Neurological Manifestations of Enterovirus 71 Infection in Children during an Outbreak of Hand, Foot, and Mouth Disease in Western Australia

Peter McMinn,1 Ivan Stratov,1 Lakshmi Nagarajan,2 and Stephen Davis3
Departments of 1Microbiology and 2Neurology, Princess Margaret Hospital for Children, Perth, and 3Magnetic Resonance Imaging Unit, Sir Charles Gairdner Hospital, Queen Elizabeth II Medical Centre, Nedlands, Australia

Enterovirus 71 (EV71) causes epidemics of hand, foot, and mouth disease associated with neurological complications in young children. We report an outbreak of EV71-associated neurological disease that occurred from February through September 1999 in Perth, Western Australia. Fourteen children with culture-proven, EV71-induced neurological disease were identified. Nine patients (64%) developed severe neurological disease; 4 of these patients developed long-term neurological sequelae. Neurological syndromes included aseptic meningitis, Guillain-Barre syndrome, acute transverse myelitis, acute cerebellar ataxia, opsomyoclonus syndrome, benign intracranial hypertension, and a febrile convulsion. Clinical and magnetic resonance imaging data indicated that immunopathology was a major factor in the pathogenesis of neurological disease in this outbreak. This finding is in contrast to reports of previous EV71 epidemics, in which virus-induced damage to gray matter was the most frequent cause of neurological disease.

Since the initial description of enterovirus 71 (EV71) in 1974 [1], outbreaks of infection with this virus have occurred periodically throughout the world [2–8]. EV71 infection manifests most frequently as the childhood exanthem known as “hand, foot, and mouth disease” (HFMD), and it is clinically indistinguishable from HFMD caused by Coxsackie virus A16 (CA16). A molecular study of the evolution of human enteroviruses has shown that EV71 and CA16 have a close genetic relationship, and, together with CA7 and CA14, they form a distinct genetic subgroup within cluster A of the Enterovirus genus of the family Picornaviridae [9]. Despite the close genetic relationship between EV71 and CA16, EV71 has a propensity to cause neurological disease during acute infection [2, 3], a feature not observed in CA16 infections. Children <4 years of age are particularly susceptible to the most severe forms of EV71-associated neurological disease, including meningitis, brain-stem and/or cerebellar encephalitis, and poliomyelitis-like paralysis. The neurological complications of EV71 infection may occasionally cause permanent paralysis or death. Several large epidemics of severe EV71 infection in young children, including numerous cases of fatal brain-stem encephalitis, have recently been reported in Southeast Asia [10–12].

In this report, we document the clinical, radiological, and laboratory features of neurological disease caused by EV71 in 14 children who presented to Princess Margaret Hospital for Children, in Perth, Western Australia, from February through September 1999, during a community-
wide outbreak of HFMD.

PATIENTS AND METHODS

Patients and data collection. From February through September 1999, 14 cases of EV71-associated neurological disease were identified at Princess Margaret Hospital for Children. Children included in this study presented with an acute neurological illness associated with EV71 isolation from stool, nasopharynx, skin vesicle, or CSF samples and/or seroconversion to EV71 in a serum neutralization assay.

Clinical definitions. The neurological diagnosis of EV71-associated neurological disease in each patient in this study was made by a pediatric neurologist and was aided in several cases by MRI evidence. Aseptic meningitis was defined as an illness clinically compatible with CSF pleocytosis (>5 leukocytes/mL) plus negative results of bacterial cultures. Guillain-Barré syndrome (GBS) was defined as rapid onset of polyneuropathy with characteristic evolution of motor weakness and evidence of delayed nerve conduction. Acute transverse myelitis was defined as an illness with an acute onset and with clinical features indicative of nontraumatic disease of the gray and white matter of the spinal cord over a long vertical extent. Acute cerebellar ataxia was defined as acute-onset truncal ataxia, with or without other cerebellar signs, after the exclusion of other conditions that might have produced a sudden onset of ataxia. Opsomyoclonus syndrome is a distinctive clinical entity characterized by bursts of irregular and rapid eye movements (opsoclonus), myoclonus, and ataxia. Benign intracranial hypertension was defined as a syndrome of raised intracranial pressure in the absence of a space-occupying lesion or obstruction of CSF circulation.

