Journal of Experimental Biology and Agricultural Sciences, December - 2022; Volume – 10(6) page 1366 – 1375

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

The epidemiological and neurological risk factors of Japanese encephalitis virus in the population of Assam, Northeast India

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Received – July 17, 2022; Revision – October 19, 2022; Accepted – November 29, 2022
Available Online – December 31, 2022

DOI: http://dx.doi.org/10.18006/2022.10(6).1366.1375

KEYWORDS

CSF

Epidemiology

Japanese Encephalitis

Vector

Assam

ABSTRACT

Japanese encephalitis is one of the world's most common public health issues, particularly it is prevalent in the north-eastern Indian states of Assam. This study aimed to find out the risk factors linked to clinical and epidemiological characteristics. A total of 245 cases were found as PCR-positive in Assam. The most common clinical symptoms were fever (87%), seizure (65%), altered sensorium (60%), cold with shivering (74%), vomiting (68%), throat irritation (31%), cough (67%), chest pain (10%), joint pain (18%), mouth ulcer (18%), diarrhea (29%), pain in the abdomen (42.9%), runny nose (64%), redness in eyes (78%), jaundice (25%), and blood in the sputum (25%). Further, the neurological symptoms included vision problems (66.5%), hearing difficulties (55 %), neck stiffness (62%), limb numbness (65%), dizziness (77%), headaches (75.5%), speaking difficulties (63%), hydrophobia (47%), and abnormal behavior (66%). The epidemiological risk factors included contact with pigs (57%), bats (21%), cattle (32%), and rates (66%). In addition, 24.5% of patients observed the death of animals/birds. The protection measure included window screening, sleeping under a mosquito net, and use of insect repellent while sleeping in open compounds (29%) and floods (63%) are considered important risk factors. JE-positive cases include daily habits like working in agriculture fields (28%), in standing water (16%), swimming in nearby lakes (24%), traveling outside their village (40%), and wearing shirts while working in the field (20%), storing water in open containers in or outside the house (62%). These were the epidemiological factors that affected the abundance of the potential mosquito vectors of the JE infection.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

JE infection is caused by the JE virus which is a single standard RNA genome and the genome size is 11 kb (Habib et al. 2022). The JEV viral structure contains mainly three underlying structural and nonstructural proteins (NS) i.e. nucleocapsid or center protein (C), non-glycosylated film protein (M), and glycosylated envelope protein (E), as well as seven non-structural (NS) proteins i.e. NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS.7 (Ashraf et al. 2021). While structural proteins are always in charge of virus assembly, viral entry into host cells, and finally viral egress from those cells. The NS proteins play a key role in viral replication and immune response evasion by the host (Xiong et al. 2022).

Basically JE influences youngsters or adolescents as the immunization program is mainly developed for mature-aged people. The hereditary examinations recommend that JEV start from the Malay Archipelago area. The infection advanced, presumably a long time back, into various genotypes (I-IV) and spread across Asia (Solomon et al. 2003). JEV keeps on attacking other topographical regions and becoming a serious general medical issue. JEV, a mosquito-borne flavivirus (Furuya-Kanamori et al. 2022), belongs to the family Flaviviridae and causes single-stranded positive-sense RNA infection (Karabatsos 1985). Pigs and birds, for instance, significantly contribute to the development and proliferation of the infection (Ladreyt et al. 2019). One percent of human JEV causes develop into JE. However, CDC (2013) reported that more than half of survivors in these cases including 20-30% of fetal cases are alive with significant neurologic or mental sequelae or mental stress disorder (CDC 2013). JEV is one of the most serious general medical conditions and also causes severe neuropsychiatric sequelae (Solomon 2006). In agricultural nations like India, kids are primarily impacted by the infection and the rate is assessed as 0.30 to 1.5 per 100,000 population (Gajanana et al.1995; Parida et al. 2006). Climate change causes an increase in potential mosquito vectors, along with this, intensifying hosts, horticultural practices, individuals' socio-cultural activities, and some other factors can also contribute to the spread of JE. During the long summer months from June to August, the frequency of motion sickness increases (Simon et al.2022). Children are more likely to develop symptoms of infection in places where Japanese encephalitis is endemic. Among all the risk factors, travelers are also the most prevalent to infect with this JE infection. So, travel guidelines for travelers towards Asia should maintain as a strict rule (Mileno 2020).

Studying the risk factors related to JE infection is necessary to identify a clear concept of virulence and pathogenesis. Further, to understand the risk factors, it is very much necessary to track the vaccine profile. The viral encephalitis infection in the northeastern part of India has not been studied among human hosts. Therefore this study was carried out to study the important symptoms, the concept of virulence, pathogenesis, and vaccine status in Assam, NE state of India.

