

Epidemiological and clinical profile of Japanese Encephalitis in Bengdubi garrison in West Bengal

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Abstract

Introduction: Japanese encephalitis (JE) is viral encephalitis that causes high morbidity and mortality and is endemic in large parts of West Bengal. Subclinical infections greatly outnumber overt cases of JE. The objective of this study was to detect cases of inapparent infections and the disease burden of JE in Bendubi garrison considered to be 'endemic' for JE.

Methods: JE disease burden was evaluated by detection of anti-JEV IgM in serum and cerebrospinal fluid (CSF) in 510 cases of acute encephalitis syndrome (AES) reporting to the base hospital in Bengdubi while inapparent infections were evaluated by detection of serum anti-JE IgG antibodies in a matched sample of 511 individuals.

Results: Fourteen (2.7%) of the 510 symptomatic cases that had presented to the hospital with fever or/and symptoms of acute encephalitis, tested positive for JEV infection. In the asymptomatic cohort, 429 out of 511 (83.9%) tested positive for the IgG antibody to JEV, giving an apparent: inapparent ratio of 1: 31.

Conclusion: The overall incidence of JE cases was lower than that reported from other 'endemic' regions of West Bengal. Residents of the area developed IgG antibodies and hence longterm immunity against JE, probably as a result of repeated subclinical infections. Anti-mosquito measures, JE vaccination and environmental modifications can break the transmission cycle and eventually eradicate JE completely.

Keywords: Japanese encephalitis, Bengdubi, Acute encephalitis syndrome, Epidemiology, IgM, IgG, Zoonotic

Introduction

Japanese encephalitis (JE) is a leading cause of viral encephalitis in Asia.⁽¹⁾ The incidence of Japanese encephalitis infection has increased over the past thirty years in parts of India, Nepal and Southeast Asia, with outbreaks being reported from regions that were previously not considered endemic for this disease.⁽²⁾ The reasons for this expansion are varied and include population shifts, migratory avian patterns and vector movement to wider areas.⁽¹⁾ The disease affects mostly children.⁽³⁾ Around 30,000–50,000 cases of JE and up to 15,000 deaths are reported annually across Asia. These statistics may not be representative of the actual burden of disease because of poor surveillance and reporting.⁽⁴⁾ About 25%–30% of JE cases are fatal, and 50% result in permanent neuropsychiatric sequelae.⁽⁵⁾

Subclinical infections greatly outnumber overt cases of JE. Thus, the present system of reporting JE cases may not reflect the actual level of transmission. A community based study in a cohort of school children in the 5 to 9 age-group, in a highly endemic PHC in South Arcot district, Tamil Nadu, showed that an average of 4000 subclinical infections occurred per 10,000 children, while concurrently, an average of 15 JE cases were reported, giving an apparent: inapparent ratio of 1: 270.⁽⁶⁾ A ratio of 1:400 has been estimated in West Bengal.⁽⁷⁾ Thus cases of JE may represent only the tip of the iceberg compared to the large number of inapparent infections (Fig. 1).

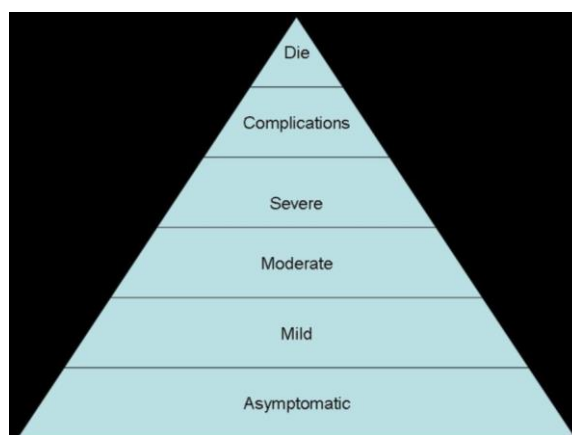


Fig. 1

The JE virus is a virus from the family Flaviviridae and its ecology has been widely studied. The virus exists in a zoonotic transmission cycle among mosquitoes, pigs, bats, and water birds belonging to the family Ardeidae (cattle egrets and pond herons). Humans become infected when bitten by an infected mosquito and are a dead-end host because of low viremia, preventing the virus from being transmitted further.⁽⁸⁾ The major mosquito vectors of JEV vary in different geographic regions; the most common are those of the *Culex* genus.⁽⁹⁾ Pigs are the main contributors in the transmission cycle with respect to human infection, because these animals often stay close to human dwellings. Ardeid birds are important maintenance hosts⁽¹⁰⁾ (Fig. 2).