MRI. MRI was performed for 6 children within 12 days of the onset of illness, by use of a 1.5-T Siemens Vision scanner (Siemens). Follow-up MRI was undertaken for 3 children within 3–8 months after the initial MRI study was done. Brain imaging sequences included axial dual T1-weighted turbo spin-echo images, sagittal T1-weighted spin-echo sequences, coronal fluid-attenuated inversion recovery (FLAIR) sequences, and diffusion-weighted images. Postgadolinium images were obtained for patients who had brain parenchymal abnormalities. Spinal imaging included sagittal T1 and turbo spin-echo T2 sequences, axial gradient-echo T2 sequences, and sagittal and axial postgadolinium T1 images when spinal cord parenchymal lesions were demonstrated.

Enterovirus isolation and identification. Isolation of virus from clinical samples was attempted by use of human embryonic lung fibroblast (MRC-5) cells (ATCC CCL 171) and human rhabdomyosarcoma (RD) cells (ATCC CCL 136). Virus isolates were identified by microneutralization in RD cells by the use of 10 antibody units of rabbit EV71-specific polyclonal antiserum 385JS (gift of Margery Kennett, National Poliovirus Reference Laboratory [NPRL], Melbourne) against 100 × TCID[50] of virus.

Serology. Assay of EV71-specific antibody in serum samples was undertaken by means of microneutralization in RD cells with the use of acute-phase and convalescent-phase serum samples in 2-fold dilutions (commencing at a dilution of 1:20) against 100 × TCID[50] of the EV71 isolate 4F/4/99 or against the child’s own virus isolate. The isolate 4F/4/99 was used as a reference strain for this outbreak because it had been typed independently by the NPRL. Cultures were examined at 3 and 5 days postinoculation, and the endpoint was the highest dilution of a serum sample that completely inhibited cytopathic effect, in comparison with control wells that contained no serum samples.

RESULTS

The Outbreak

During the period from February through September 1999, 14 cases of EV71-associated neurological disease were identified by use of virus isolation and serological testing (tables 1 and 2). The neurological manifestations of EV71 infection included aseptic meningitis, GBS, acute transverse myelitis, acute cerebellar ataxia, opso-myoclonus syndrome, benign intracranial hypertension, and a febrile convulsion. Twelve (86%) of the 14 children were <4 years of age at the time of onset of EV71 infection.

Clinical Manifestations of Neurological Cases: Prodromal Illness and Exanthem

Twelve (86%) of the 14 patients with EV71-associated neurological disease had a prodromal illness for 1–7 days prior to the onset of neurological manifestations. Clinical features included fever, coryza, malaise, headache, and diarrhea. Nine (64%) of the children with neurological disease developed a rash during their illness (table 1). One child had herpangina at the time of presentation.