2 Materials and Methods

2.1 Subject and study area

Assam is a state in Northeast India with a population of around 3 crores, according to the 2012 census. More than 85% of the population resides in rural areas, and more than 52% of the labor force is employed in agriculture. This study checked for JEV infection in Assam from June 2020 to June 2022, among the two hundred and forty-five clinically suspected viral encephalitis patients. The serums of the JEV-positive samples were collected from Gauhati Medical College and Hospital (GMCH). The serum from 245 and serum with CSF from 118 patients was collected and stored at -80^{0}C till tested at Gauhati University. JEV IgM detection was done by using ELISA units at GMC Hospital, Assam. The examples showing units > 50 were deciphered as JE infection explicit IgM antibody positive; such sure cases were taken as JE patients with late infection disease.

2.2 Data collection for epidemiology

The data related to the clinical features of the last two weeks of infections, neurological symptoms, personal behaviors, and epidemiological risk factors along with the vaccine profiles were recorded.

2.3 Statistical Analysis

Results from serological and molecular tests were combined with demographic and epidemiological data (sex, age), and entered into a spreadsheet made with Microsoft Excel software. Measuring means and frequencies was part of a descriptive statistical analysis. Statistics were considered significant for P-values under 0.05.

3 Results

In Assam, from June 2020 to June 2022, a total of 293 individuals were clinically suspected of viral encephalitis cases and also found positive for JEV IgM in the Gauhati Medical College Hospital, Assam, India. From these patients, different information related to neurological and epidemiological characteristics was recorded in this study. Also, both serum and CSF were collected from the patients. Out of 293 patients with IgM JEV positive, 245 were also found positive in PCR testing. These patients were included in this study and among these selected patients, 127 and 118 were male and female patients respectively (Table 1). The age group of the selected patients was reported between 11 and 20 and found to have the highest levels among all the study groups. Further, JEV infection was found in 38.6% of males and 45% of females, and this rate was found to be the most frequent (Table 2).

Table 1 Demographic profile of the enrolled patient's sex

Sex	Clinically-suspected cases reported (n=293)	JE positive cases (n=245);83.6%	Frequency of positive cases
Male	148 (50.5%)	127 (51.9%)	85.8%
Female	145 (49.5%)	118 (48.1%)	81.3%

Table 2 Frequency polygon distribution of age based on sex

Age (years)	Sex		Midpoint interval	Frequency
	Male	Female		
1-10	19 (15%)	19 (16.1%)	19	3
11-20	49 (38.6%)	53 (45%)	51	1
21-30	27 (21.2%)	29 (24.5%)	28	3
31-40	11 (8.6%)	5 (4.2%)	8	2
41-50	11 (8.6%)	7 (6%)	9	2
51-60	5 (4%)	3 (2.5%)	4	0
61-70	5 (4%)	2 (1.7%)	3.5	0
Total	127 (100%)	118 (100%)	-	-

Clinical features were presented in Table 3, where the data is represented by comparing the male and female populations. It was found that almost 87% of the JEV-positive patients had a history of fever and found it statistically significant ($p<0.5$). Seizures were found as an important feature of JE infection and 65% of patients had shown positive rates, which is statistically significant ($p <0.05$). Also, it was found that 76% had generalized and 24% had focal seizures. All the patients were carefully asked about their altered sensorium state during hospitalization, and 60% were found to be active with sensorium.

Table 3 Clinical features of the patients during the current illness

Factors	JEV PCR Positive Male (N=127) Frequency (%)	JEV PCR Positive female (N=118) Frequency (%)	P Value
Fever			
Present (N=213) 87%	116 (91%)	97 (83%)	<0.05
Absent (N=32) 13%	11 (9%)	21 (17%)	
Unknown (N=0) 0%	0 (0%)	0 (0%)	
SEIZURE			
Present (N=160) 65%	90 (70%)	70 (60%)	<0.05
Absent (N=80) 33%	36 (28%)	44 (38%)	
Unknown (N=5) 2%	1 (2%)	4 (2%)	
SEIZURE type			
Focal (N=58) 24%	36 (28%)	22 (19%)	0.3
General (N=187) 76%	91 (72%)	96 (81%)	
Unknown (N=0) 0%	0 (0%)	0 (0%)	
ALTERED SENSORIUM			
Present (N=146) 60%	78 (61%)	68 (58%)	0.19
Absent (N=99) 40%	49 (39%)	50 (42%)	
Unknown (N=0) 0%	0 (0%)	0 (0%)	
Cold/cold with shivering			
Present (N=182) 74%	102 (80%)	80 (68%)	<0.05
Absent (N=42) 17%	24 (19%)	18 (15%)	
Unknown (N=21) 9%	1 (1%)	20 (17%)	
VOMITING			
Present (N=166) 68%	95 (75%)	71 (60%)	<0.05
Absent (N=71) 29%	31 (24%)	40 (34%)	
Unknown (N=8) 3%	1 (1%)	7 (6%)	

