

## Hospital-Based Surveillance for Japanese Encephalitis at Four Sites in Bangladesh, 2003–2005

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**Abstract.** We investigated the epidemiology and etiology of encephalitis at four tertiary hospitals in Bangladesh during 2003–2005. Patients who met a clinical case definition for acute encephalitis and had cerebrospinal fluid (CSF) pleocytosis were eligible for enrollment; a standardized sampling pattern was used to enroll eligible patients. Recent Japanese encephalitis virus (JEV) infection was defined by presence of IgM antibodies against JEV in CSF or serum. Twenty (4%) of 492 cases had laboratory evidence of recent JEV infection; two died. All JE cases occurred during May–December, and cases were identified among all age groups. All cases resided in rural areas. Fifteen patients were re-assessed 4–6 weeks after hospitalization; 5 (33%) patients had physical disabilities and 7 (47%) reported cognitive difficulties. Infection with JEV is clearly an etiology of encephalitis in Bangladesh. Population-based studies to quantify burden of disease could assess options for targeted immunization programs.

### INTRODUCTION

Japanese encephalitis virus (JEV), a mosquito-borne flavivirus, is a leading cause of viral encephalitis in Asia.<sup>1–3</sup> Over the past three decades, the incidence of Japanese encephalitis (JE) has increased in parts of India, Nepal, and southeast Asia, with outbreaks of JE occurring in several areas that were previously not endemic for this disease.<sup>2,4–9</sup> The reasons for this increased geographic distribution are uncertain, but may include population shifts, and changes in agricultural practices, animal husbandry, migratory bird patterns, and movement of vector mosquitoes to wider areas.<sup>1</sup> It is estimated that JEV causes at least 50,000 cases of encephalitis each year in Asia, resulting in approximately 10,000 deaths with 15,000 survivors developing neurological and psychiatric sequelae.<sup>6</sup> There is no effective antiviral treatment. Importantly, JE is a vaccine-preventable disease.<sup>2,10–12</sup>

Japanese encephalitis has been reported in India and Myanmar, the two countries that border Bangladesh.<sup>5,8,13,14</sup> India has introduced a cost effective live-attenuated JE vaccine (SA 14-14-2) in a area hyperendemic for this disease area in 2006 after the JE epidemic in 2005.<sup>8,15</sup> More than 44 million children were immunized during 2006–2008. By 2010, 102 million children are targeted to be immunized in 111 districts endemic for JE in 11 states in India.<sup>16</sup> However, except for an outbreak in 1977 in the central part of Bangladesh,<sup>17</sup> JE has not been recognized and no systematic assessments of disease occurrence have been carried out since that outbreak. We conducted a hospital-based study during June 2003–July 2005 in Bangladesh to assess the etiologies of encephalitis including JEV, and report the results of JE assessment.

### METHODS

**Study site.** The study was performed at four tertiary care hospitals in different geographic areas (Figure 1). The study

began in June 2003 at three sites (Dhaka, Mymensingh, and Rajshahi) and in December 2004 at a fourth site (Sylhet) (Figure 1). The study continued at all sites until July 2005.

**Patient enrollment.** Study physicians visited the hospital wards daily to review the admission logbooks and identify patients meeting our clinical case definition of acute encephalitis with indication for lumbar puncture, based on the judgment of the patient's attending physician. The clinical case definition of acute encephalitis included new onset of fever (temperature  $\geq 38^{\circ}\text{C}$ ) or history of fever during the present illness along with altered mental status, (e.g., confusion, disorientation, coma) and/or a neurological deficit (i.e., focal or diffuse neurological dysfunction or new onset of seizures) with onset of the neurological symptoms within five days prior to hospitalization.

Enrollment in the study required that the patient met the clinical case definition and that he or she had cerebrospinal fluid (CSF) pleocytosis (defined as  $> 4$  leukocytes/mm<sup>3</sup> for patients  $> 6$  weeks of age and  $> 14$  leukocytes/mm<sup>3</sup> for the patients  $\leq 6$  weeks of age). Lumbar punctures were performed by attending physicians as a part of routine clinical care. However, patients or their guardians had to provide consent for collection of an additional amount of CSF during the lumbar puncture to be included in the study. Because of high patient volume, patients were assessed for pleocytosis using a sampling scheme. Beginning with the first patient on the line list, patients were assessed for pleocytosis until a patient demonstrated pleocytosis and was enrolled. The next three patients irrespective of CSF cell counts on the list were skipped and the next patient was selected for assessment. This process continued through the line list until a patient with pleocytosis was found and enrolled. Patients meeting the clinical case definition and whose CSF demonstrated pleocytosis were eligible for JEV testing (Figure 2).

