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# Increasing trend of Japanese encephalitis cases in West Bengal, India – a threat to paediatric population

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### ABSTRACT

**Objective:** To detect the Japanese encephalitis virus (JEV) as the etiologic agent from the acute encephalitis syndrome (AES) cases mainly amongst the children and young adults from vaccinated and non-vaccinated districts of West Bengal. **Methods:** For the detection of JEV, a total of 828 sera were referred from vaccinated and non vaccinated districts of West Bengal during 2005–2011. Japanese encephalitis (JE) positive cases were confirmed by ELISA and RT-PCR method. **Results:** Out of 828 cases, 245 samples were positive by ELISA method and 46 samples were positive by RT-PCR method. Out of 291 total positive cases, 162 (55.6%) were below 20 years of age. Initially in 2005, JE cases were highest amongst the children and young adults (0–20 years). After vaccination, although the JE cases declined gradually in the vaccinated districts, but again from 2010, JE cases from the said age group showed an increasing trend from those districts. JE cases were also reported from other endemic zones of this state, which were still non-vaccinated. **Conclusions:** In West Bengal, JE cases are still predominated among children and young adults till the year 2011. Mass scale vaccination programme and investigation on the circulating strains are essentially required to find out the reasons of increasing tendency of JE cases in this state.

## 1. Introduction

Japanese encephalitis (JE) is one of the major public health problems in many parts of South East Asia. About 3 billion people live in countries where the Japanese encephalitis virus (JEV) is endemic<sup>[1]</sup>. In Asia, annual incidence of the disease is about 50 000 cases<sup>[2]</sup>. Every year, 10 000–15 000 people die due to this disease and about 15 000 people, who survive, develop neuro-psychological complications<sup>[3,4]</sup>.

JE is endemic in many parts of India including the state of West Bengal<sup>[5]</sup>. In India, the first case of JE was recorded from Vellore, Tamil Nadu in 1955<sup>[6]</sup>. Until 1970, JE was confined within southern part of India. In West Bengal, the first major outbreaks of JE occurred in the districts of Bankura and Burdwan in 1973<sup>[7]</sup>, where about 700 cases and 300 deaths occurred. From 1978 to 2007, 103 389 cases and 33 729 deaths due to JEV infection were recorded from various parts of India<sup>[8]</sup>. Every year, 1 500–4 000 cases are

reported from this country<sup>[8]</sup>.

Studies done so far revealed that, JE was common amongst the children<sup>[5,9]</sup>. In India, JE cases have been recorded mainly amongst the children from different states like West Bengal, Uttarpradesh and Tamil Nadu, affecting the children between 5 and 15 years<sup>[10–12]</sup>. However, in areas where JEV is recently introduced, adults are also getting the infection. In children, JE accounted for over half the cases of encephalitis and was associated with a mortality rate of 39%; over half of the survivors were left with permanent neurological sequel<sup>[13]</sup>. Children up to 10 years of age are the most vulnerable group for JEV infection due to either absence or low protective immunity against the virus in them; 700 million children live at risk to this disease<sup>[10,12,14]</sup>.

There are currently 3 vaccines available for the prevention of JE. In spite of vaccination programme in the last six decades, 10.5 million children have been infected of which 3 million deaths have occurred<sup>[15]</sup>. In 2005, over 5 000 cases and 2 000 deaths occurred due to JEV infections in India<sup>[11]</sup>. In India, in the year 2006, children belonged to the age group of 1–15 years were vaccinated with live JE vaccine of SA-14-14-2 strain, manufactured by Chengdu Institute of Biological Products, Chengdu, China<sup>[16]</sup>. For this purpose, most affected areas of some states were selected; it included seven districts of UP, two districts of Assam and one district

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each from West Bengal and Karnataka<sup>[9]</sup>. In 2007 and 2008, vaccination programme had been initiated in Andhra Pradesh, Bihar, Haryana, Maharashtra and Tamil Nadu<sup>[9]</sup>.

In spite of vaccination programme, cases and deaths due to JEV infection are continuously being reported from vaccinated states like Uttarpradesh<sup>[17]</sup>. Although in some districts of West Bengal (Burdwan, Birbhum, Midnapore West, Howrah and Hoogly) the vaccine has been used, still cases are continuously being reported every year. In this study we aimed to detect the JE cases mainly among the children from the vaccine covered districts as well as from other districts where vaccination has not yet been done.