Factors			JEV PCR Positive Male (N=127) Frequency (%)		JEV PCR Positive female (N=118) Frequency (%)		P Value
Irritation/Pain in the throat							
Present	(N=76)	31%	31	(24%)	45	(38%)	<0.05
Absent	(N=166)	67%	96	(76%)	70	(59.5%)	
Unknown	(N=3)	2%	0	(0%)	3	(2.5%)	
Cough							
Present	(N=164)	67%	93	(73%)	71	(60%)	<0.05
Absent	(N=77)	31%	34	(27%)	43	(36%)	
Unknown	(N= 4)	2%	0	(0%)	4	(4%)	
Chest Pain							
Present	(N=25)	10%	7	(5%)	18	(15%)	<0.05
Absent	(N= 219)	89%	119	(94%)	100	(85%)	
Unknown	(N=1)	1%	1	(1%)	0	(0%)	
Joint Pain							
Present	(N=44)	18%	17	(13%)	27	(23%)	<0.05
Absent	(N=192)	78%	109	(86%)	83	(70%)	
Unknown	(N=9)	4%	1	(1%)	8	(7%)	
Mouth Ulcers							
Present	(N=45)	18%	15	(12%)	30	(25%)	<0.05
Absent	(N=198)	81%	112	(78%)	86	(73%)	
Unknown	(N=2)	1%	0	(0%)	2	(2%)	
Diarrhea							
Present	(N=71)	29%	39	(31%)	32	(27%)	0.6
Absent	(N=174)	71%	89	(69%)	85	(73%)	
Unknown	(N=0)	0%	0	(0%)	0	(0%)	
Pain in the abdomen							
Present	(N=105)	42.9%	51	(40%)	54	(46%)	<0.05
Absent	(N=138)	56.3%	77	(60%)	61	(52%)	
Unknown	(N= 2)	0.8%	0	(0%)	2	2%)	
Runny nose							
Present	(N=156)	64%	83	(65%)	73	(62%)	0.1
Absent	(N= 89)	36%	44	(35%)	45	(38%)	
Unknown	(N=0)	0%	0	(0%)	0	(0%)	
Redness of eyes							
Present	(N=151)	78%	82	(65%)	69	(59%)	<0.05
Absent	(N= 47)	19%	43	(33.4%)	44	(37%)	
Unknown	(N=7)	3%	2	(1.6%)	5	(4%)	
Jaundice							
Present	(N= 62)	25%	36	(27%)	26	(23%)	0.4
Absent	(N= 173)	71%	86	(68%)	87	(74%)	
Unknown	(N=10)	4%	6	(5%)	4	(3%)	
notice any blood in sputum/vomiting/urine/faces							
Present	(N= 61)	25%	34	(27%)	27	(23%)	0.8
Absent	(N=184)	75%	93	(73%)	91	(77%)	
Unknown	(N= 0)	0%	0	(0%)	0	(0%)	

*p<0.05=Significant data

Further, 74% of JE patients show a history of cold or cold with shivering. Clinical findings also showed that 68% of patients were positive for vomiting. But a few patients (31%) had pain in the throat and most of the patients (67%) did not have any irritation in the throat. About 67% of JE-positive patients had a cough while 31% of patients did not have a cough. In this study, chest pain among the JE patients was not a major risk factor because 89% did not exhibit these symptoms and only a few (10%) have this type of symptom. The majority of the patients did not have joint pain, and only 18% of JE patients showed this symptom. Mouth ulcers were absent in 81% of patients. Most of the JE-positive patients had no issues with diarrhea (71%). Also, pain in the abdomen was reported in 56.3%, and 42.9% of respondents. Further, 64% of patients had runny noses, and 78% showed redness in the eyes. Jaundice was found positive only in 25% of JEV patients and blood in the sputum, vomiting, urine, or faces was observed only in 25% of

patients. JE patients with various neurological symptoms were also recorded when they were ill (table 4). Among the selected patients, 66.5% had problems with vision, 18% of patients had problems with photophobia, 10% of patients had double vision problems, and 49% showed blurred vision. Most of the patients had problems with hearing (55%). Further, 62% of positive JE patients had a major issue with their necks because they could not move them or neck stiffness occurred. A factor known as numbness in the limbs

