Laboratory-based Japanese Encephalitis Surveillance in Nepal and the Implications for a National Immunization Strategy


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Abstract. We report on two years of Japanese encephalitis (JE) surveillance in Nepal and the implications for a national immunization strategy. From May 2004 to April 2006, 4,652 patients with encephalitis were evaluated. A serum or cerebrospinal fluid specimen was collected from 3198 (69%) patients of which 1,035 (32%) were positive by Japanese encephalitis IgM ELISA. Most cases (N = 951, 92%) were from the 24 Terai districts (i.e., southern plains, 12.3 million persons) with the majority (N = 616, 65%) from four western Terai districts (population = 1.8 million). The case fatality ratio was 14.7% and 6.3% and the proportion of cases under 15 years old was 52% and 62% in the four western and 20 non-western Terai districts, respectively. Japanese encephalitis immunization targeting residents one year of age and older in the western districts and one through 14 years old in the non-western Terai districts may have reduced Japanese encephalitis cases by 84% and deaths by 92%, nationally.

INTRODUCTION

Japanese encephalitis (JE) virus belongs to the family Flaviviridae and genus Flavivirus. Infections are transmitted between animal hosts and mosquito vectors with humans as accidental hosts. Human infection ranges from asymptomatic to life-threatening encephalopathy with frequent neuropsychiatric sequelae in survivors.1

Since 1978, Nepal has experienced epidemics and postmonsoon, seasonal increases of encephalitis cases in the Terai, the flat southern plains that borders India and includes nearly 12.5 million people. Based on aggregated, clinical reports collected from hospitals during the period of 1978 to 2003, nearly 26,700 suspected JE cases with 5,400 deaths have been identified.2

In most Asian countries, it is difficult to measure the disease burden from JE because of varying or unstated case definitions, limited availability of laboratory diagnostic testing especially in rural areas, and problems with ascertaining disease outcome.3 The Department of Health Services of Nepal’s Ministry of Health and Population recognized these as critical issues when developing JE surveillance and, in May 2004, instituted new JE surveillance guidelines. It entailed enhanced case-based surveillance using a standardized definition for Acute Encephalitis Syndrome (AES), an increase in surveillance sites, and an increased access to laboratory facilities performing JE testing.

We report here the results of the first 2 years of hospital-based JE surveillance in Nepal. Using these data, the age-specific incidence, mortality, and case fatality ratio for JE cases detected at government hospitals located throughout Nepal are calculated. The implication of these findings for developing a national immunization strategy to reduce JE morbidity and mortality is given.

MATERIALS AND METHODS

Country description. Nepal has a mostly rural, poor population of 25 million. It is administratively divided into 5 development regions running from west to east, which includes 75 districts. It is also divided topographically into 3 ecological zones from north to south. The Himalayan Mountains are in the north along the border with China, the Terai are along the southern border with India, and the Himalayan foothills are sandwiched between the two zones.

Nepal has previously immunized children against JE. In 1999, 224,000 doses of SA 14-14-2 JE vaccine were given to children 1 to 15 years old in 3 districts (i.e., Bardiya, Banke, and Kailali) of the 4 hyperendemic western districts.4,5 In 2000 to 2001, 378,112 persons 6 months through 10 years of age residing in 3 of the 4 hyperendemic western districts (Kailali, Banke, and Dang) and in 2 adjacent districts (Kanchanpur and Rupandehi) received a complete regimen of 3 doses of a cell culture inactivated vaccine grown in hamster kidney cells.4 Despite these attempts to control JE, this disease remains highly endemic in Nepal.

Disease surveillance. This Nepal model for JE surveillance is based on experience implementing syndromic encephalitis surveillance and from integrating many JE surveillance activities with the infrastructure developed for Acute Flaccid Paralysis (AFP) surveillance. From AFP surveillance, for example, data management was integrated into the computer database used for monitoring AFP and specimens were transported by the same reverse cold chain used to ship stool specimens for poliovirus detection. Technical assistance and quality control of laboratory procedures were provided by the WHO technical staff in Nepal.