## 2. Materials and methods

### 2.1. Study area

West Bengal is located at the eastern region of India (Figure 1). Kolkata (formerly Calcutta) is the capital of this State is also one of the metropolitan cities of India. The state has an area of 88 752 km<sup>2</sup>. At January 2011, the population of the state is 91 347 736 of which 10 112 599 (11.07%) belong to the age group 0–6 years. Agriculture is the main economic source of this state.

Except the northern hilly region, other parts of this state are warm and humid for the maximum time of the year. The main seasons are summer, monsoon, autumn, late autumn and winter. The summer lasts from mid–March to mid–June, with the temperature ranging from 38 °C to 45 °C. The monsoon arrives by the middle of June and lasts upto September<sup>[18]</sup>.

### 2.2. Vaccination programme

In West Bengal, JE vaccination programme was initiated in 2006 along with some other states of India. The vaccine was given to the children of Burdwan district, belonging to the age group of 1–15 years in the year 2006. In 2007 and 2008, vaccine was administered to the children of Birbhum and West Midnapore districts respectively, whereas the district Howrah and Hoogly were covered by JE vaccine in the year 2009. In the year 2010 and 2011, no vaccination campaign had been initiated.

### 2.3. Selection criteria

Patients admitted with acute encephalitis syndrome (AES) in different medical college and district hospitals, with high fever ( $\geq 39$  °C) and any two of the following symptoms, *i.e.* headache, neck rigidity, vomiting, unconsciousness, convulsions, seizure, stupor, delirium, altered sensorium or presence of kerning's sign, were considered for this study. Informed consent was obtained from the patients or from the parents or legal guardians of minors before the collection of the samples. Cerebral malaria and bacteriological aetiology were ruled out by the clinicians of the hospitals concerned.

### 2.4. Sample collection

During the study period from 2005–2011, a total of 828 AES cases were referred by the clinicians from different districts hospitals of West Bengal to our department for detection of JEV in them with detail socio–demographic information and clinical history. From 2006, the vaccine was given to the children between 1 year and 15 years of age, by the government. In the year 2010, the children upto 15 years of

age would attain the age of upto 20 years. So in this study, the cases upto 20 years of age were considered. The study was duly approved by the research ethics committee of ICMR Virus Unit, Kolkata.

### 2.5. Sample processing and analysis

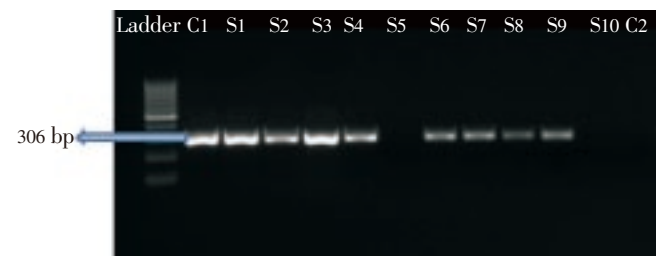
The collected blood samples and CSF were transported maintaining the cold chain system to this unit. Sera were separated and the samples were stored at –80 °C until testing.

For diagnosis of JEV infection, enzyme–linked immunosorbent assay (ELISA) was performed. The kit was purchased from National Institute of Pune, India<sup>[16]</sup>. The kit follows IgM antibody captured ELISA technique using specific antigen for JE. Optical density was measured at 492 nm using an ELISA reader (Titertek Multiskan Plus, Lab systems Finland, Type– 314).

For the detection of actual JE positive cases, ELISA negative samples were further screened for the isolation of viral RNA followed by RT–PCR test. Viral RNA was isolated by using Qiagen viral RNA isolation kit (Qiagen, GmbH, Hilden, Germany) following the manufacturer's protocol. The RT–PCR test was performed following the cost effective RT–PCR method<sup>[19]</sup>. The PCR products were run on 1.5% agarose gel.

## 3. Results

Results of agarose gel (1.5%) electrophoresis showing JE specific band at 306 bp (Figure 1).



**Figure 1.** Results of agarose gel (1.5%) electrophoresis showing JE specific band at 306 bp. Lane L: 100 bp DNA ladder; Lane C1: Positive control; Lane S1–S10: JEV specific band from patients' samples; Lane C2: Negative control (without template).