Clinical Manifestations of Neurological Cases: Neurological Syndromes

Aseptic meningitis. Five children with aseptic meningitis were identified (table 1). At presentation, 4 of the 5 children were noted to have a truncal rash, which was petechial in 3 cases. Patient 11 presented initially with aseptic meningitis, which was complicated by the development of severe pain in the region of the left shoulder and by the patient’s refusal to use her left arm. Clinical examination revealed flaccid paralysis
Table 1. Clinical and virological features of 14 children with enterovirus 71 (EV71)–associated neurological disease.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age</th>
<th>Clinical presentation</th>
<th>Rash</th>
<th>Protein, g/dL</th>
<th>WBC count, cells/μL</th>
<th>Site of EV71 isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>12 y</td>
<td>Guillain-Barré syndrome</td>
<td>MP 4 w previously</td>
<td>2.26</td>
<td>1</td>
<td>Feces</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>9 mo</td>
<td>Acute transverse myelitis</td>
<td>None</td>
<td>1.41</td>
<td>0</td>
<td>Feces</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>1 y and 6 mo</td>
<td>Opso-myoclonus syndrome</td>
<td>HFMD, 3 w previously</td>
<td>0.13</td>
<td>1</td>
<td>Feces, throat</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>1 y and 2 mo</td>
<td>Guillain-Barré syndrome</td>
<td>MP, on trunk</td>
<td>2.18</td>
<td>4</td>
<td>Feces</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>2 mo</td>
<td>Meningitis</td>
<td>Petechial, on trunk</td>
<td>1.09</td>
<td>94</td>
<td>CSF, throat, feces</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>1 y and 7 mo</td>
<td>Acute transverse myelitis</td>
<td>None</td>
<td>2.16</td>
<td>1</td>
<td>CSF, throat</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>1 y and 6 mo</td>
<td>Meningitis</td>
<td>Petechial, on trunk</td>
<td>0.37</td>
<td>94</td>
<td>Throat</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>1 mo</td>
<td>Meningitis</td>
<td>Petechial, on trunk</td>
<td>ND</td>
<td>550</td>
<td>CSF, feces</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>2 y and 9 mo</td>
<td>Acute cerebellar ataxia</td>
<td>None</td>
<td>0.5</td>
<td>18</td>
<td>CSF, feces</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>3 y and 4 mo</td>
<td>Acute cerebellar ataxia</td>
<td>HFMD</td>
<td>ND</td>
<td>7</td>
<td>Feces</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>3 y and 4 mo</td>
<td>Meningitis, monoplegia</td>
<td>None</td>
<td>0.4</td>
<td>900</td>
<td>Feces, throat</td>
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<tr>
<td>12</td>
<td>F</td>
<td>2 mo</td>
<td>Meningitis</td>
<td>MP, on trunk</td>
<td>1.23</td>
<td>63</td>
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<tr>
<td>13</td>
<td>F</td>
<td>3 y and 3 mo</td>
<td>Febrile convulsion</td>
<td>MP, herpangina</td>
<td>ND</td>
<td>ND</td>
<td>Feces</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>6 y and 1 mo</td>
<td>Benign intracranial hypertension</td>
<td>None</td>
<td>0.45</td>
<td>20</td>
<td>Throat</td>
</tr>
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</table>

**NOTE.** HFMD, hand, foot, and mouth disease; MP, maculopapular; ND, not determined.

*a Normal protein, 0.15–0.45 g/dL.

of the left arm in the C5–C6 distribution. All 5 children recovered fully within 2 weeks of admission to the hospital.

**Guillain-Barré syndrome.** Patient 1 presented 2 weeks after the onset of progressive muscular weakness, which was more marked distally than proximally and was associated with areflexia. Findings of nerve conduction studies were consistent with a demyelinating polyneuropathy. The patient still has residual weakness in both feet 15 months after the onset of illness. Patient 4 presented with rapid development of symmetrical muscle weakness and a markedly elevated CSF protein level; she was also considered to have developed GBS, although the diagnosis was not formally established by nerve conduction studies. The patient gradually regained shoulder and upper-limb strength; she was discharged from the hospital 6 weeks after the onset of illness. Eight months later, she fully regained neurological function.

**Acute transverse myelitis.** Patient 2 presented with fever (temperature, 38.7°C) and rapid-onset flaccid paralysis (over 24 h) that involved all limbs. An MRI study undertaken 3 days after the onset of illness showed diffuse flame-shaped signal abnormality centered in the dorsal columns of the cervical cord (figure 1A) that was associated with mild cord expansion from C3 to T2 (figure 1B). The abnormalities that appeared on MRI were consistent with acute transverse myelitis. The patient had regained some muscle function by 1 month, and at 1 year he still had asymmetric spastic quadriparesis. Follow-up MRI done at 8 months showed minimal residual gliosis of the cervical spinal cord, without cavitation or atrophy (data not shown).