Sixty-four referral hospitals located throughout Nepal were enrolled in laboratory-based JE surveillance. AES cases were defined as any patient presenting with acute onset of fever and a deterioration in mental status (e.g., confusion, disorientation, coma, or inability to talk) and/or new onset of seizures excluding simple febrile seizures.6 Using a structured reporting form, information on age, gender, district of residence, and whether the patient had been immunized against JE were recorded. Clinic data including date of disease onset

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and symptoms (e.g., fever, neck rigidity, convulsions) as well as outcome at discharge (i.e., cured, referred, death, unknown) were recorded. Five ml of serum or 2 mL of cerebrospinal fluid (CSF) were obtained from each patient. The serum and CSF samples were labeled with the patient identification number and stored at 2° to 8°C until transported in cold boxes with ice packs to a diagnostic laboratory. Specimen transportation took no more than 4 hrs by plane or road. Laboratory results were entered into a computer database by a data entry clerk and rechecked independently by a second clerk.

Because this was a national surveillance activity, written informed consent was not required by the Ministry of Health and Population.

**Diagnosis.** Upon arrival at the referral laboratory, serum and CSF samples were again stored at 2° to 8°C or, if batch testing was not planned for the next week, they were kept at −70°C. Laboratory confirmation was made from a single serum or CSF sample by detection of anti-JE immunoglobulin M (IgM) antibody titers using an IgM antibody capture enzyme-linked immunosorbant assay.7,8 Serum and CSF specimens were tested at the National Public Health Laboratory (NPHL) of the Department of Health Services in Kathmandu or the B. P. Koirala Institute of Health Sciences (BPKIHS) in Dharan. Test reagents were provided to NPHL and BPKIHS by the Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand.

**Analysis.** The annualized, age-specific incidence and mortality rate was the number of JE IgM-positive cases or deaths during the 2 years divided by 2 and then divided by the age-specific population multiplied by 100,000. Population estimates were taken from the 2001 national census, the last census in Nepal. Age categories were infants (< 1 year), toddlers (1–4 years old), children (5–14 years old), adolescents (15–19 years old), adults (20–34 years old), and older adults (35 years and older). The case fatality ratio (CFR) was defined as the percentage of JE IgM-positive deaths among total number of JE IgM-positive cases. Since mortality data were not available for each patient, the denominator only included persons from whom we received these data.

To visualize the geographic distribution of laboratory identified JE cases, we plotted one dot for each case by district of residence on a map of Nepal. Each dot was randomly assigned within the case’s district of residence.

To estimate the potential effectiveness of the proposed immunization strategy, we identified the number of JE IgM-positive cases and deaths that would have been prevented had the population been immunized against JE according to the suggested strategy at the start of surveillance in May 2004. We assumed that the vaccine had 98.5% effectiveness, the estimate of vaccine efficacy for persons aged one to 15 years old, 12 to 15 months after immunization.9 The expected number of cases or deaths if persons would have been immunized against JE was calculated as observed cases or deaths during the 2 years of surveillance multiplied by 1 minus 0.985.

**RESULTS**

From May 2004 through April 2006, 4,652 AES cases were detected. From these cases, we obtained a serum (N = 2792) or CSF (N = 406) sample from 69% (N = 3198). Of the sampled patients, 32% (N = 1035) tested positive for JE including 34% (N = 962) of serum samples and 18% (N = 73) of CSF samples. Six (0.6%) of the positive cases reported that they had been immunized against JE. The remaining patients were unsure of their immunization status (N = 285, 28%) or reported no prior JE immunization (N = 744, 72%).

Japanese encephalitis cases were found in 43 of 75 districts, but 92% (N = 951) of JE cases occurred in the 24 southern Terai districts (Figure 1). The remaining cases (N = 84) occurred sporadically throughout other areas of Nepal (popu-
The annualized incidence was higher for males at 4.3 cases per 100,000 (537/2/6,296,635) than females at 3.4 cases per 100,000 (414/2/6,085,654) per year.

Of the 24 districts, 4 districts in western Terai (i.e., Kailali, Bardiya, Banke, and Dang) accounted for the majority of cases (N = 616, 65%) and deaths (N = 66, 88%). The incidence and mortality rate per 100,000 persons and the CFR for the 4 western Terai districts was 16.7%, 1.8%, and 14.7%, respectively (Table 1). The same values were 1.6%, 0.04%, and 6.3%, respectively, for the other 20 Terai districts.

The age distribution of cases differed between the 4 western and 20 non-western Terai districts. In the 4 western Terai districts, the annualized incidence rates were highest among children 1 to 4 years old (24.9 per 100,000), subsequently decreasing among older children and adults, but peaking again at 17.4 per 100,000 among adults aged 35 years and older. In the other 20 endemic districts, disease incidence peaked at 2.9 per 100,000 for children 5 to 14 years of age and decreased linearly for each age group thereafter. In the 4 western Terai districts, 52% (N = 323) of cases were less than 15 years old, whereas in the 20 other Terai districts, 62% (N = 209) of cases were less than 15 years old.