During 2005–2011, among 828 AES cases JEV infections were detected in 291 samples, of which 245 were positive by ELISA method and 46 were positive by RT–PCR method. The yearly distribution of JE cases are presented in Table 1. The males were more affected than the females (Table 2).

**Table 1**

Yearly distribution of Japanese encephalitis cases in West Bengal, India.

Year	No. tested	No. positive		Percentage positivity (%)
		IgM positive	PCR positive	
2005	158	49	6	34.8
2006	52	7	2	17.3
2007	68	17	2	27.9
2008	163	38	4	25.8
2009	72	28	1	40.2
2010	104	40	9	47.1
2011	211	66	22	41.7

**Table 3**

District wise distribution of Japanese encephalitis positive cases in West Bengal, India.

Districts	2005		2006		2007		2008		2009		2010		2011	
	Total	In children	Total	In children	Total	In children	Total	In children	Total	In children	Total	In children	Total	In children
Burdwan*	12	12	0	0	0	0	0	0	0	0	1	0	5	2
Birbhum*	25	23	5	5	0	0	3	0	0	0	0	0	2	1
Mid (W)*	0	0	4	3	1	1	0	0	0	0	2	1	2	1
Howrah*	0	0	0	0	0	0	0	0	4	2	3	0	2	0
Hoogly*	2	2	0	0	1	1	3	2	4	0	3	1	2	2
Bankura	0	0	0	0	0	0	0	0	2	0	0	0	2	0
Kolkata	0	0	0	0	2	0	4	0	2	1	2	0	2	1
24 PG (S)*	0	0	0	0	0	0	5	2	6	3	7	3	0	0
Mid (E)	0	0	0	0	3	2	5	3	0	0	15	7	7	4
Murshidabad	0	0	0	0	3	0	4	1	0	0	7	4	7	3
Nadia	0	0	0	0	4	2	4	2	9	7	2	2	4	2
Malda	16	14	0	0	5	2	14	3	0	0	7	3	12	10
Dinajpur (N)	0	0	0	0	0	0	0	0	0	0	0	0	3	2
Dinajpur (S)	0	0	0	0	0	0	0	0	2	0	0	0	4	4
Coochbehar	0	0	0	0	0	0	0	0	0	0	0	0	6	2
Jalpaiguri	0	0	0	0	0	0	0	0	0	0	0	0	27	14
Darjeeling	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Total	55	51	9	8	19	8	42	13	29	13	49	21	88	48

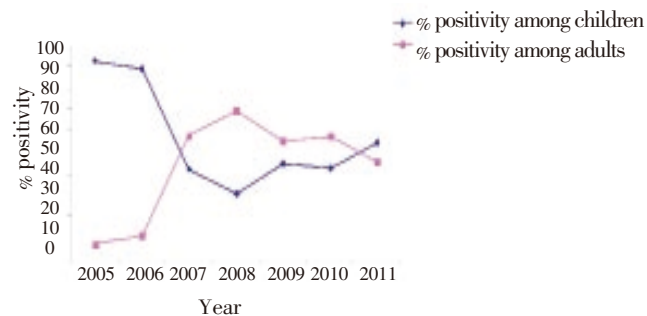
The vaccinated districts are marked as \*.

**Table 2**

Age and sex wise distribution of Japanese encephalitis cases in West Bengal, India from 2005–2011.

Age group (years)	Sex	2005	2006	2007	2008	2009	2010	2011	Total
		0–10	Male	16	3	1	3	5	6
	Female	9	0	2	3	3	5	7	29
11–20	Male	17	3	2	6	5	7	22	62
	Female	8	2	3	4	3	7	11	38
21–30	Male	1	1	3	4	3	4	5	21
	Female	2	0	2	4	1	3	7	19
31–40	Male	2	0	3	6	2	3	4	20
	Female	0	0	0	2	2	3	5	12
41–50	Male	0	0	1	2	0	3	5	11
	Female	0	0	1	4	3	2	2	12
≥51	Male	0	0	1	4	0	4	4	11
	Female	0	0	0	4	2	2	7	13

Out of total 291 positive cases, 162 cases (55.6%) were below 20 years of age. In 2005 and 2006, about 92.7% and 88.8% JE positive cases belonged to that age group. In 2007, 2008, 2009, 2010 and 2011 JE positive cases amongst children and young adult of upto 20 years of age were 42.1%, 30.9%, 44.8%, 42.8% and 54.5%, respectively (Figure 2).



