Japanese Encephalitis Outbreak, India, 2005

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An outbreak of viral encephalitis occurred in Gorakhpur, India, from July through November 2005. The etiologic agent was confirmed to be Japanese encephalitis virus by analyzing 326 acute-phase clinical specimens for virus-specific antibodies and viral RNA and by virus isolation. Phylogenetic analysis showed that these isolates belonged to genogroup 3.

An epidemic of viral encephalitis was reported from July through November 2005 in Gorakhpur, Uttar Pradesh, India. It was the longest and most severe epidemic in 3 decades; 5,737 persons were affected in 7 districts of eastern Uttar Pradesh, and 1,344 persons died. An estimated 50,000 cases and 10,000 deaths annually cause of childhood viral encephalitis in the world; it causes 1,000 replicates with the MEGA version 2.1 program. The phylogenetic tree was constructed with the neighbor-joining method with bootstrap analysis of 310 sequencer (Applied Biosystems, Foster City, CA, USA) with the primer pairs JED3S: ATG CGC GGA TCC GAC AAA CTG GCC CTG AA (1839–1867) and JED3C: GGG GAA GCT TCG TGC TTC CAG CTT TGT CC (2193–2165) on the basis of the sequence in domain III of the E gene of strain JaOArS982.

Japanese encephalitis virus (JEV) is the most common etiologic agent was confirmed to be Japanese encephalitis virus by analyzing 326 acute-phase clinical specimens for virus-specific antibodies and viral RNA and by virus isolation. Phylogenetic analysis showed that these isolates belonged to genogroup 3.

Virus isolation was attempted in C6/36 cells (4) from RT-PCR– and IgM-positive serum and CSF samples according to standard protocol (5). Double-stranded sequencing of domain III of the E gene of JEV was performed on an ABI 310 sequencer (Applied Biosystems, Foster City, CA, USA) with the primer pairs JED3S: ATG CGC GGA TCC GAC AAA CTG GCC CTG AA (1839–1867) and JED3C: GGG GAA GCT TCG TGC TTC CAG CTT TGT CC (2193–2165) on the basis of the sequence in domain III of the E gene of strain JaOArS982.

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Analysis indicated an overall positivity of 50% of serum samples and 30% of CSF samples. The antibody profile of the serum samples showed 23% IgM, 19% IgG, and 7% both IgM and IgG positivity, compared with 26% IgM, 4% IgG, and 1% both IgM and IgG positivity in CSF samples. A total of 9% of CSF samples were positive for JEV-specific RNA (355-bp amplicon) as determined by RT-PCR. All these RT-PCR–positive CSF samples were also positive for IgM. None of the serum samples were positive by RT-PCR for viral RNA. Adding RT-PCR– and IgM-positive samples to C6/36 cells yielded 7 JEV isolates.
from IgM-positive CSF samples only, as confirmed by ELISA and RT-PCR. The antibody profile of the RT-PCR—and isolation-positive samples is depicted in Table 1.

Further analysis of a 355-nucleotide sequence in domain III of the E gene of these isolates showed >95% homology with JEV on BLAST search. On comparison with 24 other geographically diverse JEV isolates (Table 2), all JEV isolates sequenced in this study were closely related (≥99% homology). The isolates from this outbreak showed a nucleotide sequence identity of 95.6% and 94.6% with prototype JEV (Nakayama strain) and the first Indian JEV (isolated from Vellore in 1956), respectively. The dendrogram showed that the JEV isolates responsible for the 2005 Gorakhpur epidemic belong to genogroup 3 (G3) but form a cluster separate from earlier Indian isolates (Figure).

Conclusions

The Gorakhpur district of Uttar Pradesh, which shares a border with Nepal and Bihar, has been experiencing periodic outbreaks of JEV since 1978. The virus cannot usually be isolated from clinical specimens, even with the best laboratory facilities, probably because of low levels of viremia and the rapid development of neutralizing antibodies. The diagnosis is therefore usually based on the presence of antibodies. The IgM capture ELISA for serum and CSF has become the accepted standard for diagnosing

| Serial number | IgM | IgG | RT-PCR | Virus isolation |
|---------------|-----|-----|--------|-----------------|
| 1             | +   | –   | +      | –              |
| 2             | +   | –   | +      | –              |
| 3             | +   | –   | +      | –              |
| 4             | +   | –   | +      | –              |
| 5             | +   | –   | +      | –              |
| 6             | +   | –   | +      | –              |
| 7             | +   | –   | +      | –              |
| 8             | +   | –   | +      | –              |
| 9             | +   | –   | +      | –              |
| 10            | +   | –   | +      | –              |
| 11            | +   | –   | +      | –              |
| 12            | +   | –   | +      | –              |
| 13            | +   | –   | +      | –              |
| 14            | +   | –   | +      | –              |
| 15            | +   | –   | +      | –              |
| 16            | +   | –   | +      | –              |
| 17            | +   | –   | +      | –              |
| 18            | +   | –   | +      | –              |
| 19            | +   | –   | +      | –              |
| 20            | +   | –   | +      | –              |

*RT, reverse transcription; Ig, immunoglobulin.