From October 2004 until the end of the study, patients who had a positive CSF or blood culture, or a positive CSF latex agglutination test result<sup>18</sup> for *Streptococcus pneumoniae*; *Neisseria meningitidis* serogroups A, B, or C; or *Haemophilus influenzae* type b were excluded from JEV testing (Figure 2).

**Ethical considerations.** Study physicians obtained written informed consent before sample and data collection from either patients or their guardians. Assent was obtained for

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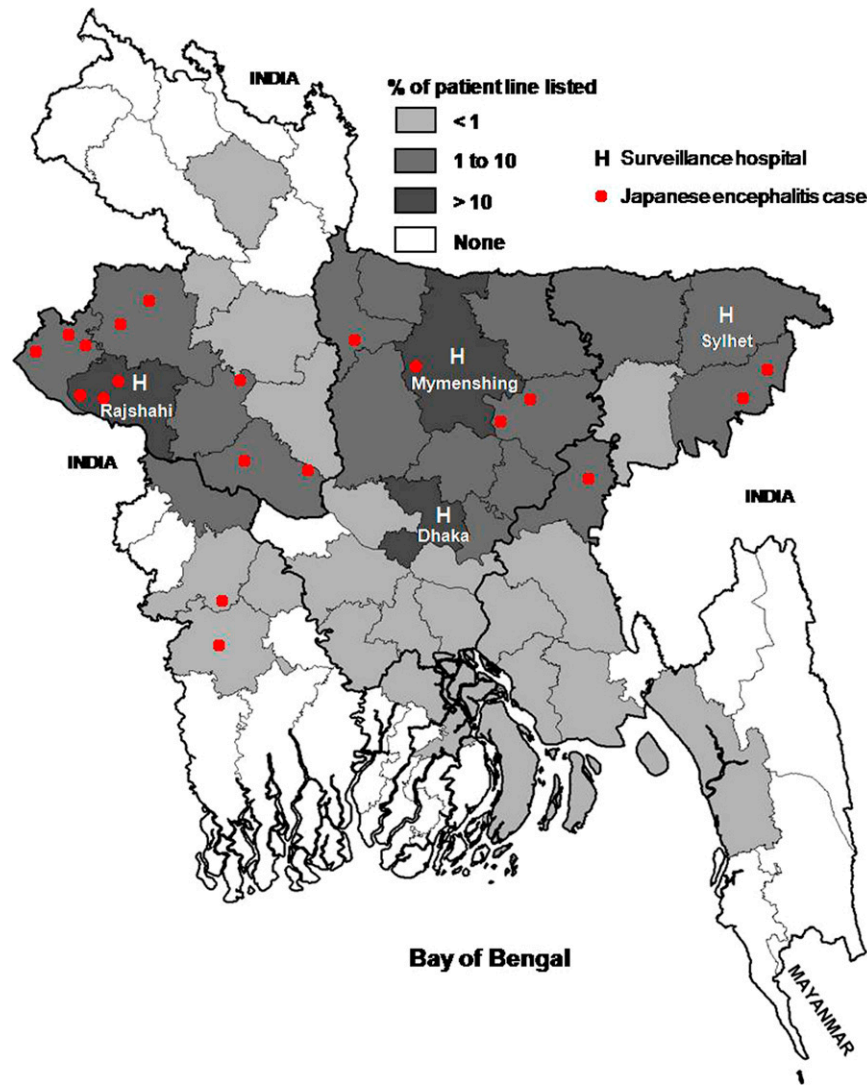


FIGURE 1. Districts of Bangladesh showing percentage of all line-listed acute encephalitis cases and distribution of Japanese encephalitis cases. This figure appears in color at [www.ajtmh.org](http://www.ajtmh.org).

patients 7–17 years of age. The study protocol was reviewed and approved by institutional review boards at International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B) and the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.

**Data collection.** Using a standardized questionnaire, the study physician collected data on sociodemographic status, exposure to wild and domestic animals, mosquitoes, and other insect bites, travel (outside home district or outside Bangladesh) within one month before the onset of illness, clinical symptoms and signs including Glasgow coma score, results of local laboratory tests, type and duration of treatment, duration of hospitalization, and outcome of illness including mortality, and neurological and functional level of activity at time of hospital discharge.