Table 4 Neurological Symptoms of JEV patients during the illness of JEV

Factors	JEV PCR Positive Male (N=127); Frequency (%)	JEV PCR Positive female (N=118); Frequency (%)	P Value
Problems in Vision			
Present (N=163); 66.5%	75 (N=59%)	88 (N=75%)	<0.05
Absent (N=69) ; 28.2%	46 (N=32%)	23 (N=19.5%)	
Unknown(N=13) ;5.3%	6 (N=9%)	7 (N=5.5%)	
Bothered-by light/Photophobia			
Present (N=45); 18%	32 (N=25%)	13 (N=11%)	<0.05
Absent (N=186);76%	88 (N=69%)	98 (N=83%)	
Unknown(N=14); 6%	7 (N=6%)	7 (N=6%)	
Double vision/Diplopia			
Present (N=24);10%	18 (N=14%)	6 (N=5%)	<0.05
Absent (N=212);87%	101 (N=80%)	111 (N=94.2%)	
Unknown(N=9);3%	8 (N=6%)	1 (N=0.8%)	
Blurred vision			
Present (N=121);49%	73 (N=57%)	48 (N=41%)	<0.05
Absent (N=81); 33%	47 (N=37%)	34 (N=29%)	
Unknown(N=34) ;18%	7 (N=6%)	36 (N=30%)	
Any difficulties in hearing			
Present (N=136);55%	74 (N=57%)	62 (N=53%)	<0.05
Absent (N=97);40%	49 (N=38%)	48 (N=41%)	
Unknown(N=12);5%	4 (N=5%)	8 (N=6%)	
Unable to move neck/Neck stiffness			
Present (N=152);62%	87 (N=69%)	65 (N=55%)	<0.05
Absent (N=92); 37.5%	40 (N=31%)	52 (N=44%)	
Unknown (N=1); 0.5%	0 (N=0%)	1 (N=1%)	
Experience numbness in limbs			
Present (N=159);65%	93 (N=73%)	66 (N=56%)	<0.05
Absent (N=85); 34.5%	33 (N=26%)	52 (N=44%)	
Unknown(N=1);0.5%	1 (N=1%)	0 (N=0%)	
Dizziness			
Present (N=188);77%	106 (N=83%)	82 (N=69%)	<0.05
Absent (N=57);23%	21 (N=17%)	36 (N=31%)	
Unknown(N=0);0%	0 (N=0%)	0 (N=0%)	
Headaches			
Present (N=185);75.5%	103 (N=81%)	82 (N=70%)	<0.05
Absent (N=55);22.5%	23 (N=18%)	32 (N=27%)	
Unknown(N=5);2%	1 (N=1%)	4 (N=3%)	
Difficulties in speaking			
Present (N=155);63%	91 (N=72%)	64 (N=54%)	<0.05
Absent (N=90);37%	36 (N=28%)	54 (N=46%)	
Unknown(N=0);0%	0 (N=0%)	0 (N=0%)	
Any repulsion to drinking water/Hydrophobia			
Present (N=115);47%	65 (N=51%)	50 (N=42%)	0.18
Absent (N=124);51%	56 (N=44%)	68 (N=58%)	
Unknown(N=6);2%	6 (N=5%)	0 (N=0%)	
Any abnormal behavior			
Present (N=162);66%	86 (N=68%)	76 (N=64%)	0.14
Absent (N=77);31%	38 (N=30%)	39 (N=33%)	
Unknown(N=6);3%	3 (N=2%)	3 (N=3%)	

* p<0.05=Significant data

was also observed in 65% of the JE patients. In the case of dizziness, a total of 77% of patients have positive responses towards dizziness. Further, 75.5% of patients had headaches while admitted to the hospital. Among 63% of positive JEVs, there was a problem with speaking. Hydrophobia was recorded in 47% of positive patients. As JE is mainly a neurological disorder, 66% of patients showed abnormal behaviors.