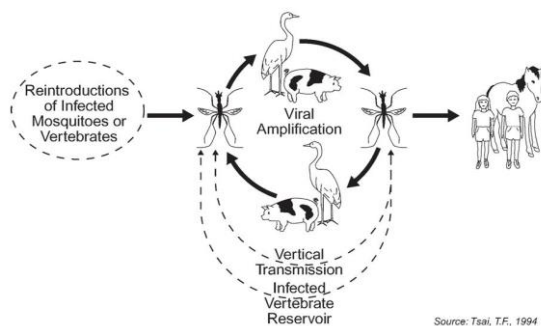


Fig. 2

Bengdubi garrison is a semi-urban area spread over approximately 40 square kilometers and has vast uninhabited areas of thick undergrowth interspersed with low-lying marshlands and forest cover. Its periphery has numerous villages with poor environmental sanitation and a fair population of cattle and pigs. Thus there is ample opportunity for the JE viral transmission cycle to flourish in this region.

Serologic evidence of JEV infection in endemic rural areas is found in nearly all inhabitants by early adulthood. Most symptomatic infections in endemic areas occur in young children (aged 2-10 years) and elderly people. In nonendemic areas, JEV infection has no age predilection.⁽¹¹⁾ IgG antibodies to JE in sera of asymptomatic individuals in endemic areas indicate subclinical exposure to the virus. Presence of IgM anti-JEV antibodies in CSF and/or sera indicates recent JEV infection. Paired sera samples are preferable to single readings.⁽¹²⁾

Does the virus pre-exist in areas where epidemics occur, and if so, does it induce immunity in residents of the area? This study was undertaken in order to locate cases of in apparent infections and thereby long-term immunity in an endemic region by detection of serum anti-JE IgG antibodies and to assess the disease burden by detection of anti-JEV IgM in serum and cerebrospinal fluid in cases of acute encephalitis syndrome (AES) reporting to the base hospital in Bengdubi. The final aim was to analyze the data in order to determine if preventive measures like vaccination must be offered to all residents of this region in order to reduce the burden of infection and the resultant morbidity.

Materials and Methods

The study was hospital-based prospective surveillance study carried out from December 2007 upto October 2009 in a zonal tertiary care hospital in Bengdubi (West Bengal), considered an endemic region for JE. The study population was divided into the following:

- (i) Symptomatic individuals who had been admitted to the hospital with Acute encephalitis syndrome

(AES) or undiagnosed fever of more than five days' duration;

- (ii) Asymptomatic individuals who had been resident in the area for more than two years

A total of 510 cases of AES were included in the study and they were tested for the presence of recent infection with JEV utilizing IgM antibody detection in paired serum samples (collected five days apart) and CSF. Presence of JEV infection was defined by the occurrence of IgM antibodies in the CSF or the serum. For single serum and CSF specimens, an elevated serum IgM levels of 40 IU/ml of anti-JEV IgM, was considered diagnostic of CNS infection. For the paired samples, an increase of 15 IU/ml in two sequential tests, two weeks apart, was considered evidence of recent JEV infection.

A matched sample of 511 individuals who had resided in the corresponding region for more than two years, was taken for studying in apparent infection rate in the asymptomatic population through IgG antibody detection. This population was matched for age, sex, socio economic background and rural/urban distribution with the symptomatic populace to neutralize any confounding variables.

Cases positive for malaria, tuberculous/pyogenic meningitis or meningo-encephalitis, cryptococcus, hepatitis B or C, dengue fever or those with a history of travel outside the catchment area of the study in the preceding two months for a period exceeding 15 days were excluded from the study.