For the 4 western Terai districts, infants had the lowest annualized mortality rates (0.0 deaths per 100,000) and CFR (0.0%), although the number of cases (N = 4) was also low. The highest mortality rate (3.2 per 100,000) and CFR (25.0%) was for persons 35 years and older. For the other endemic districts, the mortality rate never exceeded 0.1 deaths per 100,000; the CFR was highest for persons 15 to 19 years old (10.0%) and for persons 35 years and older (10.0%). There were no deaths reported among children less than 5 years old.

**DISCUSSION**

**Japanese encephalitis epidemiology in Nepal.** In both surveillance years, we found JE to be limited to the months of June through October, which coincides with the monsoon season. We also observed that JE is not uniformly distributed throughout Nepal, but is primarily confined to 24 districts of the southern plains or Terai that border from east to west with India. That JE is primarily limited to this ecological zone may be due to a greater density of mosquito vectors than in the hill and mountain zones. Of these 24 districts, 4 western Terai districts are considered hyperendemic; that is, JE incidence is significantly higher in this area than in other areas of Nepal. The geographic distribution of disease has been reported by other investigators.4,10,11 Outside Terai, we de-
ected sporadic cases including cases in Kathmandu as reported earlier. It was outside the scope of this study to determine if these sporadic cases were caused by transmission taking place within those districts, by people traveling during the incubation period from endemic districts to these non-endemic districts, or by patients seeking services at larger referral hospitals located in non-endemic areas, as may be the case in Kathmandu.

Contrary to previous reports emphasizing JE in pediatric populations, we found JE in Nepal to also produce morbidity and mortality in adults. Noteworthy was the high mortality in adults over 35 years old in the 4 western Terai districts. We suggest that persons from the hills and mountains may have migrated to this area for work and may have been non-immune to JE. To the best of our knowledge, this has not been investigated. This observation also implies a limitation to studies and vaccine trials that only consider children.

We observed that about one-third of AES cases were JE IgM-positive. A study of JE in Bali using similar diagnostic procedures detected 30% more JE infections when testing an acute and convalescent CSF sample from patients. Even if our case load was increased by 30%, there would still be a significant number of cases that were JE negative, suggesting that agents other than JE may be responsible for some AES cases.

Before examining the implication of these results, we comment on some of the limitations of their data. Firstly, we were unable to obtain diagnostic specimens for all cases. Those patients without specimens were similar to those with specimens in terms of age (mean 22 vs. 18 years), gender (46% vs. 43% female), and residence (52% vs. 44% from 4 western Terai districts). However, we did observe that patients without specimens had a higher CFR than patients with specimens. This may suggest that patients without samples were admitted with severe, life-threatening disease that precluded obtaining a sample. If these cases were JE, we have underestimated the number of JE cases and deaths. Secondly, if our diagnostic test was unreliable, our test results may not be the true rates. Confirmatory and blinded samples from Nepal, using AFRIMS results as the standard, found sensitivity of 99% among 677 JE positive samples and a specificity of 76% among 101 JE negative samples. The high sensitivity and moderate specificity imply that these results are worth reporting. Thirdly, our diagnosis was based on a single serum or CSF sample. A positive result on serum specimen can provide at most a diagnosis of probable JE. Only a positive result on CSF specimen is considered diagnostic for JE as a cause of AES. However, the WHO has suggested that a single serum sample from an acutely encephalitic patient is adequate for surveillance efforts. Finally, it has been proposed that samples taken during the first week after disease onset may be falsely negative because this may be too early in the natural history of disease to detect anti-JE IgM antibodies. About 67% of our specimens were collected during this period, and we may have false negatives.

**Implications for an immunization strategy.** Based on studies conducted in Nepal, the Government of Nepal has approved the use of the SA 14-14-2, a live-attenuated JE vaccine. All 3 studies suggest that this vaccine may be efficacious and safe. After the administration of 224,000 vaccine doses in the year 1999, a case-control study conducted in the western Terai suggested 99% (95% Confidence Interval [CI]: 95%–100%) protection for children when vaccine was administered only days to weeks before the seasonal increase in JE cases. A second study found 98.5% protection (CI: 90.1%–99.2%) 12 to 15 months after immunization and a third study conducted 5 years after the immunization program found 96.2% protection (CI: 73.1%–99.9%). No significant adverse events after immunization were reported in children or adults.

