Endemic Japanese Encephalitis in the Kathmandu Valley, Nepal

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Abstract. Japanese encephalitis (JE) is endemic in the Terai region of Nepal. There is little information on the occurrence of JE outside the Terai and particularly in the densely populated Kathmandu valley. Acute encephalitis syndrome (AES) cases were detected using a sentinel surveillance system that has been functioning since 2004. JE was confirmed using anti-JE IgM ELISA. All laboratory-confirmed JE cases that occurred in the Kathmandu valley during 2006 were followed up for verification of residence and travel history. JE was confirmed in 40 residents of the Kathmandu valley, including 30 cases that had no history of travel outside the valley during the incubation period. Incidence was 2.1/100,000 and the case fatality was 20% (8/40). Currently, JE prevention is focused on the Terai region in Nepal; given the evidence, this should be reviewed for the possible inclusion of the Kathmandu valley in the national JE prevention and control program.

INTRODUCTION

Japanese encephalitis (JE), a flavivirus infection transmitted by mosquitoes (most commonly Culex species), is prevalent throughout Southeast Asia. JE was first confirmed in Nepal in 1978 after an outbreak in the western part of the country along the border with India.1 Since then, JE infection has been reported in animal reservoirs and in humans throughout the Terai region, which borders India2–5; however, there is little data regarding the occurrence of JE outside the Terai region. As a result, the national JE prevention and control program, which includes mass immunization of the entire population > 1 year old with a single dose of a JE vaccine in all endemic districts followed by the introduction of a single dose in the routine immunization program for 12- to 23-month-old children, has focused on the Terai. In this study, we followed up all laboratory-confirmed JE cases during 2006 who reported residency in the Kathmandu valley to provide evidence of JE endemicity in this area of Nepal.

MATERIALS AND METHODS

The Nepal Ministry of Health and Population has been conducting surveillance for acute encephalitis syndrome (AES), as per WHO-recommended standards for surveillance,6 since May 2004 through a national network of 93 surveillance sites (including 8 sites within the Kathmandu valley) tracking vaccine preventable diseases (polio, measles/rubella, maternal and neonatal tetanus, and Haemophilus influenzae type b) and supported by the World Health Organization Immunization Preventable Diseases Unit (WHO-IPD). Potential JE cases were captured through this network. Although sporadic cases were reported from districts outside the Terai and Kathmandu valley, this article focuses on those cases reported from the Kathmandu valley in 2006 and compares the incidence in the valley with the incidence in the 24 districts of the Terai and inner Terai known to be JE endemic.

Study population and data collection. The study population included any person of any age who 1) presented to any AES reporting site throughout the country at any time from 1 January through 31 December 2006 with the acute onset of fever and a change in mental status (symptoms such as confusion, disorientation, coma, or inability to talk) and/or a new onset of seizures (excluding simple febrile seizures), or was clinically diagnosed as AES, JE, or viral encephalitis; and 2) was confirmed to have JE antibody by IgM capture ELISA on a serum or CSF specimen. The subset of potential cases for this study included those patients who reported an address within the Kathmandu valley (i.e., within Kathmandu, Lalitpur, or Bhaktapur districts). All patients (or next of kin if the patient was deceased or unavailable) with a reported Kathmandu valley address were visited at home or contacted by telephone to confirm their residence and travel history during the 30 days before the onset of the above symptoms given the incubation period of 5–15 days.

As part of the national AES surveillance system, serum, or cerebrospinal fluid (CSF) specimens were routinely obtained from patients with clinically diagnosed AES. Blood samples were collected by nurses or laboratory personnel and transported to the laboratory in the same hospital for serum separation by centrifugation at 3,000 rpm for 5–10 minutes. CSF specimens were collected by the attending physicians and were transported to the laboratory for cell count and glucose and protein analyses. Both serum and CSF samples were stored at 2–8°C until transported, maintaining reverse cold chain, to the B.P. Koirala Institute of Health Sciences (BPKIHS) in Dharan or the National Public Health Laboratory (NPHL) in Kathmandu. Patients with no specimens or who had specimens that were determined to be anti-JE IgM negative were classified as “AES” of unknown etiology.

