurred maximum in cool and dry period (upto 43.4 % in January), consistently significant in hot and wet to cool and wet periods (upto 33.75 % in September) however, maximum night trap count was recorded in hot and dry period i.e., 16.67 counts in June ($p<0.05$, LSD=0.94). The population of *Culex tritaeniorhynchus* was prominent in cool and wet season i.e., October to July (66.98 %), and surprisingly it was abundant throughout the year (Table 4.5, Table 4.6). The maximum mosquito per trap per night for *Culex tritaeniorhynchus* (28.67), was recorded in November which coincides with peak paddy cultivation period ($P<0.05$, LSD=2.61) (Table4.5, Table4.6, Figure4.3). This investigation proved that occurrence of *Culex vishnui* i.e., highly counted species (78.66%) (LSD=11.25 $p<0.05$) during April to July coincides with significant AES and JE ($R=0.868$, $p<0.01$, two-tailed) and JE ($R=0.820$, $p<0.01$, two-tailed) incidences in the study area (Table 4.6, Table 4.8).

Potential JEV vectors identified in this study are those that prefer to breed in paddy field especially during the rice growing season i.e., February to April (cool and dry period), July to August (hot and wet period) and December to February (cool and dry period). This observation positively corresponds to the relative abundance and month wise JE and AES cases in the study area; It was observed that the three species of *Culex* actively occurred during the rice growing season but *Culex vishnui* dominated in cool and dry period (53.33 %) and hot and wet period (78.66 %) (Table 4.6). JEV infections peaked during the rice growing period i.e., February (cool and dry period) to early September (hot and wet period), and gradually decline towards post monsoon season in early November (cool and wet period). It was also established that *Culex tritaeniorhynchus*, *Culex pseudovishnui* and *Culex vishnui* are the three species which prefer to breed in stagnant water like rice fields[14]. During this entire period i.e., February to November, out of the total night traps, 2421, 410 were identified as *Culex tritaeniorhynchus*, 336 as *Culex pseudovishnui* and 1675 as *Culex vishnui*. Accordingly, 69 % of the total catch was *Culex vishnui* followed by *Culex tritaeniorhynchus* (16.94 %). These findings match with the observations of Khan et al., (1996), however, Kanojia et al., (2007) found *Culex tritaeniorhynchus* to be more abundant during JE transmission period that coincides with the paddy cultivation season[15].

Table 4.6: Seasonal distribution of *Culex sp.* in study site (2019)

Species / Season	***Culex tritaenorrhynchus***	***Culex pseudovishnui***	***Culex vishnui***
Cool and dry	22.99	24.09	53.33
Hot and dry	9.75	11.51	77.34
Hot and wet	11.56	10.02	78.66
Cool and wet	66.98	28.37	13.02

4.3.5 Relative density

In this investigation, five genera namely *Anopheles*, *Culex*, *Aedes*, *Armigeres* and *Mansonia* were identified from the trapped mosquito's population. *Culex* sp. were isolated and preserved separately for advanced studies. *Culex sp*. has three JE potential sub-species namely *Culex tritaineorhynchus, Culex vishnui*, *Culex pseudovishnui*. 2414 female mosquitoes belonging to the species – *Culex tritaeniorhynchus*, *Culex pseudovishnui*, and *Culex vishnui* were collected during dark hours. It was observed that, among the three species, overall relative density was high for *Culex vishnui* (85.46 % in July) followed by *Culex pseudovishnui* (43.4 % in January) and *Culex tritaeniorhynchus* (65.65 % in November) (Table 4.5) for 12 months study period. Relative density of mosquitoes was significant during the period pre-monsoon to post-monsoon i.e., March to September, except for *Culex tritaeniorhynchus* which shows its peak in November.

Culex vishnui significantly dependent on rainfall which coincide with inundation of the rice fields (rainfall, $R=0.67$ at $p<0.05$ and rainy days, $R=0.74$ at $p< 0.01$) (Table 4.7). Earlier studies also report that rainfall is a crucial factor for proliferation of JE vectors especially *Culex vishnui* that concentrates during the month of April to September in the North Bank Plain Agroclimatic Zone[16]. Three JE vectors i.e., *Culex tritaenorynchus, Culex pseudovishnui*, and *Culex vishnui* were distributed mostly at three places – cattle-sheds, residential huts and concrete residential houses depending on their feeding nature of the female mosquitoes.