Given the geographic distribution of JE cases, our surveillance data suggests an immunization campaign targeting the 24 southern districts with further prioritization to the 4 western Terai districts where JE morbidity and mortality is at its highest for all ages. It appears that a campaign immunizing all persons greater than 1 year old with SA 14-14-2 JE vaccine would be appropriate for these districts. Such a campaign in these 4 districts would have reduced JE cases by 63% and deaths by 87% if started prior to May 2004 (Table 2). For the remaining 20 districts, a program immunizing all persons greater than 1 year would be ideal, but the large population (<10 million) for financial and logistical reasons precludes such an activity at this time. Still, a campaign limited to 1-year-olds through 14-year-olds in the 20 districts with SA 14-14-2 would have prevented an additional 21% of cases and 5% of deaths, nationally. Given the seasonality of disease, this campaign should be completed by April, providing 1 month before the seasonal increase in disease to ensure that all recipients have sufficient time to develop a vaccine induced immune response.

In summary, an immunization strategy targeting all persons greater than or equal to 1 year old in the 4 hyperendemic western Terai districts and 1 to 14 years old in the other 20 Terai districts would have reduced JE cases by 84% and deaths by 92%, if completed before May 2004. To ensure that the disease is controlled in these populations in the following years, it is suggested that routine immunization be instituted.

**Table 2**

Observed and expected reduction in IgM-positive, Japanese encephalitis cases and deaths if selected populations were immunized against JE for the period 01 May 2004 to 30 April 2006

<table>
<thead>
<tr>
<th>Population</th>
<th>Observed events*</th>
<th>Expected events if immunized†</th>
<th>Percent of all cases and deaths prevented‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Deaths</td>
<td>Immunized</td>
</tr>
<tr>
<td>≥ 1 year old in the 4 hyperendemic Terai districts</td>
<td>612</td>
<td>66</td>
<td>Yes</td>
</tr>
<tr>
<td>1 to 14 year olds in 20 endemic Terai districts</td>
<td>199</td>
<td>4</td>
<td>Yes</td>
</tr>
<tr>
<td>&lt; 1 in 24 districts and ≥ 15 year olds in 20 districts§</td>
<td>140</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td>951</td>
<td>75</td>
<td>152</td>
</tr>
</tbody>
</table>

* Cases and deaths observed from 01 May 2004 through 30 April 2006
† Expected cases and deaths if immunized or not immunized with Japanese encephalitis vaccine before 01 May 04 and assuming 98.5% vaccine effectiveness (i.e., Expected cases = observed cases * [1-0.985] and expected deaths = observed deaths * [1-0.985])
‡ Percent of all cases (N = 951) and deaths (N = 75) prevented through immunization (i.e., % cases prevented = (observed cases–expected cases)/951)*100 and % deaths prevented = (observed deaths–expected deaths)/75)*100)
§ Age range for cases was 4 months to 84 years and age range for deaths was 2 to 80 years.
for children aged 9 through 11 months in the 24 districts. Unfortunately, efficacy has not been tested in children less than 8 months of age and there is a risk that maternal antibodies may interfere with development of immunity.

This immunization strategy may have limitations. First, the effectiveness of SA 14-14-2 was 98.5% (95% CI: 90.1%–99.2%) at 12 to 15 months after immunization and the second study was 96.2% (CI: 73.1%–99.9%) at 5 years. At the lower confidence interval of effectiveness, up to 10% of immunized persons may not be protected after 12 to 15 months and 27% after 5 years. Second, this strategy does not protect against JE occurring outside the Terai or within the Terai among cases 15 years old or older residing in the 20 non-western districts. To protect these persons, an immunization campaign would need to cover an additional 17 million individuals. Finally, efficacy has not been tested in children less than 8 months of age, and there is a risk that maternal antibodies may interfere with development of immunity. For infants, environmental controls including bed nets and appropriate clothing to protect against mosquito bites should be investigated.

To the best of our knowledge, this is the most extensive laboratory-based, JE surveillance activity in the Southeast Asia region of the WHO and demonstrates how other immunization preventable diseases could be integrated into AFP surveillance. Nepal’s JE surveillance system provides critical data for documenting the disease burden and rationale for an immunization strategy. As JE immunization strategies are implemented, surveillance will continue to define changes in disease epidemiology and immunization policy.

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