When the study was done along with the clinical factors, a few important factors were noticed as presumptive risk factors (Table 5). It was found that 57% had contact with pigs in the last four weeks. There are also many people related to pig farming, so there is a chance of transmission of JE from pigs. Only 21% of patients had contact with bats and 32% had contact with cattle. Also, it was found that 66% of positive cases were in contact with the rates while 24.5% of JE-positive cases were in contact with dead animals. Further, the majority of patients (85%) did not have window screening in their homes, and 81% did not have insect repellents to protect themselves from vectors. Most of the patients used mosquito nets but 29% of patients slept outside their houses in the open compound in the last month of their infections. Further, 63% of patients had faced flood problems during the time of infection or recently occurred. Earlier studies from various parts of the world discovered that during flood times, incident rates of JEV

Table 5 Epidemiological Risk Factors of the JEV Patients among male and female groups

Factors	JEV PCR Positive Male (N=127)	JEV PCR Positive Female (N=118)	P Value
In the last four weeks have you been in contact with Pig			
YES (N=140); 57%	90	50	<0.05
NO (N=101); 41%	33	68	
UNKNOWN (N=4); 2%	4	0	
In the last four weeks have you been in contact with Bats			
YES (N=52); 21%	36	16	<0.05
NO N=182); 74%	83	99	
UNKNOWN (N=11); 5%	8	3	
In the last 4 weeks have you been in contact with Cattle			
YES (N=79); 32%	53	26	<0.05
NO (N=153); 62%	67	86	
UNKNOWN (N=13); 6%	7	6	
In the last four weeks have you been in contact with Rats			
YES (N=161);66%	92	69	<0.05
NO (N=68); 28%	28	40	
UNKNOWN (N=16); 6%	7	9	
Was there any animals/bird death			
YES (N=60); 24.5%	45	15	<0.05
NO (N=146); 59.5%	61	85	
UNKNOWN (N=39); 16%	21	18	
Do you have window screening in your house?			
YES (N=32); 13%	23	9	<0.5
NO (N=207); 85%	98	109	
UNKNOWN (N=6); 2%	6	0	
Do you sleep under the mosquito Net?			
YES (N=194);79%	108	86	<0.5
NO (N=51);21%	19	32	
UNKNOWN(N=0); 0%	0	0	
Use insect repellents?			
YES (N=44); 18%	34	10	<0.5
NO (N=199); 81%	92	107	
UNKNOWN (N=2); 1%	1	1	
Sleep outside your house in the open compound anytime in the last month?			
YES (N=72); 29%	51	21	<0.5
NO (N=173);71%	76	97	
UNKNOWN (N=0); 0%	0	0	
Recently flood occurred in the area?			
YES (N=155); 63%	90	65	<0.5
NO (N=90); 37%	37	53	
UNKNOWN (N=0); 0%	0	0	

Table 6 Personal behaviors of the patients during infection

Factors	JEV PCR Positive Male (N=127); Frequency (%)	JEV PCR Positive Female (N=118); Frequency (%)	P Value
Work in agricultural land			
YES (N=69); 28%	46	23	<0.5
NO (N=171); 71%	78	93	
UNKNOWN (N=3); 1%	3	0	
Work in standing water			
YES (N=39);16%	30	9	<0.5
NO (N=206); 84%	97	109	
UNKNOWN	0	0	
Swim in a nearby lake/pond			
YES (N=59); 24%	26	33	<0.5
NO (N=186);76%	101	85	
UNKNOWN	0	0	
Travel outside your village			
YES (N=97); 40%	72	25	<0.5
NO (N=148);60%	55	93	
UNKNOWN	0	0	
Usually, wear a shirt while working in the field			
YES (N=50);20%	34	16	<0.5
NO (N=45);18%	45	0	
Not applicable (N=150);62%	48	102	
Store water in open containers in or outside your house			
YES (N=153);62%	92	61	<0.5
NO (N=92);38%	35	57	
UNKNOWN	0	0	
Usually, take Bath			
River /Stream (N=14);5.7%	5	9	<0.5
Pond (N=13);5.3%	5	8	
House (N=218);89%	117	101	

*p<0.05=Significant data

infection rise because the conditions become more favorable for mosquitoes. In table 6, it was found that personal behaviors also have a significant role in JEV infections. In the case of working conditions, 28% of patients worked on agricultural land and 16% of patients worked on standing water. Also, 24% of patients swam near lakes or ponds while 40% of JEV patients used to travel outside their villages. Only 20% of patients usually wore shirts while working in the fields. Further, 62% of patients used to store water in open containers in or outside their houses while 89% of the patients used to take baths in houses, 5.3% used to take baths in ponds, and 5.7% used to take baths in rivers or streams.