**Patient follow-up.** All surviving patients with JE were asked to return to the hospital and complete a follow-up interview 4–6 weeks after enrollment. During this visit, the study physician completed a follow-up questionnaire for documentation of short-term outcomes, residual neurological problems since discharge, the subject’s overall quality of life, and current

cognitive status. If the patient failed to return for the follow-up visit, the study physician or health worker attempted to visit the patient’s home to evaluate the status of the patient and encourage the patient to return for the follow-up visit.

**Specimen collection, local laboratory testing, storage, and shipment.** The study physicians collected CSF during a lumbar puncture performed for clinical assessment and serum samples during enrollment and the follow-up interview for JEV testing at CDC. The serum and CSF samples were aliquoted at study sites. The specimens were stored at  $-20^{\circ}\text{C}$  in the study hospitals for no longer than one week and then transported to ICDDR,B in a cold box and frozen at  $-70^{\circ}\text{C}$ . Aliquots were sent to CDC in dry ice.

**Laboratory testing for JEV and case criteria.** Serum and CSF samples from patients enrolled in the study were tested at the Arboviral Diseases Branch, CDC (Fort Collins, CO). Specimens were tested using JEV and dengue virus (DENV) IgM-capture enzyme-linked immunosorbent assays.<sup>19</sup> Specimens positive for IgM against JEV or DENV in serum or CSF were tested for JEV- and DENV-neutralizing antibodies by plaque reduction neutralization tests using a