Table 4.7. Correlation between *Culex vishnui, Culex tritaenorynchus* and *Culex vishnui* abundance and climate variables – rainfall and no. of rainy days

	Cx.tritaenorynchus	***Cx.pseudovishnui***	***Cx.vishnui***
Rainfall	0.002	0.422	0.668^{*}
No. of rainy days	0.007	0.351	0.740^{**}

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

**Cx., Culex; No., number*

4.3.6 Influence of environmental variables on distribution of JE vectors

The principal aim of this study was to determine the role of seasonal and environmental variables in abundance of dominant JE vectors at a spatiotemporal scale and hence positively influencing the incidences of JE and AES in the study area. Modulations of meteorological variables were directly involved in modulating the populations of JE vectors in rice fields and hence elevating the JE transmission rates in the study area which was established by regression analysis (Table 4.4, Table 4.6). This investigation finally revealed that occurrence of JE vectors has a strong dependency on rainfall events, temperature, and humidity. Table 4.5 clearly depicts the relative abundance of *Culex vishnui* in July i.e., hot and wet period, with a high mosquito count of 178.33; during this period rainfall (rainfall days), temperature and humidity was comparatively higher than any other months throughout the year, this association of dominance of *Culex vishnui* with climate variables was statistically significant (R=0.949, F=9.141, p=0.01) (Table 4.8). While in case of *Culex tritaenorynchus,* AES and JE cases was evident in two different months (Table 4.5; Table 4.6), i.e., July (18.33) and November (28.67), with significant and direct relation with temperature, rainfall, and humidity (R=0.966, F=14.036, p<0.01) (Table 4.8). It coincides with paddy cultivation[15]. However, *Culex pseudovishnui* dominance was not significantly related to the climatic variables (R=0.822, F=2.077, p>0.01) (Table 4.8), the population inclined in April (18) which gradually declined and reached its minimum in December (2.67) (Table 4.5).

It was evident that ample amount of rainfall provides a preferable breeding environment for the vectors, especially for *Culex vishnui* and *Culex tritaenorynchus*[17]; the rainfall pattern also influenced the vector population size and larval habitats[18]. Moreover, humidity contributed to a longer survival and dispersal of the JE vectors which also increased the probability of higher transmission of JE virus and indices of AES and JE in the study area; similar findings were reported by Linquist et al., (2013)[19]. In this investigation, it was established that there is a strong dependency of JE occurrence on climatic variables and hence AES and JE infestation is a recurring issue in Northeast India that experiences four types of weather i.e., i) cool and dry (January to March), ii) hot and dry (April to June), iii) hot and wet (July to September), and iv) cool and wet periods (October to December). From epidemiological data and experimental findings in this study, it was also evident that JE is very frequently observed in Sonitpur in the North Bank Plain Agroclimatic Zone, Assam throughout the year, especially monsoon and post-monsoon months. It is evident that *Culex vishnui* is the most frequently occurring JE vector species that concentrated during the period pre-monsoon to post-monsoon i.e., April to November, and preferred an average optimum temperature of 29°C, relative humidity up to 85.3 % and breeding evidently in the inundated rice fields with rainfall up to 28 cm (rainfall, R=0.67 at p<0.05 and rainy days, R=0.74 at p< 0.01). Climatic variables modulated the active occurrence of JE vectors and all the three vectors noticeably coexisted in the study area with the highest densities of *Culex vishnui*.

Table 4.8: Regression among *Culex vishnui, Culex tritaenorynchus and Culex pseudovishnui* abundance and relative humidity (evening and morning), temperature (max and min) and rainfall

	R	F-value	p-value
Cx.vishnui	0.949**	9.141	0.015
Cx.tritaenorynchus	0.966**	14.036	0.006
Cx.pseudovishnui	0.822	2.077	0.221

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

T_{max}=Maximum temperature; T_{min}=Temperature minimum; Rh_m=Relative humidity of morning; Rh_e= Relative humidity of evening

Additionally, regression analysis between monthly JE cases and meteorological variables (i.e. temperature, rainfall, no. of rainy days, and humidity) with month- wise lag effect confirmed that 1–3 months might delay JE infections corroborating Singh et al., (2020)[20]. It was observed that a 1-month lag period in JE infection was significantly influenced by temperature (T_{max}) and relative humidity (R_{he}) at 6.00 h in the morning ($p < 0.05$) (Table 4.9). Relative humidity of both morning (6.00 h) and evening (18.00 h) were significantly correlated with JE occurrence with a lag of 2 and 3 months, respectively. This study revealed that the JE cases depend on meteorological factors and may follow a lag period of 1–3 months. These lag periods may act as an early indicator of JE infection, and the rate of JE infection may be slowed down by modulating the temperature, rainfall, and humidity.