From each patient, a serum sample was immediately obtained at admission. Because it may not have been positive in a JE-infected person, a second serum sample was collected at discharge or on the 07th day of illness onset. The sera were stored at 4°C for a maximum of 1-3 days only before they were tested for IgM and IgG antibody. CSF samples for virological testing were sent to the hospital laboratory as soon as possible.

IgM and IgG antibodies to the JE virus in sera and cerebrospinal fluid (CSF) were tested by using 'Antibody-Capture Solid-Phase' enzyme-linked immunoassay (ELISA). The sera of the first subgroup of the study population viz. the symptomatic patients was tested for IgM antibodies to JEV in paired sera samples (taken at least one week apart) and in CSF (if the cases presented with acute encephalitis).

Results

During the study period from Dec 2007 to Oct 2009, a total of 1021 cases and matched samples were tested for IgM and IgG antibodies against JEV. The sample of 510 symptomatic individuals included 329 patients who met the clinical case definition for acute encephalitis and had cerebrospinal fluid (CSF) pleocytosis. 181 patients with fever not responding to antibiotics and antimalarials for a period of more than

five days were also tested for serum antibodies (IgM) to JEV.

Fourteen (2.7%) of the 510 symptomatic cases that had presented to the hospital with fever or/and symptoms of acute encephalitis, tested positive for recent JEV infection, i.e. presence of IgM antibody in the CSF and serum (Fig. 3). Of these, eleven (3.3%) were out of the 329 patients suffering from acute encephalitis and three (1.6%) of the positive cases for JEV infection were from the 181 patients who had been admitted to the hospital with fever for more than five days duration, and had had a poor response to treatment with antimalarials and/or antibiotics. In all of the cases with acute encephalitis, both the CSF and the serum samples were taken for IgM antibody testing for the JEV. In patients presenting with fever, only the serum samples were tested for antibody to the JEV.

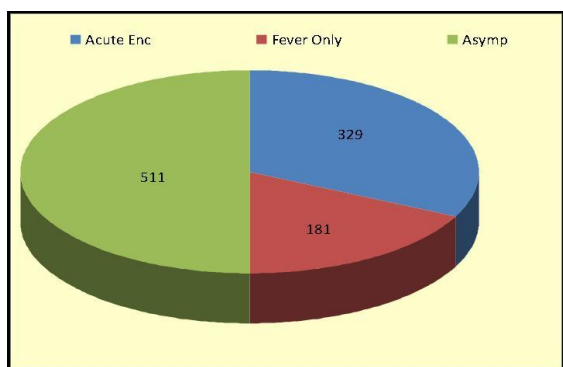


Fig. 3

A second sample size of 511 asymptomatic individuals, matched with the symptomatic cohort for age, sex, geographical location, socio economic background was tested for serum IgG antibodies to JEV. In the asymptomatic cohort, four hundred and twenty nine individuals out of 511 (83.9%) tested positive for the IgG antibody to JEV (Fig. 4), giving an apparent: inapparent ratio of 1: 31.

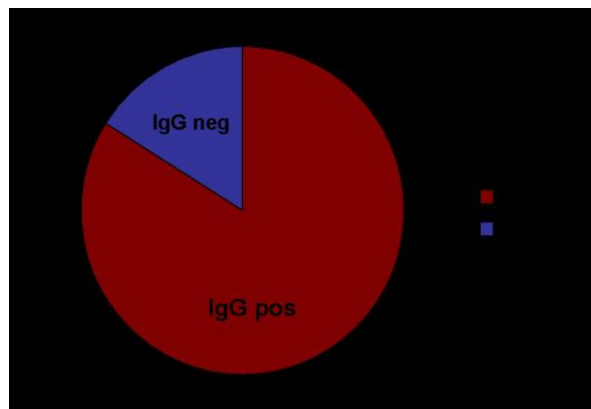


Fig. 4

When the data was analyzed for gender differences, among the fourteen recent JEV infections (IgM positive), ten (71.4%) were males. In the matched sample of asymptomatic individuals tested for IgG antibody, 366 out of the 429 cases of inapparent infection were males (85.3%). Cases of recent infection (IgM positive) were identified in all the age groups with four (28.5%) cases less than 05 years of age, seven (50%) positive cases in children between 5-15 years and three cases (21.4%) in the age group more than 15 years.