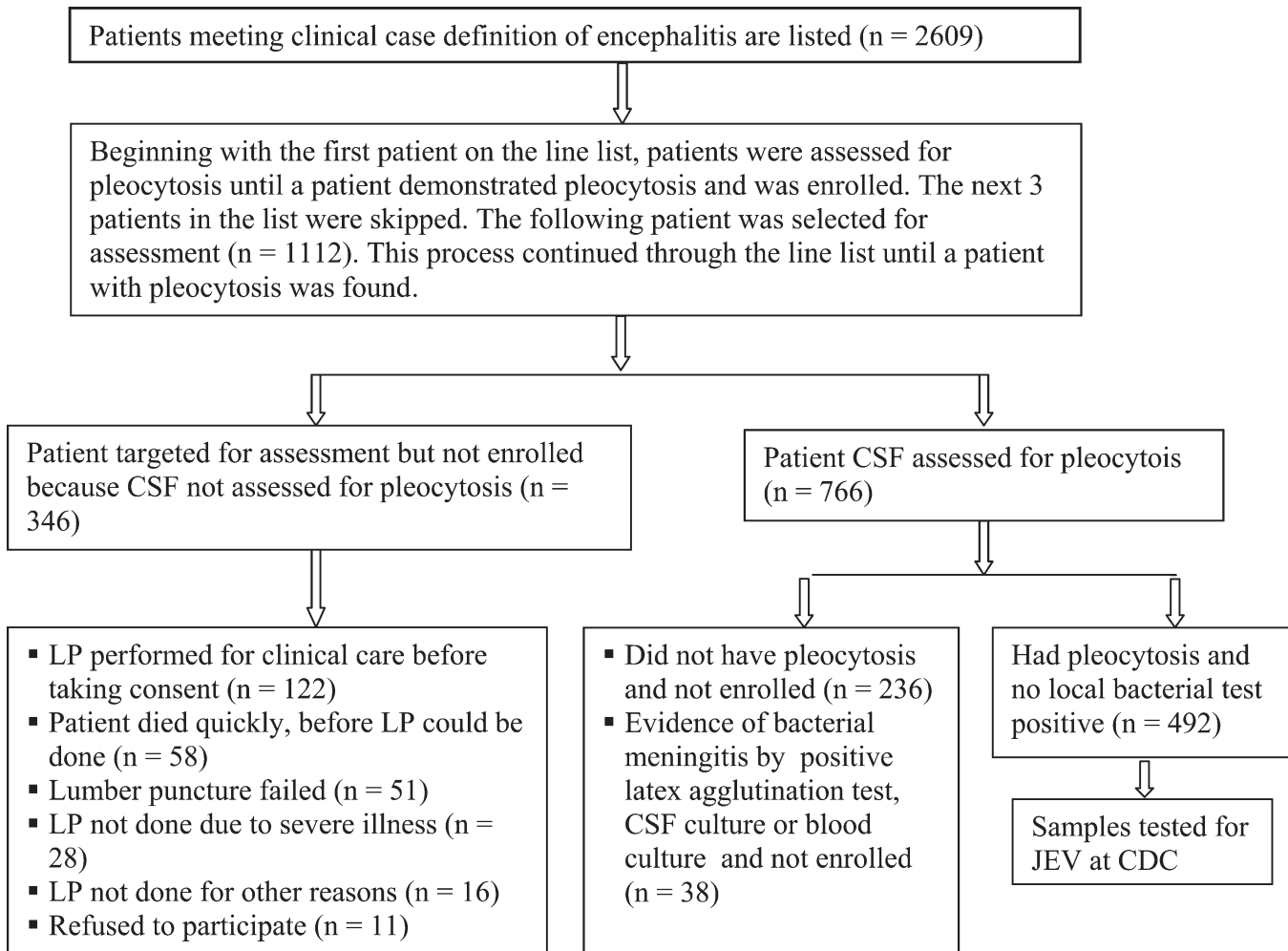


FIGURE 2. Flowchart for acute encephalitis cases, Bangladesh, June 2003–July 2005.

90% cut-off value (PRNT<sub>90</sub>).<sup>20</sup> Laboratory confirmation of recent JEV infection was defined as the presence of 1) IgM against JEV in CSF, or 2) IgM against JEV in serum with a JEV PRNT<sub>90</sub> titer  $\geq 20$  and a JEV PRNT<sub>90</sub> to DENV PRNT<sub>90</sub> titer ratio  $\geq 4$ .

**Statistical analysis.** The data were entered in Epi-Info version 6 software (CDC, Atlanta, GA) and analyzed by using STATA/SE 10 statistical software.<sup>21</sup> We calculated medians (range) for continuous data. Proportions were measured for categorical data.

## RESULTS

During the study period, 2,609 patients met the clinical case definition of acute encephalitis, had indication for lumbar puncture, and were included on the line list (Figure 2). Among the line listed cases, 808 (31%) were from Dhaka, 878 (34%) were from Mymensingh, 768 (29%) were from Rajshahi, and 155 (6%) were from Sylhet.

A total of 1,112 patients from the line listing were assessed for pleocytosis. Of these, 492 (44%) patients had pleocytosis, were enrolled, and had samples tested for JEV (Figure 2). Twenty (4%) of the 492 patients evaluated had laboratory evidence of recent JEV infection.

Cases of JE were identified in 3 (2%) of 131 of study patients in Dhaka, 4 (3%) of 156 study patients in Mymensingh, 11 (6%) of 173 study patients in Rajshahi, and 2 (6%) of 32 study patients in Sylhet.

Overall, the median age of JE cases was 18 years (range = 1.5 months–55 years). Cases were identified among all age groups with 11 (55%) cases greater than 15 years of age (Table 1). Eleven (55%) cases were males. All cases lived in rural areas and 12 (61%) resided in the northwestern part of the country (Figure 1). None of the cases traveled outside Bangladesh within the month before illness. Eighteen (90%) of the cases occurred during May–October, and no cases were identified during January–April (Figure 3). Fourteen (70%) JE cases were identified in the first year, and six (30%) JE cases were identified in the second year (Figure 3). In the first year, 14 (5%) of 285 encephalitis patients were diagnosed with JE. In the second year, 6 (3%) of 207 encephalitis patients were positive for JEV.

Patients with JE had fever (100%), altered consciousness (100%), convulsion (85%), headache (79%), severe weakness or lethargy (57%), stiff neck (55%), and vomiting (35%) (Table 2). The median (range) of their Glasgow coma scores was 8 (4–15), and 11 (55%) patients had a Glasgow coma score  $\leq 7$  during clinical evaluation at the time of recruitment.

TABLE 1

Demographic characteristics of 20 cases of Japanese encephalitis, Bangladesh

Characteristic	Value
Median age, years, (range)	18 (1.5 months–55 years)
Age group, years, no. (%)	
≤ 5	2 (10)
6–10	4 (20)
11–15	3 (15)
16–20	4 (20)
21–40	5 (25)
> 40	2 (10)
Sex, no. (%)	
M	11 (55)
F	9 (45)
Occupation, no. (%)	
Farmer	2 (10)
Student	9 (45)
Housewife	4 (20)
Children less than school age, no. (%)	2 (10)
Day laborer/petty business/unemployed, no. (%)	3 (15)

Six (30%) patients left the hospital with neurological sequelae that included focal weakness (n = 4) and paralysis (n = 1).