4 Discussion

The single-stranded, enclosed RNA virus known as Japanese encephalitis virus (JEV), has been linked to severe neurological problems, particularly in young infants, and is a health concern throughout Asia (Robert and Gandhi 2020). The main natural hosts for JEV are pigs, bats, and birds, though humans can also contract the disease. JEV needs a few specific host proteins for their multiplication inside the host cells and these are available in mammals (Kumar et al. 2022). The disease transmission rate and study of the research center affirmed JE differed notably by prefecture, with transcendence in young generations in Assam. The JEV vaccinations helped adults to eradicate the infection. Results of this study showed 83.6% of serologically confirmed JE cases in 2 consecutive years (2020–2022), with reports of 293 clinically-suspected viral encephalitis patients in Assam. A viral-specific JEV IgM antibody test showed a positive confirmation of JEV. Both the CSF and serum were used to study the positive rates of admission to hospitals. The proof of ongoing JE infection in patients with the clinical elements of meaning encephalopathy and an epidemiological foundation of the illness upheld the affirmation of JE cases without CSF testing.

Previous studies reported that JE is more prevalent among the young age groups, and it is necessary to find age-wise infection information (Li et al. 2016). This may be because of their high openness to tainted mosquito nibbles with lower insusceptibility. The event was more noteworthy in the long-term age group, which may be because of the age-related hyperactivity of the youngsters.

This study showed higher involvement of the adult population aged between 11 and 20. Similarly, the male population (51.9%) is more susceptible as compared to the female population (48.1%). This might be due to the higher exposure of male patients to

mosquito vectors or the use of less protective measures being used by male populations. A new set of treatments to stop JE may be developed as a result of our better understanding of the JE pathophysiology (Ashraf et al. 2021). The clinical features observed in the study were found to be significant. But variations can be observed among the male and female populations. Although the vast majority have, for all intents and purposes, no side effects, others can endure mind contamination that can cause cerebral pains, spasms, retching, confusion, and seizures. In the current study, the most widely recognized factor was fever and these results are in agreement with the previous studies of Sen et al. (1976) and Patgiri et al. (2014).

In this study, symptoms like fever, seizure, cold, vomiting, pain in the throat, cough, chest pain, joint pain, redness in the eyes, mouth ulcer, and pain in the abdomen are reported and these findings corroborated by the findings of Xu et al. (2022). However, abdominal pain might be associated with dietary habits or any other bacterial infections that might be given importance while the patients are admitted to the hospital. Among the studied vectors, the predominant vector in South India is *Culex tritaeniorhynchus*, (Arunachalam et al. 2004) and in a few other JE-impacted regions in India (Kanojia et al. 2003). Overall in all parts of India JE reported about every year. From that point forward, the infection was found to be dynamic in practically all aspects of India, and episodes have been accounted for consistently. Stop inoculation remains the best preventive procedure for JEV control because of its complicated eco-the study of disease transmission. The Disease's expansion to the gullible non-endemic region of the Country and the conditions in India's Northern and north-eastern regions were both constantly attributed to the infection's travel. As of late, India saw one more huge flare-up in Malkangiri in 2012 and Manipur in July 2016 (Dwibedi et al. 2015).

As JE is also a neurologic disorder (Ghosh and Basu 2009), various neurological features have also been studied among the patients. However, it was observed that patients are at high-risk with problems ($p<0.05$) like vision, being bothered by light or photophobia; double vision; blurred vision; difficulties in the hearing; unable to move neck/neck stiffness; experiencing numbness in limbs; dizziness, headaches, and difficulties in speaking. At the end of four weeks, patients had been in contact with pigs, bats, cattle, and rats, which are considered to have a higher risk of causing JEV ($P<0.05$). Also, 24.5% of patients had seen animals and birds in dead conditions. Window screening, mosquito nets, and insect repellents can also be considered protective measures against JE infection. As we know that mosquitoes lay eggs in open water storage containers, it was also found that patients who slept in open compounds before the infection occurred are more prone to JE infections ($p<0.05$). Further, severe and outrageous flooding during the summer of May has caused broad harm in Assam consistently for many years. Floods enhance JE infection in two ways, first, it gives more than adequate rearing locales to equipped vectors of the infection, for example, *Culex annulirostris*. Second, it gives the territory to water birds, which can move Japanese encephalitis over significant distances. As a result, flooding is identified as a major source of concern for JE infection ($p<0.05$).

The illness is generally regarded as rustic, and proximity to rice fields and pigs is associated with an increased risk of transmission. Significant mosquito vectors prefer to breed in rice fields, and pigs are thought to be important in enhancing infection and leading to death (Henriksson et al. 2021). This study found that working on agricultural land, working in standing water, swimming in nearby lakes/ponds, and wearing shirts or fully covered clothes while working in the field have a significant effect on this JE infection ($p<0.05$). As we know, standing water is the main breeding spot for mosquitoes. Mosquitoes breed by laying eggs in stale water. So in this study, it's also found that storing water in open containers in or outside your house is a major risk factor for an increase in JE infection ($p<0.05$).