The mean CSF leukocyte count of cases positive for CSF IgM antibody was 121 cells/mm³ (range = 7 – 900 cells/mm³) with lymphocyte predominance (mean = 72%, range = 30 – 100%). The CSF glucose concentrations varied between 45 to 190 mg/dL (mean 65 mg/dL); CSF protein levels were mildly raised (mean = 60 mg/dL, range = 20 – 100 mg/dL).

Most of the JE cases that were identified were clustered in the pre monsoon and the monsoon seasons (Table 1). Nine (64.1%) of the cases who tested as IgM positive were detected in the months of Jul to Sep (Fig. 5).

Table 1: Seasonal variation of JE cases

Months	Serological Assay		
	IgM Positive (%)	First Year (n=279)	Sec Year (n=231)
May	7.1	1	0
Jun	7.1	0	1
Jul	14.2	1	1
Aug	28.5	3	1
Sep	21.4	1	2
Oct	7.1	1	0
Nov	7.1	0	1
Dec	7.1	1	0

Patients with recent JE infection (IgM positive) had fever (100%), altered consciousness (100%), convulsions (21.4%), headache (71.4%), severe weakness (92.8%), stiff neck (62.4%) and vomiting (50%) (Table 2).

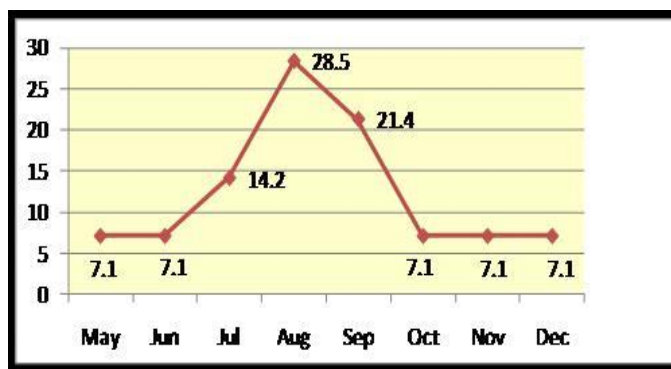


Fig. 5

Table 2: Clinical features of JE cases

Symptoms	Proportion (%) n = 14
Fever	14 (100)
Altered Sensorium	14 (100)
Convulsions	3 (21.4)
Headache	10 (71.4)
Severe Lethargy	13 (92.8)
Neck Stiffness	8 (62.4)
Vomiting	7 (50)
Nausea	8 (57.1)
Muscle Pain	5 (35.7)
Limb Weakness	9 (64.2)
Signs	Proportion (%)
Temp > 37.8 °C	14 (100)
Neck Rigidity	10 (71.4)
Extensor Plantar	6 (42.8)
Abnormal Pupillary Light Reflex	8 (57.1)
Kernig's Sign	5 (35.7)
Tremors/Rigidity/Myoclonus	4 (28.5)

In contrast, all 429 cases of inapparent infection were in the age group more than 15 years. Thus the age distributions differed markedly for the populations tested for IgM and IgG antibodies to the JEV. The largest cluster of acute infections was in children less than 15 years of age while IgG antibodies were detected only in the adult population, suggesting development of long-term immunity in areas of JEV transmission.

Discussion

This is a pioneering study to document the presence of human JE virus transmission cycle in a catchment area of a garrison located in the endemic region of West Bengal. Although several epidemiological surveillance studies have been carried out in other parts of the country, especially South India,^(6,13,14) there is a lack information regarding the presence of JE infection and immunity among the residents of this area. Our study hospital was located in a semi urban area and all the cases were from semi urban, semi rural and rural agricultural areas where the

requisite conditions for JE transmission (i.e. vector, reservoir host and virus) may all be present.