Table 4.9. Association between meteorological variables and monthly total JE cases showing the lag effect at 1–3 months

Time	T_{max} – JE cases		T_{min} – JE cases		Rainfall – JE cases		No. of rainy days – JE cases		Rh_m – JE cases		Rh_e – JE cases	
	R	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**	**R**	**p-value**
1 month lag	0.753	0.035	0.767	0.029	0.137	0.927	0.146	0.918	0.587	0.184	0.786	0.021
2 months lag	0.545	0.244	0.545	0.244	0.504	0.31	0.554	0.231	0.746	0.039	0.818	0.012

3 months lag	0.652	0.11	0.652	0.11	0.631	0.131	0.691	0.075	0.799	0.029	0.777	0.025

T_{max}=Maximum temperature; T_{min}=Temperature minimum; Rh_m=Relative humidity of morning; Rh_e= Relative humidity evening

4.3.7 Co-existence of JE vector populations

It was also evident from the night traps that the three JE vectors species, *Culex tritaenorynchus, Culex pseudovishnui*, and *Culex vishnui* coexisted, irrespective of their relative densities in the site of trapping. A correlation was derived after a critical observation of 12 months; *Culex vishnui* had no correlation with the increase or decrease of populations of *Culex tritaenorynchus* and *Culex pseudovishnui* populations ($p<0.01$). Occurrence of one mosquito species was not dependent or affected by the dominance of another species, the three coexisted independently however, and the *vishnui* species may have a higher dominance than the other two species due to superior breeding abilities, as greater number of female mosquitoes or predominant existence in the study area for a long period have been seen. Another crucial reason for higher populations of *Culex vishnui* may be due to habitat diversity and asynchronous breeding. However, Khan, (1996)[21] in his study found that occurrence of *Culex vishnui* had a highly significant correlation with other JE vector mosquito species ($p <0.01$). Accordingly, incidences of AES and JE were also highly significant with the occurrence of mosquitoes especially *Culex vishnui* in the study area (AES, r=0.87 and JE, r=0.82, $p< 0.01$) (Table 4.10).

Table 4.10: Correlation matrix showing relationship among abundance of three JEV vectors and AES and JE infections

	Culex tritaenorynchus	***Culex pseudovishnui***	***Culex vishnui***	**AES**	**JE**
Culex pseudovishnui	0.553				
Culex vishnui	0.217	0.573			
AES	0.337	0.386	0.868**		
JE	0.365	0.379	0.820**	0.992**	

**. Correlation is significant at the 0.01 level (2-tailed).

4.4 Conclusion

Japanese Encephalitis is surfacing as a life-threatening disease, especially in the Indian subcontinent because this zone experiences a sub-tropical climate that promotes high emergence of JE and AES infestations. Knowledge about the nature and ecology of dispersal of JE vectors is insufficient. This study mechanistically explored the eco-biology of JE vectors, and mode and frequency of occurrence of Acute Encephalitis Syndrome (AES) and JE infestations. Statistical analyses showed the impact of environmental variables on JE infestation by *Culex tritaeniorhynchus, Culex vishnui,* and *Culex pseudovishnui*. *Culex vishnui* had a high abundance in residential areas during hot-wet period (85.46 %), at temperatures between 31°C–34°C and relative humidity of 80 %–85.3 %. JE infestation dominated near rainfed rice-fields (rainfall, R=0.67 at $p<0.05$ and rainy days, R=0.74 at $p< 0.01$). SPR was up to 32.28 % and CFR was as high as 42.86 %, JE infestation concentrated in adult male humans near rice-fields (15.66 %).

As a result of this study, it can be deduced that the North Bank Plain Agroclimatic Zone is susceptible to JE infestation, due to abundance of dominant JE vectors. Implementation of vector control and surveillance programme during monsoon and post monsoon months in the Northeast India may adopted as the need of the hour because JE is currently an epidemic disease in Asian countries, mostly in India and China. Modulations in temperature and rainfall patterns over years shifted the breeding period of *Culex vishnui*. There is a tendency of rise of JE virus load in JE hotspot regions, especially near rice-fields. Thus, comprehensive epidemiological investigations will help in preventing a silent pandemicity of JE virus.

Vaccination with respect to long-term protective effect, and immunization programs will benefit the residents of India are pocketed in rural areas, and population surviving at below poverty line (BPL) in unhygienic places in the urban, vaccinations may be primarily focused in these areas[22] . Further studies should be undertaken to analyse the JE virus load and its transmission in these regions and possible control measures in the JE reported areas. It may be concluded that inclusive study and extended research on frequency of Acute Encephalitis Syndrome (AES) and JE infestations and the causal vectors will help in creating base line information and hence sustainable solutions in eradicating JE in the most affected regions of the world. It is also emphasized that research investigations on JE and its vectors will support the health care organizations to understand the impact of environment on JE infestation and the precise cause of spatiotemporal occurrence of JE vector species in the JE epidemiological pockets.

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