Patient 6 presented with fever (temperature, 38°C) and symmetrical flaccid paralysis that involved all limbs and respiratory muscles and developed within 30–60 min. An MRI study undertaken 3 days after the onset of illness showed a diffuse lesion from the lower medulla throughout the spinal cord to the level

Table 2. Neutralizing antibody titers of 10 children with EV71–associated neurological disease.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Virus used for neutralization</th>
<th>Acute-phase sample</th>
<th>Convalescent-phase sample</th>
<th>Interval between samples, w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4F/4/99b</td>
<td>&lt;20</td>
<td>160</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>4F/4/99b</td>
<td>80</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>2M/3/99 (feces)d</td>
<td>160</td>
<td>40</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>4F/4/99b</td>
<td>&lt;20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
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<td>4F/4/99b</td>
<td>40</td>
<td>320</td>
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<td>4F/4/99b</td>
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<td>7</td>
<td>4F/4/99b</td>
<td>20</td>
<td>160</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>11F/6/99 (feces)d</td>
<td>&lt;20</td>
<td>80</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>4F/4/99b</td>
<td>20</td>
<td>&lt;20</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>4F/4/99b</td>
<td>&lt;20</td>
<td>20</td>
<td>20</td>
</tr>
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<td>11</td>
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<td>&lt;20</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>12</td>
<td>4F/4/99b</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>5</td>
</tr>
<tr>
<td>13</td>
<td>12F/7/99 (feces)d</td>
<td>&lt;20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>4F/4/99b</td>
<td>2560</td>
<td>10,240</td>
<td>5</td>
</tr>
</tbody>
</table>

*a 100 × TCID[50] of virus was used in the neutralization assay.
b 4F/4/99 is a fecal isolate of EV71 (from patient 4). It was typed independently at the National Polio Reference Laboratory (Melbourne) and was used as an epidemic reference strain.
c Titers are expressed as reciprocals.
d The child’s own EV71 strain and site of isolation.
of T9 (figure 2A), with swelling of the cervical cord and poorly defined peripheral enhancement at the margin of the lesion. Follow-up MRI at 3 months showed atrophy (myelomalacia) from the medulla to T5 (figure 2B), that was consistent with severe acute transverse myelitis with lower brain-stem involvement. The patient remains quadriplegic and ventilator dependent as of 1 year after admission to the hospital.

**Acute cerebellar ataxia.** Patient 9 presented with truncal ataxia, bilateral cerebellar signs, and loss of speech; she had evidence of intact comprehension. An MRI study done 7 days after the onset of illness showed right-side cerebellar swelling and cortical abnormalities, with increased signal on T2-weighted imaging (figure 3A) and low-grade cytotoxic edema on diffusion-weighted imaging (figure 3B). The mutism and ataxia diminished within a few days but were slow to resolve completely (6–8 weeks). Follow-up MRI done at 3 months showed resolution of the cerebellar swelling and cytotoxic edema, with persistent low-grade signal changes in the cerebellar cortex consistent with postinflammatory gliosis (data not shown).

Patient 10 also presented with truncal ataxia, slurred speech, and bilateral intention tremor, but he recovered fully within 3 days. The illness was accompanied by HFMD. An MRI study undertaken 12 days after admission was suggestive of mild, diffuse cerebellar swelling with effacement of the fourth ventricle (data not shown).

**Opso-myoclonus syndrome.** Patient 3 presented with opso-myoclonus syndrome 3 weeks after the development of HFMD. Although the patient's neurological illness responded to corticosteroids, she remained steroid dependent 1 year after the onset of illness.

**Other neurological syndromes.** Patient 13 presented after she had a single generalized seizure; she had fever (temperature, 40.7°C) and herpangina. The patient recovered fully, and no neurological sequelae were identified during follow-up.