Laboratory methods. Laboratory confirmation was done at BPKIHS or NPHL as previously reported by Innis and others.7 Briefly, ELISA plates (supplied by the Armed Forces Research Institute of Medical Sciences [AFRIMS]) were coated with goat anti-human IgM antibody. Diluted serum or CSF samples and positive and negative controls were added in duplicate wells and incubated for 2 hours at 18–32°C. Next, diluted JE antigen was added and incubated at 18–32°C for 2 hours and then human anti-flavivirus IgG conjugate was added and incubated for 1 hour at 37°C. The reaction was visualized with the addition of ortho-phenylene diamine substrate and measured in an ELISA reader at 492 nm (HUMAREADER, Human, Germany). The samples with ≥ 40 EIA units were considered positive. External quality assessment was done at AFRIMS in Bangkok, Thailand.

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RESULTS

In 2006, 1,481 AES cases were reported nationwide through the national surveillance network. Of the 1,291 clinical cases with serum or CSF samples, 292 (23%) were confirmed as JE at the NPHL or at the BPKIHS. The 999 cases with samples but no evidence of anti-JE IgM antibody and the 190 cases with no sample were classified as AES–unknown, because etiology has not been determined. The 292 JE-positive cases were distributed across 42 districts, including the 3 districts in the Kathmandu valley (Figure 1), with incidence peaking in August and September (Figure 2). In the 24 JE-endemic districts of the Terai and inner Terai, specimens were collected from 741 (84%) of 886 AES cases, including 206 (28%) CSF, 503 (68%) single acute serum, and 32 (4%) paired serum specimens. The incidence of laboratory-confirmed JE in the Terai and inner Terai was 1.6/100,000. In the Kathmandu valley, 350 AES cases were detected, of which 318 (91%) had at least one serum or CSF specimen, and 48 (15%) of these were confirmed anti-JE IgM positive. The specimens collected included 123 (39%) CSF, 167 (52%) single acute serum, and 28 (9%) paired serum specimens. Among the 48 JE cases in the Kathmandu valley, 40 cases

![Figure 1. Distribution of 292 laboratory-confirmed JE cases by ecological region, Nepal, 2006.](image)

![Figure 2. AES of unknown etiology and laboratory-confirmed JE cases in the 24 districts of the Terai and inner Terai (A) and in the Kathmandu valley (B), by month, Nepal, 2006.](image)
were verified to be residents of the valley, and 30 of these had no history of travel outside the valley during the 30 days before the onset of illness (Figure 3). Considering the 40 cases with confirmed residency in the valley, the overall incidence of JE in the Kathmandu valley was 2.1/100,000 and was 3.1/100,000 and 1.6/100,000 in the <15- and 15+-year populations, respectively. These 40 cases were predominantly men (72.5%), and their ages ranged from 4 months to 78 years (Table 1). No cases had been immunized against JE. The case fatality was 20% (8/40).

DISCUSSION

In Nepal, the majority of JE cases occur in the lowland plains or Terai ecological region that borders India during and after the annual monsoon season from May to October. In 2006, the peak month of JE case detection was 1 month later in the Kathmandu valley than in the Terai. This is probably because of the later onset of the monsoon season in the valley. In both 2004 and 2005, 88% of the confirmed JE cases occurred in the Terai; in 2006, 73% of cases occurred in the Terai (WHO, unpublished data). The shift in the proportional distribution across ecologic regions in 2006 is likely caused by the mass immunization campaign using the live attenuated SA 14-14-2 vaccine that was conducted in the Terai in July/August 2006 that targeted all of the population >1 year of age in four highly endemic districts and the >1- to <15-year population in two endemic districts. Another possible reason for the shift may be because the Terai experienced a large JE epidemic in 2005 (591 laboratory-confirmed cases) that may have conferred widespread immunity resulting in fewer susceptibles in the Terai in 2006. The Kathmandu valley did not seem to experience the same epidemic level (there were 36 laboratory-confirmed JE cases reported from the valley in 2005, but addresses and travel history were not confirmed).