While past evaluations propose that the gamble for an explorer to Asia of contracting JE was one in a million (Hills et al. 2010), today there is a gamble of openness to JE infection and suggestive sickness. The risk of getting JE increases during the active transmission season. Thus, travel medicine specialists give risk avoidance and mindfulness counseling to those voyagers at their most serious risk. In this study, we observed that patients who travel outside their villages have a high risk of getting a JEV infection. The results of our study also showed that taking a bath in a river, or pond, can be considered a risk factor for JEV infection. This epidemiological study reflects that the JEV is a burden on Assam and it's a severe public health issue. The present study touched on many important aspects of JEV that developed in the past few years. The burden of the viral JE infection in the North Eastern part of India is not well characterized to date. So, the results of this study will significantly contribute to the development of policies and immunization programs to control JE in NE states Assam which helps in reducing the mortality rates.

Conclusion

The data from the NE region of India is used to understand neurological problems and underlying infections associated with the JE infection. It is necessary for those who reside in JE endemic areas, to have the necessary immunizations at the appropriate times because this infection poses a high risk to any group of people. It is also important to research the vaccine history to comprehend infection eradication. Additionally, this disease is transmitted via vector so proper management of important vectors is also necessary which seems difficult because pig and rice farming are

crucial for the Nation's economic prosperity, so some future research should be planned for the proper management of vectors.

Declaration of interest statement

None

Funding

The Department of Health Research, New Delhi, India, provided funding for this study.

Ethical approval

The ethical clearance has been taken from Gauhati Medical College Hospital, Guwahati.

Acknowledgements

The authors acknowledged the Department of Health Research (DHR), New Delhi for giving the funds. A special thanks to the Gauhati Medical College Hospital, Guwahati for allowing the samples to collect. Also, we are thankful to Bioengineering and technology laboratory Dept, Gauhati University for providing the working space.

References

Arunachalam, N., Samuel, P. P., Hiriyan, J., Thenmozhi, V., & Gajanana, A. (2004). Japanese encephalitis in Kerala, south India: can Mansonia (Diptera: Culicidae) play a supplemental role in transmission?. *Journal of medical entomology*, *41*(3), 456–461. https://doi.org/10.1603/0022-2585-41.3.456

Ashraf, U., Ding, Z., Deng, S., Ye, J., Cao, S., & Chen, Z. (2021). Pathogenicity and virulence of *Japanese encephalitis* virus: Neuroinflammation and neuronal cell damage. *Virulence*, *12*(1), 968–980. https://doi.org/10.1080/21505594.2021.1899674.

Centers for Disease Control and Prevention (CDC) (2013). Japanese encephalitis surveillance and immunization-Asia and the Western Pacific, 2012. *Morbidity and mortality weekly report*, *62* (33), 658–662.

Dwibedi, B., Mohapatra, N., Rathore, S. K., Panda, M., et al. (2015). An outbreak of Japanese encephalitis after two decades in Odisha, India. *The Indian journal of medical research*, *142* (Suppl 1), S30–S32. https://doi.org/10.4103/0971-5916.176609

Furuya-Kanamori, L., Gyawali, N., Mills, D. J., Hugo, L. E., Devine, G. J., & Lau, C. L. (2022). The Emergence of Japanese Encephalitis in Australia and the Implications for a Vaccination Strategy. *Tropical medicine and infectious disease*, *7*(6), 85. https://doi.org/10.3390/tropicalmed7060085

Gajanana, A., Thenmozhi, V., Samuel, P. P., & Reuben, R. (1995). A community-based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic. *Bulletin of the World Health Organization*, *73*(2), 237–244.

Ghosh, D., & Basu, A. (2009). Japanese encephalitis-a pathological and clinical perspective. *PLoS neglected tropical diseases*, 3(9), e437. https://doi.org/10.1371/journal.pntd.0000437

Habib, M., Rahman, A.U., & Hussain, A. (2022). Japanese encephalitis virus. In R. Z. Abbas, A. Khan, P. Liu & M. K. Saleemi (eds), *Animal Health Perspectives* Vol. 2 (pp 126-130), Faisalabad, Pakistan, Unique Scientific Publishers, . https://doi.org/10.47278/book.ahp/2022.51

Henriksson, E., Söderberg, R., StrömHallenberg, G., Kroesna, K., et al. (2021). Japanese Encephalitis in Small-Scale Pig Farming in Rural Cambodia: Pig Seroprevalence and Farmer Awareness. *Pathogens (Basel, Switzerland)*, *10*(5), 578. https://doi.org/10.3390/pathogens10050578