Our study demonstrates JEV as an etiological agent of fever and encephalitis in 2.7% of symptomatic cases, as evidenced by the presence of IgM antibody in serum and CSF. Studies carried out in the Indian subcontinent and South East Asia exhibit a wide range of confirmed cases which vary from 0.14%⁽¹⁵⁾ through 61.7%⁽¹⁶⁾ to 86.2%.⁽¹⁷⁾

In our study 2.7% cases of acute encephalitis were due to JEV infection. This is in contrast to the findings by Kumar et al⁽¹⁸⁾ who reported that JE virus contribution to acute encephalitis was 23% in endemic regions in India. This difference can be attributed to governmental efforts of strict anti larval and anti mosquito measures in the form of regular sprays and fogging and education of the residents about personal preventive measures against mosquito bites.

We detected that 1.6% of the cases with fever only, admitted to our hospital also tested positive for IgM antibody to the JEV. A detailed Medline/ Pubmed search did not show any study carried out in the Indian subcontinent on the incidence or prevalence of JEV infections in such cases. The positive cases in our study could have been at the prodromal stage of the illness and early detection significantly reduced the morbidity in such cases. The average length of stay of JE cases presenting with fever only was eight days as compared to those with acute encephalitis that averaged 23 days. Our findings underline the need for an early diagnosis and a high index of suspicion in all cases of fever in JE endemic areas to allow for reduced morbidity and mortality.

The majority of the studies focusing on the gender prevalence revealed the presence of a skew towards greater rates of infection among the males. In our surveillance study, among the cases with recent JEV infections (IgM positive), 71.4% were males. In the matched sample of asymptomatic individuals, 85.3% of IgG positive individuals were males. This compares favorably with Rayamajhi, who, in his study found a positive fraction of 69% among the males and 31% among the females,⁽¹⁹⁾ which is about same as in our study. Another study from the Indian subcontinent also

showed males to be affected in 58% of the cases.⁽²⁰⁾ This trend can be explained by the wider ranging area of the males in the rural and semi rural area as also the nature of military duties which entail long periods of exposure to the out doors. These findings are consistent with the pattern of livelihood followed by the male members in the rural belts of the North East, where wood foraging, paddy cultivation and employment in large tea estates comprise the primary economic activities.⁽²⁰⁾

The prevalence of seropositivity for serum IgG antibodies in the matched cohort (n=511) of the affected geographical locations (residing at the location for more than 2 years), was 83.9%. This is high as compared to the study by the WHO, which illustrated that the prevalence could be 34-58% in most regions of sub Himalayan plains.⁽¹⁾ This points to the fact that subclinical infections among residents of this region were causing the development of longterm natural immunity against JE and this was probably also responsible for low incidence of JE cases.

Cases of JE identified in this study represented all age groups. In the JE endemic areas of South East Asia, most people are infected with JE before 15 years of age.⁽²⁰⁾ In our study group, cases of recent infection were identified in all the age groups; four (28.5%) were less than 5 years of age and seven (50%) positive cases were children from 5-10 years of age. Hence 78.5% of the positive cases in our study were less than 10 years of age, well in conformity with the other studies.⁽¹⁸⁾ An Indian study by Gupta Neeru et al⁽²¹⁾ showed that 31.25% of the cases were less than five years of age; also, more than 4/5th of the cases (84%) were between one to twelve years of age.

In contrast, all 429 cases that tested positive for the IgG antibody were adults. An increased rate of IgG seropositivity in adults in the endemic areas may reflect upon the development of a robust immune response over years of repeated sub clinical infection. Thus, the increased prevalence of IgG positivity among adults in the endemic areas, as has been demonstrated by Kudo et al and Burke et al.^(22,23)

In the present study, the CSF studies were carried out on 329 patients hospitalized with acute encephalitis syndrome. Among these, eleven (3.3%) tested positive for IgM antibody to the JEV. The mean CSF leukocyte count of cases positive for CSF IgM antibody was 121 cells/mm³ (range = 7 – 900 cells/ mm³) with a lymphocyte predominance (mean = 72%, range = 30 – 100%). The CSF glucose concentrations varied between 45 to 190 mg/dL (mean 65 mg/dL); CSF protein levels were mildly raised (mean = 60 mg/dL, range = 20 – 100 mg/dL).