Patient 14 presented with fever (temperature, 38.6°C), bilateral abducens nerve palsies, and blurred optic disks. She was unable to walk without assistance. A CT scan revealed generalized edema of the cerebrum (data not shown). Diplopia resolved after 2 days, but the optic disk margins remained blurred for 4 subsequent days. The patient's gait remained mildly unsteady for a total of 8 days, and she was discharged 11 days after the onset of illness.

**Laboratory Features**

**Virus isolation and identification.** Twenty-four enterovirus isolates were recovered from 16 children who presented...
Figure 2. MRI of a 19-month-old girl (patient 6) with enterovirus 71–associated neurological disease. A, Midsagittal turbo spin-echo T₂-weighted MRI scan done 3 days after the onset of acute transverse myelitis, showing a high-signal lesion from the midmedulla (arrow) to the spinal cord (arrowheads) at the level of T9 (out of view), with diffuse expansion of the cord. B, Midsagittal turbo spin-echo T₂-weighted MRI scan done 3 months after the onset of acute transverse myelitis, showing atrophy of the medulla and spinal cord to T5 (myelomalacia).

to Princess Margaret Hospital for Children with acute neurological illnesses during the HFMD outbreak. Twenty-two enterovirus isolates (obtained from 14 of these children) were identified as EV71 by means of neutralization assay (table 1). In addition, echovirus type 4 was isolated from the stool sample of a 12-year-old girl who had acute cerebellar ataxia, and echovirus type 9 was isolated from the stool of a 10-year-old boy who had aseptic meningitis.

Serology. Acute-phase and convalescent-phase serum samples from 10 cases of EV71-associated neurological disease were examined for EV71-specific antibody by means of serum neutralization assay (table 2). Serological analysis with the epidemic prototype strain 4F/4/99 revealed the presence of EV71-specific neutralizing antibodies in 50% of the acute-phase serum samples (titers, 1:20–1:2560) and 70% of convalescent-phase serum samples (titers, 1:20–1:10,240).

The failure to detect antibody to 4F/4/99 in the serum samples from patients 3, 11, and 12 is of particular interest, because these children all had culture-proven EV71 infection and neurological disease. In these instances, serum neutralization tests were repeated with the use of each child’s own EV71 isolate. Two children (patients 3 and 11) produced antibody to their own virus strains. However, patient 12 produced no neutralizing antibody, either to the epidemic reference strain or to her own virus.

DISCUSSION

EV71 is an endemic enterovirus with a worldwide distribution [3, 13] and a propensity to cause epidemics with a high frequency of HFMD and/or neurological disease [2–4, 6]. There is significant variation in the spectrum of neurological disease associated with EV71 epidemics. Certain outbreaks have been associated with a predominance of aseptic meningitis (97%) [8], a high frequency of poliomyelitis-like paralysis (21%) [4, 5, 13], and, more recently, fatal brain-stem encephalitis [12,
Enterovirus 71 in Western Australia

Figure 3. MRI of a 3-year-old girl (patient 9) with enterovirus 71-associated neurological disease. A, Coronal fluid–attenuated inversion recovery MRI scan of the cerebellum done 7 days after the onset of acute cerebellar ataxia and mutism, showing a diffuse high-signal lesion confined to the cortical gray matter of the right cerebellar hemisphere. The white matter and supratentorial brain are spared. B, Axial diffusion-weighted image of the cerebellum performed during the same examination as in A, showing cytotoxic edema confined to the cortical gray matter.

Although the clinical presentation of acute neurological disease in the Perth outbreak was similar to that observed in previous epidemics [2–4, 6, 8], immunopathological mechanisms, rather than primary gray matter disease, appeared to be responsible for many cases of neurological disease in our cohort.