**Figure 2.** Percentage positivity of JE cases among children and adults during 2005–2011 in West Bengal, India.

The district wise distribution of JE positive cases during the study period, are given in Table 3. During the year 2005–2006, *i.e.* before vaccination, maximum number of positive cases was detected from the district Birbhum. About 92% and 100% of total JE positive cases from this district belonged to the age group of 0–20 years in the year 2005 and 2006 respectively. After vaccination in 2006, JE positive cases amongst the children ( $\leq 20$  years of age) were not recorded from this district except in the year 2011. Similarly, in other vaccinated districts, the numbers of JE cases mainly amongst the above said age group were initially declined after vaccination but are again increasing from the year 2010. On the other hand, higher numbers of JE positive cases are still being recorded from the non vaccinated districts like Malda, Midnapore (East), Murshidabad, South 24 Parganas and Nadia. In the year 2011, highest numbers of JE cases were reported from the district Jalpaiguri. The highest number of positive cases was recorded in the year 2011.

**4. Discussion**

JE is considered as a serious disease due to its complexity and lack of any specific treatment. Pig–mosquito–pig and bird–mosquito–bird cycle is responsible for the maintenance of the virus in nature. Man is the ‘dead end’ host.

The virus causes large epidemics at intervals and has been endemic in many rural areas of West Bengal. Although the JE cases are being reported at the tip of iceberg in this state, but still a huge number of positive cases has been found from the year 2009. In endemic areas young and young adult age groups ( $\leq 20$  years) are mainly affected. The economy of most of the people in the rural areas is mainly dependent on cultivation. Moreover, people of that age group usually take active part in crop field for the cultivation. The vector usually breeds in the stagnant water in the paddy field and the majority from this age group gets exposed to the vector directly. To raise the economic standard; poorer section of the community has accepted piggery and mini poultry as an accelerated source of income. The domestic bird, animal and cattle happen to be the reservoir of JEV[21]. The majority

of the people of these districts live under the same roof with those animals, which bring animals in close association with human being, and thus the community becomes exposed to JEV infection through mosquitoes. Here household crowding, low socioeconomic status and lack of air conditioning appear to be the risk factors for acquiring JE.

Effective vaccine against JEV is now available. Due to the widespread use of JE vaccine JE cases has been declined in China, Korea, and Japan<sup>[20]</sup>. The targeted age group for this study was below 20 years age. During the period of study, maximum numbers of JE cases amongst the children and young adults were detected in the year 2005 followed by 2006. In this state, the vaccination programme was initiated in 2006. In our study, Although in some districts of West Bengal like Burdwan, Birbhum, Midnapore (W), Howrah and Hoogly, the vaccine was given up to 15 years of age between the year 2006 and 2009, but in the year 2010 and 2011, JE cases were reported amongst the children and young adults from those vaccine covered districts. Again from the year 2009 onwards, high number of positive cases was reported from those vaccine covered districts. It might be due to partial vaccination in those districts although the possibilities of the change in genotype of the circulating strains can not be excluded. Recently in Uttarpradesh, India genotype I strain of JEV was isolated from Gorakhpur, a JE endemic region of the state<sup>[17]</sup>. The children and young adult population of this region was vaccinated with SA–14–14–2 strain of JEV (genotype III). But, with the introduction of genotype I in this region, the circulating strain affected good number of people of this region.

The maximum numbers of positive cases were found from the districts of Midnapore (East), Malda, Murshidabad, Nadia and South 24 Parganas, where no vaccination has yet been initiated. In 2011, JE cases were recorded from the district of Jalpaiguri and Coochbehar, which are located in the hilly and cold climatic regions. These two districts are adjacent to the state of Assam, where an outbreak of JEV had been recorded in July 2011<sup>[22]</sup>. Possibly, the JE affected people of those two districts were infected by the vector mosquitoes that migrate from the neighboring state.

An intense molecular study on the circulating strain of JE is required to find out the reasons of increasing tendency of JE cases. Immediate mass vaccination with a booster dose is required to all the children of the highly endemic regions of this state. Not only the children and young adults, but the adults were also affected by JEV recently. Government should think about the vaccination programme in adults as early as possible. Otherwise the increasing trend of JEV activity in the state of West Bengal, India will become a serious public health threat in near future.

### Conflict of interest statement

We declare that we have no conflict of interest.

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