These findings were commensurate with the standards laid down for diagnosis of acute viral encephalitis/ encephalomeningitis and are consistent with other studies.⁽²⁴⁾ There are many possible causes of acute encephalitic syndrome; thus, a laboratory

confirmation is essential for the accurate diagnosis of JE, which is not a simple process because of the very low viremia. Diagnosis is therefore targeted toward the detection of antibodies in cerebrospinal fluid.

Cases of cross-reactivity of antibodies to other flaviviruses cause confusion in the diagnosis of JEV. The World Health Organization (WHO) has drawn up surveillance standards for the detection of JEV, recommended case definitions of JE, and set up criteria that are to be fulfilled to diagnose a case as JE.⁽²⁵⁾ IgM capture ELISA has been the most widely used diagnostic method for JE detection.⁽²⁶⁾ Hence this method was the bedrock of our analysis. At present, however, the latest advances in diagnostic methods for early detection of JEV include the dipstick method,⁽²⁷⁾ JEV-CheX,⁽²⁸⁾ and reverse transcriptase PCR.⁽²⁹⁾

Our findings agree well with the epidemiological data available on the incidence and prevalence of JE infection in India. Serological surveys carried out between 1955 and 1972 showed that JEV infections occurred in scattered areas in Gujarat, Maharashtra, Orissa, Assam and Arunachal Pradesh, with highest prevalence in the three southern States of Tamil Nadu, Andhra Pradesh and Karnataka. The first major epidemic, involving over 700 cases and 300 deaths, occurred in Burdwan and Bankura districts of West Bengal in 1973 followed by a second epidemic in 1976. Between 1977 and 1979 there were extensive epidemics involving new areas and several districts in West Bengal and Andhra Pradesh, Bihar, Assam and Uttar Pradesh.⁽²⁰⁾ Since then, the disease has become 'endemic' in large parts of West Bengal, with extensive outbreaks in epidemic years, which frequently coincide with years having heavy or unusual rainfall patterns.⁽³⁰⁾

Conclusion

Japanese Encephalitis is a serious public health problem with significant morbidity and mortality in 'endemic' areas. Our study demonstrated that JEV infection is clearly an etiology of encephalitis in Bengdubi garrison, which falls in an 'endemic' zone for JE.

Our study has been significant in that it demonstrated that JE is a cause of acute encephalitis syndrome and /or fever in only a minority of patients in Bengdubi and that too especially in children. This could be due to recent anti-mosquito efforts of the local government as part of the Malaria Control Programme. Residents of Bengdubi develop IgG antibodies over time, most likely due to repeated subclinical infections. As a result, cases of JE are rarer in patients more than 15 years old.

Our study, though reporting a lower rate of JE infection, did stress the importance of considering JE as a viable differential in all cases of encephalitis and recalcitrant fever. Thus, promoting the use of JE diagnostic tests in zonal hospitals in known endemic areas will improve the accuracy and speed of JE

diagnosis and facilitate earlier therapeutic intervention, thus reducing mortality. Also, based on our findings, the following steps to curb JE can be safely proposed:

1. Immunization programs for 'endemic' regions,
2. Pig immunization and the separation of pig rearing (piggeries) from human settlements,
3. Changes in agricultural practices in surrounding areas, (e.g., enhanced mechanization and decrease of irrigated land),
4. Improved environmental engineering (e.g., better housing and sanitation),
5. Integrated vector control methods to control mosquito breeding and spread.

India currently has no national vaccination program against JE, but the Ministry of Health has recently drawn up a plan in which children 1–12 years of age will be immunized. In Tamil Nadu and Uttar Pradesh, immunization programs are already running; thus, JE incidence might stabilize in those regions. The findings from this study would influence decision-making on policies regarding prevention, especially for the potential introduction of JE vaccine in endemic regions like Bengdubi in West Bengal.

In the future, prospective studies must be carried out for assessing the clinical and epidemiological impact of vaccination against JE where this has been implemented.

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