The tropism of EV71 for spinal cord and brain-stem gray matter is well established [5, 12, 16]. In our study, patient 9 developed acute cerebellar ataxia and mutism and had changes shown on MRI that were consistent with primary gray matter disease, including the presence of cytotoxic edema in the right cerebellar hemisphere on diffusion-weighted imaging. Cerebellar mutism is a rare, transient syndrome of unknown pathogenesis, most frequently associated with the trauma caused by surgical removal of midline cerebellar tumors [17]. To our knowledge, this is the first case of cerebellar mutism associated with enterovirus infection.

In contrast to gray matter disease, the role of immunopathological mechanisms in the pathogenesis of EV71-associated neurological disease has been poorly documented. Patients 1 and 4 presented with acute onset of symmetrical flaccid paralysis and elevated CSF protein levels and were considered to have developed GBS after acute EV71 infection. In patient 1, the diagnosis of GBS was established by the demonstration of delayed signal conduction in peripheral nerves. GBS has been attributed to EV71 in the past [2, 3], although its diagnosis in these patients was based on the demonstration of symmetrical paralysis and elevated levels of CSF protein alone—features which are also present in some cases of acute paralytic poliomyelitis [18].

Patient 3 developed opso-myoclonus syndrome 3 weeks after the onset of HFMD. Opso-myoclonus syndrome is thought to be an autoimmune disease resulting from a lesion in the dentate nucleus of the cerebellum. Although it previously has been linked to enterovirus infection [19, 20], to our knowledge it has not been associated with EV71 infection. The evidence for an autoimmune etiology of opso-myoclonus syndrome in patient 3 included (1) the late onset of opso-myoclonus syndrome in relation to HFMD, (2) the high titer of EV71 antibodies at the onset of neurological disease, and (3) the responsiveness of the condition to corticosteroids.

The clinical presentation of patient 6 was that of acute transverse myelitis. However, the MRI data were consistent with acute necrotizing myelitis, a severe disease of the spinal cord characterized by necrosis of gray and white matter, most marked in areas of perivascular lymphocytic infiltration [21, 22]. It has been suggested that cytokine-mediated small-vessel vasculitis is the primary mechanism in the pathogenesis of this disease [21, 22]. This child developed extensive brain-stem and spinal cord necrosis that left her quadriplegic and ventilator-dependent. It is of interest that EV71 was isolated from the CSF at the time of the acute illness; this suggests that the virus
played a direct role in the pathogenesis of her disease. Patient 2 appeared to develop a milder form of necrotizing myelitis than did patient 6.

Cerebritis due to EV71 has not been demonstrated at autopsy [12, 16]. Seizures that develop during acute enterovirus infections are thought to be mainly febrile convulsions [23]. One child in our study presented with a generalized seizure associated with fever (temperature, 40°C) and herpangina. She was considered to have had febrile convulsion during acute EV71 infection and to have made a complete recovery. Unfortunately, the diagnosis was not supported by performance of lumbar puncture, neuroimaging, or electroencephalographic studies. Patient 14 developed benign intracranial hypertension during acute EV71 infection. Benign intracranial hypertension has not previously been associated with acute EV71 infection, although it has been described in association with acute Coxsackie virus B4 infection [24].

There is currently no effective antiviral therapy available for EV71-associated neurological disease. Intravenous immunoglobulin (IVIG) was administered to 4 children in our study without appreciable benefit, despite the presence of (low-titer) neutralizing antibody to EV71 in this preparation (P. McMinn and I. Stratov, unpublished observation). A report detailing the treatment of 34 patients with culture-proven EV71 infection of the CNS [14] showed that IVIG is of little benefit in the management of this disease. In addition, the new antipicornavirus agent pleconaril (ViroPharma), shown to have strong inhibitory activity against most enterovirus strains in cell culture, has not, to date, been proven to be effective against EV71 strains examined at concentrations testable in vitro (D. Pevear, ViroPharma, personal communication).

In view of the lack of effective treatment for EV71-associated neurological disease and a disturbing increase in the magnitude and severity of EV71 epidemics in the Asia-Pacific region in recent years, a major research effort should be focused on the development of a vaccine against EV71 infection.

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References