Fifteen patients, including five of the six patients who had residual neurological findings upon hospital discharge, were evaluated 4–6 weeks after recruitment. At that time, five (33%) patients had residual neurological problems (e.g., dysarthria, limb weakness, hearing impairment, and urinary incontinence). Seven (47%) reported difficulties with thinking/reasoning skills (Table 3). Two patients died; one at the hospital and one at home.

The mean CSF leukocyte count 109 cells/mm<sup>3</sup> (range = 7–980 cells/mm<sup>3</sup>) with a predominance of lymphocytes (mean = 75%, range = 30–100%). The CSF glucose concentrations ranged from 46 to 191 mg/dL (mean = 66 mg/dL); CSF protein levels were generally mildly elevated (mean = 58 mg/dL, range = 24–102 mg/dL).

DISCUSSION

This is the first study to document human JE viral infection in Bangladesh since 1977. We cannot determine if the virus has re-emerged or if it has been present, but not recognized, since the 1977 outbreak because of the limited diagnostic capac-

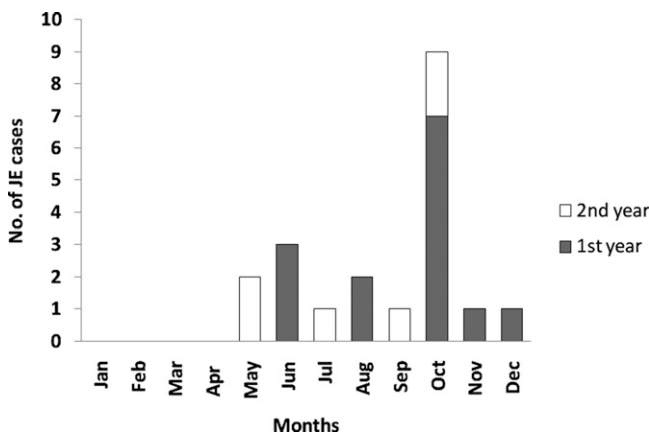


FIGURE 3. Number of Japanese encephalitis cases, by month of symptom onset, Bangladesh, June 2003–July 2005.

TABLE 2

Clinical features of 20 cases of Japanese encephalitis, Bangladesh

Features	Value
Symptoms, no. (%)	
Fever	20 (100)
Altered consciousness	20 (100)
Convulsion	17 (85)
Headache	15 (75)
Severe weakness or lethargy	13 (65)
Stiff neck	11 (55)
Vomiting	7 (35)
Nausea	6 (30)
Muscle pain	3 (15)
Limb weakness	3 (15)
Signs, no. (%)	
Temperature > 37.8°C	8 (40)
Neck rigidity	13 (65)
Babinski's sign (extensor plantar response)	8 (42)
Abnormal pupillary light reflex (sluggish/absent)	7 (35)
Kernig's sign	6 (30)
Tremor/rigidity/myoclonus	5 (25)
Glasgow coma score, median (range)	8 (4–15)
Grade	
15	0 (0)
13–14	1 (5)
8–12	8 (40)
3–7	11 (55)
Discharged with complication (focal weakness, paralysis and cognitive function impairment),* no. (%)	6 (30)
Duration of hospitalization in days, median (range)	9 (4–26)
Death, no (%)	2 (10)

\* Focal weakness (n = 4), paralysis (n = 1), and cognitive function impairment (n = 3).

ity for JE in Bangladesh.<sup>22</sup> Our study to assess etiologies of encephalitis in Bangladesh demonstrated evidence of acute JEV infection in 4% of enrolled cases. The cases were identified at all of the four participating study hospitals. However, 11 (6%) of encephalitis patients had JE at Rajshahi, but only three (2%) were identified in Dhaka, the site with the largest urban catchment area. All age groups were represented in the study, but more than 50% of the JE cases were greater than 15 years of age. Most JE cases occurred during May–October during monsoon and post-monsoon seasons.