Table 2. Japanese encephalitis viruses compared for sequence analysis*

| Sl no. | Virus ID no. | Year of sample collection | Country | Source | GenBank accession no. |
|--------|-------------|---------------------------|---------|--------|-----------------------|
| 1      | G6924       | 1956                      | India   | Mosquito | U70394                |
| 2      | 826309      | 1962a                     | India   | Human brain | U70403                |
| 3      | NA          | 1982b                     | India   | Human brain | U03689                |
| 4      | 733913      | 1973                      | India   | Human brain | Z34095                |
| 5      | GP78        | 1978a                     | India   | Human brain | AF075723               |
| 6      | 782219      | 1978b                     | India   | Human brain | U70402                |
| 7      | 7812474     | 1978c                     | India   | Human brain | U70387                |
| 8      | P20778      | 1958                      | India   | Human brain | Z34096                |
| 9      | NO          | 1995                      | Australia | Human serum | L43566                |
| 10     | SA14        | 1954                      | China   | Mosquito | U14163                |
| 11     | JKT7003     | 1981a                     | Indonesia | Mosquito | U70408                |
| 12     | JKT9092     | 1981b                     | Indonesia | Mosquito | U70409                |
| 13     | JKT5441     | 1981c                     | Indonesia | Mosquito | U70406                |
| 14     | Nakayama    | 1935                      | Japan   | Human brain | U03694                |
| 15     | JaOH0566    | 1966                      | Japan   | Human brain | AY029207              |
| 16     | JaNaR0500   | 1990                      | Japan   | Mosquito | AY427795              |
| 17     | 95-187      | 1995                      | Japan   | Pig blood | AY377577              |
| 18     | JaNaR0102   | 2002                      | Japan   | Mosquito | AY377577              |
| 19     | K91P55      | 1991                      | Korea   | Mosquito | U34928                |
| 20     | WTP-70-22   | 1970                      | Malaysia | Mosquito | U70421                |
| 21     | 691004      | 1969                      | Sri Lanka | Human brain | Z34097                |
| 22     | 86VN207     | 1986                      | Vietnam | Human brain | AY376461              |
| 23     | 89VN49      | 1989                      | Vietnam | Human brain | AY376462              |
| 24     | 02VN22      | 2002                      | Vietnam | Pig blood | AY376465              |
| 25     | GP14†       | 2005                      | India   | Human CSF | NS                    |
| 26     | GP48†       | 2005                      | India   | Human CSF | NS                    |
| 27     | GP55†       | 2005                      | India   | Human CSF | NS                    |
| 28     | GP67†       | 2005                      | India   | Human CSF | NS                    |
| 29     | GP82†       | 2005                      | India   | Human CSF | NS                    |

*NA, not available; CSF, cerebrospinal fluid; NS, not submitted.
†Sequenced in this study.
The presence of only IgG antibodies in 19% of the patients indicated exposure to JEV infection in the past. This finding was expected because JEV is endemic to northern India, particularly Gorakhpur, and several large JEV epidemics have occurred in the past decade. In the present study, only 13 CSF samples (9%) were positive by RT-PCR. Seven virus isolates were obtained from IgM-positive CSF samples that did not yield RT-PCR amplicons before cultivation. Similar variations in virus detection and isolation have been reported (10,11); these findings underscore the sensitivity of cell culture systems for amplification of viable virus. Furthermore, the inability to detect genomic RNA or isolate virus from serum samples was striking and highlights the need for CSF sampling for both clinical diagnosis and epidemiologic studies.

We also investigated the molecular epidemiology of the outbreak by comparative sequence analysis of the isolates obtained in this study with reference strains of JEV. Domain III of the E gene was targeted for this purpose because this is the region under immune selective pressure, and it exhibits sufficiently rapid mutation to show evolutionary and epidemiologic relationships (12–14). We determined the partial sequence of these isolates directly from clinical samples without risk of altering the genome by passage in vitro. The dendrogram showed that the G3 of JEV is still circulating in India. However, compared with isolates from 1956 to 1988, recent isolates form a separate cluster. Frequent introduction of new virus genotypes through bird migration has led to shifts in circulating genotypes in neighboring Asian countries, including Japan, Vietnam, China, Korea, Sri Lanka, and Malaysia (3,15). Therefore, detailed and continuous epidemiologic surveillance is warranted to monitor the incursion and spread of JEV genotypes in India, which will allow effective control and management strategies to be undertaken at the earliest opportunity.

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