These points must also be taken into consideration when recognizing that in 2006 the JE incidence in the Kathmandu valley (2.1/100,000) was comparable to the incidence in the 24 JE-endemic districts of the Terai and inner Terai (1.6/100,000).

JE infection in the Kathmandu valley has been reported previously, but in these studies, the determination of the magnitude and distribution of JE in the valley was limited because cases were detected at only one hospital from September and October 1995 (2 laboratory-confirmed JE cases among 15 meningo-encephalitis cases), August to October 1996 (3 laboratory-confirmed JE cases among 7 encephalitis cases), and 1998 (9 laboratory-confirmed JE cases among 14 encephalitis cases). In our study, we were able to use the

<table>
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<th>Characteristic</th>
<th>Age* (years)</th>
<th>Sex</th>
<th>Death associated with JE</th>
<th>Previously immunized against JE</th>
<th>Specimen type collected</th>
<th>History of travel outside Kathmandu valley†</th>
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* Range: 4 months to 78 years.
† In the 30 days before onset of symptoms.

Figure 3. Distribution of laboratory-confirmed JE cases by travel history, Kathmandu valley, Nepal, 2006.
AES surveillance network to detect encephalitis cases at eight hospitals throughout the valley during an entire year to develop a more complete and comprehensive picture of JE transmission in the area. In addition, a WHO-IPD Surveillance Medical Officer and two data management staff were assigned to follow-up each case to verify their addresses and travel history during the 30 days before onset of symptoms. Based on travel histories and illness onset dates, we are confident that the source of infection for 30 JE cases was within the Kathmandu valley and that the valley was also the probable source of infection for 10 other cases. The existence of JE transmission in the valley is also supported by a report by Darsie and Pradhan and an ecologic study conducted in 2001 (Environmental Health Project, unpublished report) that provided clear evidence of the presence of JE vectors in the Kathmandu valley. In addition, a 1997 study confirmed JE virus infection in amplifying hosts (i.e., Sus scrofa domestica pig) in the valley.

JE was confirmed in a greater proportion among men (72.5%) than women (27.5%), which is consistent with a previous study conducted in Nepal. This is probably because of a differential in the exposure to the vector, because women and girls were more likely to spend the vector's prime feeding hours within the household, whereas the men spent more time outside during these hours. The incidence of JE in the Kathmandu valley in the <15-year population (3.1/100,000) was nearly twice the incidence in the 15+-year population (1.6/100,000), which is consistent with JE infection being predominantly in children. The case fatality in 2006 in the Kathmandu valley (20%) was higher than that previously reported from the 1997 JE outbreak in southwestern Nepal (13.2%) but falls within the expected range of 0.3–60%.

Limitations to this study are related to the shortcomings of a developing sentinel site surveillance system. Not all cases of AES reported to a hospital for various reasons (access to health care, death before seeking health care, and health care–seeking behavior in general), and not all health facilities in Nepal are included in the AES surveillance network. Diagnostic specimens were not collected from all AES cases. In cases where serum specimens were collected, paired acute/convalescent specimens were not commonly collected. These shortcomings would lead to underestimation of the incidence of JE. Although serum specimens are considered diagnostic for surveillance purposes, diagnostic specificity is higher with CSF specimens, and because serum collection is the more common practice in Nepal, the loss of specificity could lead to an overestimation of the incidence of JE. The typical incubation period for JE is 5–15 days. We used a more conservative period of 30 days when determining if a case had traveled outside the valley during the incubation period. It is possible that the incubation period was >30 days for some cases, leading to incorrect travel history and an overestimation of the source of JE being from within the Kathmandu valley. Another limitation of this study is the inability to comprehensively define the epidemiology of AES in Nepal because of the lack of data on other etiologies.

JE is seasonally endemic to Nepal's Kathmandu valley, which has an estimated population of 1.9 million. Currently, JE prevention is focused on the Terai region of the country; given evidence of the presence of an animal reservoir and vector, and incident human cases with no travel history, the inclusion of the Kathmandu valley in the national JE prevention and control program should be considered. In addition, immunization against JE should be recommended for tourists to Nepal even if they are only spending time in the Kathmandu valley.

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