Hills, S. L., Griggs, A. C., & Fischer, M. (2010). Japanese encephalitis in travellers from non-endemic countries, 1973-2008. *The American journal of tropical medicine and hygiene*, *82*(5), 930–936. https://doi.org/10.4269/ajtmh.2010.09-0676

Kanojia, P. C., Shetty, P. S., & Geevarghese, G. (2003). A long-term study on vector abundance & seasonal prevalence in relation to the occurrence of Japanese encephalitis in Gorakhpur district, Uttar Pradesh. *The Indian journal of medical research*, *117*, 104–110.

Karabatsos. (1985). *International catalogue of arboviruses : including certain other viruses of vertebrates* (3rd ed.). Published for the Subcommittee on Information Exchange of the American Committee on Arthropod-borne Viruses by the American Society of Tropical Medicine and Hygiene.

Kumar, S., Verma, A., Yadav, P., Dubey, S. K., et al. (2022). Molecular pathogenesis of Japanese encephalitis and possible therapeutic strategies. *Archives of virology*, 1–24.. https://doi.org/10.1007/s00705-022-05481-z

Ladreyt, H., Durand, B., Dussart, P., & Chevalier, V. (2019). How Central Is the Domestic Pig in the Epidemiological Cycle of Japanese Encephalitis Virus? A Review of Scientific Evidence and Implications for Disease Control. *Viruses*, *11*(10), 949.

Li, X., Cui, S., Gao, X., Wang, H., et al. (2016). The Spatio-temporal Distribution of Japanese Encephalitis Cases in Different Age Groups in Mainland China, 2004 - 2014. *PLoS neglected*

tropical diseases, *10*(4), e0004611. https://doi.org/10.1371/journal.pntd.0004611

Mileno M. D. (2020). Japanese Encephalitis Vaccine. *Rhode Island medical journal*, *103*(6), 49–50.

Parida, M. M., Santhosh, S. R., Dash, P. K., Tripathi, N. K., et al. (2006). Development and evaluation of reverse transcription-loop-mediated isothermal amplification assay for rapid and real-time detection of Japanese encephalitis virus. *Journal of clinical microbiology*, *44*(11), 4172–4178. https://doi.org/10.1128/JCM.01487-06

Patgiri, S. J., Borthakur, A. K., Borkakoty, B., Saikia, L., Dutta, R., & Phukan, S. K. (2014). An appraisal of clinicopathological parameters in Japanese encephalitis and changing epidemiological trends in upper Assam, India. *Indian journal of pathology & microbiology*, *57*(3), 400–406. https://doi.org/10.4103/0377-4929.138732

Roberts, A., & Gandhi, S. (2020). Japanese encephalitis virus: a review on emerging diagnostic techniques. *Frontiers in bioscience (Landmark edition)*, *25*(10), 1875–1893. https://doi.org/10.2741/4882

Sen Gupta, S. N., Sen, M. K., Das, P. K., Bhattacharya, D. P., & Rath, B. B. (1976). Clinical profile of the epidemic of Japanese encephalitis at Bankura. *The Indian journal of medical research*, *64*(10), 1393–1402.

Simon, L.V., Sandhu, D.S., Goyal, A., et al. (2022). Japanese Encephalitis. [Updated 2022 May 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan.

Solomon T. (2006). Control of Japanese encephalitis--within our grasp?. *The New England journal of medicine*, *355*(9), 869–871. https://doi.org/10.1056/NEJMp058263

Solomon, T., Ni, H., Beasley, D. W., Ekkelenkamp, M., Cardosa, M. J., & Barrett, A. D. (2003). Origin and evolution of Japanese encephalitis virus in southeast Asia. *Journal of virology*, *77*(5), 3091–3098. https://doi.org/10.1128/jvi.77.5.3091-3098.2003

Xiong, J., Yan, M., Zhu, S., Zheng, B., Wei, N., Yang, L., Si, Y., Cao, S., & Ye, J. (2022). Increased Cleavage of Japanese Encephalitis Virus prM Protein Promotes Viral Replication but Attenuates Virulence. *Microbiology spectrum*, *10*(3), e0141722. https://doi.org/10.1128/spectrum.01417-22

Xu, C., Zhang, W., Pan, Y., Wang, G., et al. (2022). A Bibliometric Analysis of Global Research on Japanese Encephalitis From 1934 to 2020. *Frontiers in cellular and infection microbiology*, *12*, 833701. https://doi.org/10.3389/fcimb.2022.833701