The total number of JE cases who came to the four hospitals during our study was likely higher than the 20 cases identified by laboratory testing through our surveillance. In our study, 492 (64%) of the 766 patients evaluated had evidence of pleocytosis and had no bacterial infection. If we applied this same proportion to the other 1,843 patients meeting the clinical case definition for encephalitis but who were not evaluated for pleocytosis, we would have expected to identify an additional 1,183 patients or a total of 1,675 patients with encephalitis and pleocytosis. Of the 492 patients tested for JEV infection, 20 (4%) were positive. If this same proportion were applied to the projected number of patients with encephalitis and pleocytosis, we would have identified a total of 68 cases of JE at the study sites.

Our study hospitals were located in urban areas, but all the JE cases were from rural agricultural areas, with 55% from areas around the Rajshahi Medical College Hospital in the northwestern part of Bangladesh. The high prevalence of JE in rural areas could be because the requisite conditions (i.e., vector, reservoir host and virus) are all present.<sup>1</sup> This finding is consistent with the finding that JE is more common in rural rice-cultivating areas throughout Asia.<sup>23,24</sup> The low-lying and/or rice-producing rural areas of Bangladesh are conducive to breeding the mosquito vectors (*Culex vishnui*, *Cx. triae-*

TABLE 3

Follow-up of 15 cases of Japanese encephalitis 4–6 weeks after enrollment in the study, Bangladesh

Finding	No. (%)
Patient unable to resume his/her previous activities	7 (47)
Reported deficits in thinking and reasoning since illness	7 (47)
Depression	4 (27)
Dysarthria	4 (27)
Limb weakness	2 (13)
Hearing impairment	2 (13)
Personality change	2 (13)
Urinary incontinence	1 (7)

*niorhynchus*, and *Cx. gelidus*) that transmit JEV.<sup>17,25</sup> Common aquatic birds, such as egrets and herons, and pigs are reservoirs for JEV. Although pigs are less common than cows and goats in this predominantly Muslim country, the pigs found in Bangladesh often forage over wide-ranging areas.

Case of JE identified in this study represented all age groups. In JE-endemic areas of southeast Asia, most people are infected with JEV before 15 years of age.<sup>2,3,6,26</sup> The occurrence of cases in Bangladesh among somewhat older persons might reflect a recent introduction of JEV, or may be caused by less intense transmission, leading to a larger population of susceptible adults than in other countries endemic for JE. In Nepal, the age group affected also included young adults and adults and may have been caused by migration of working people from non-endemic areas to endemic areas.<sup>27</sup>

All JE infections were identified during May–December, with 90% identified during May–October. The assessment of JE prevalence in Sylhet in northeastern Bangladesh was limited because patients were only enrolled from December 2004 through July 2005 and this period was only three months of a single JE transmission season. These findings are consistent with seasonality noted in other areas of southeast Asia.<sup>2,6,8,28,29</sup> This finding may be related to seasonal increases in the *Culex* mosquito population, the main vector for transmission of JEV, during and just after the rainy season (June–September).<sup>2,6,8,28–30</sup>

Similar to other studies, almost one-third of our patients with JE had neurological sequelae and almost half had subjective cognitive function impairment at the six-week follow-up interview.<sup>6,31</sup> This finding suggests that JE may represent a substantial burden in an already impoverished country because of the resultant lack of productivity and caregiver activities.

It is likely that our assessment captured only a small proportion of JE cases in Bangladesh. Many sick persons may not have come to a health facility for care or may have sought care in hospitals other than the tertiary care centers included in our study. The overall case-fatality rate of 10% was also lower than that reported in other studies,<sup>4</sup> which may be caused by late care-seeking behavior, resulting in patients dying before reaching the hospital. Additionally, some patients might have died in a hospital before a lumbar puncture could be performed or a lumbar puncture may not have been performed because of severe illness. It is likely that some of these patients had JE, which further contributes to an underestimate of case fatality.

The evaluation of other etiologies of encephalitis is ongoing. The findings of this study indicate that JEV infection is clearly an etiology of encephalitis in Bangladesh and is worthy of closer inspection as a potentially important cause of encephalitis. Better assessment of disease incidence and the

financial and functional impact of both acute illness with JE and the long-term sequelae of illness on cases and family units will aid in clarifying the overall public health burden and societal impact of JE in this population. The findings from these efforts would impact decision-making on policies regarding prevention, especially for the potential introduction of JE vaccine in Bangladesh and what populations should be targeted for vaccine programs.

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