Enterovirus 71 in Taiwan

Luan-Yin Chang*

Department of Pediatrics, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

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population surveillance;
Taiwan;
transmission;
vaccine

1. Introduction

Enteroviruses belong to the Picornaviridae, a family of single-stranded positive-sense RNA viruses. Serologic studies using neutralization tests with antisera against enteroviruses have identified more than 67 human enterovirus serotypes: poliovirus serotypes 1–3, 23 Coxsackie A virus serotypes, 6 Coxsackie B virus serotypes, 31 echovirus serotypes, and the numbered enterovirus serotypes (enteroviruses 68–71). Clinically, polioviruses and enterovirus 71 (EV71) are among the most important and severe enteroviruses and are responsible for many deaths and severe sequelae. Since the eradication of poliovirus by vaccination in Taiwan in 2000, EV71 has been considered one of the most important enteroviruses. Continuous surveillance of its occurrence, investigation into its virulence and transmission, and the development of treatment strategies, including antiviral therapies and vaccines, are all required in order to improve the future control and outcomes of EV71 infection.

2. EV71 in Taiwan Before the 1998 Epidemic

EV71 was first isolated in California, USA, in 1969.\(^1\) Since then, four epidemic outbreaks with high mortality have occurred: in Bulgaria in 1975 (44 deaths),\(^2\) in Hungary in 1978 (47 deaths),\(^3\) in Malaysia in 1997 (at least 31 deaths),\(^4\) and in Taiwan in 1998 (78 deaths).\(^5\)

The EV71 outbreak in Taiwan in 1998 was well publicized, but EV71 existed in Taiwan before 1998. Our seroepidemiological study conducted prior to...
the 1998 outbreak showed pre-epidemic (1997) EV71 seroprevalence rates to be about 60–70% in adults and children older than 6 years of age.\textsuperscript{6} Lu et al examined serial serum antibody titer levels to EV71 in 81 children born in 1988 who had yearly blood samples available for 1989 to 1994, and for 1997 and 1999.\textsuperscript{7} They discovered that there was a yearly incidence of EV71 seroconversion of 3–11% between 1989 and 1997, and that 68% of those children had serological evidence of EV71 infection by 1997.\textsuperscript{7}

EV71 was also isolated from patients in Taiwan with hand, foot, and mouth disease (HFMD) and poliomyelitis-like paralysis, as early as 1980 and 1986.\textsuperscript{5,8} We performed a retrospective case review of all discharge notes from the Department of Pediatrics, National Taiwan University Hospital from 1980 and 1981, and identified 16 cases of HFMD associated with central nervous system (CNS) involvement: nine had polio-like syndrome, four had encephalitis or encephalomyelitis, one had cerebellitis, and two had aseptic meningitis.\textsuperscript{9} Two patients with HFMD plus encephalitis died within 1 day of hospitalization, and one of them also had acute cardiopulmonary failure mimicking myocarditis,\textsuperscript{9} a clinical picture similar to that seen in the 1998 EV71 epidemic. Twenty years later, at least one male patient was still suffering from the sequelae of polio-like syndrome and was therefore exempted from military service. The clinical severity of these cases in 1980–1981 was comparable to those seen in the 1998 EV71 epidemic.

These findings indicate that EV71 had been circulating in Taiwan for at least 17 years before 1998, though its clinical significance had not been investigated. It is likely that severe cases of EV71 had occurred previously, but were not recognized. The pre-epidemic EV71 seroprevalence rates were inversely correlated with mortality and morbidity.\textsuperscript{6} Among the cohort of 81 children, Lu et al found that the annual EV71 seroconversion rates (3–4%) between 1994 and 1997 were significantly lower than the rates (7–11%) before 1994.\textsuperscript{7} It is likely that there was a lower incidence of EV71 infection between 1994 and 1997, and that the accumulation of susceptible hosts that exceeded the threshold density might have triggered the 1998 outbreak. However, changes in EV71 neurovirulence and host genetic factors are also thought to have affected clinical outcomes.

3. Surveillance Systems for Severe Enterovirus Cases in Taiwan

A physician-based sentinel surveillance system for infectious diseases was established in Taiwan in 1989 and has been operated by the Ministry of Health since then.\textsuperscript{10} This system originally involved 850 physicians, 8.7% of the primary care physicians in Taiwan. HFMD and herpangina were included in the system, following an outbreak of EV71-related HFMD in Malaysia in 1997. Because of a rapid increase in the numbers of severe and fatal cases of HFMD, a hospital-based reporting system for monitoring such cases was initiated in May 1998. From June 1998, both physician-based and hospital-based surveillance systems have been maintained simultaneously.\textsuperscript{10} The total number of HFMD/herpangina cases reported by the physician-based sentinel surveillance system was 129,106; the number of severe cases peaked in early June, around the same time as the peak in HFMD cases.\textsuperscript{3} There were 405 severe cases and 78 deaths during the 1998 epidemic.\textsuperscript{3} From 1998 to 2005, a seasonal variation in the number of severe enterovirus cases was observed, with the annual peak occurring in the second quarter. Deaths due to severe enterovirus infections varied from year to year, with most (92%) cases occurring in children younger than 4 years of age.\textsuperscript{11} Children infected with EV71 had higher risks of pulmonary edema/hemorrhage and encephalitis than those infected with other enteroviruses.\textsuperscript{11}

The Taiwan Centers for Disease Control (CDC) also established virological surveillance systems for enterovirus and influenza, with 10 viral contract laboratories for surveillance located in different areas of Taiwan in 2000, and 13 in 2007.\textsuperscript{12} Table 1 shows the numbers of severe and fatal enterovirus cases in Taiwan from 1998 to 2006, and the number of EV71 cases among the fatal cases. Table 2 shows the five most common enterovirus serotypes in Taiwan from 1998 to 2006.\textsuperscript{13} EV71 was the most common circulating serotype in 1998, 2000 and 2001 (Table 2),\textsuperscript{13} and more severe and fatal enteroviral cases occurred in these years than in the other years. We therefore predict that more severe and fatal enterovirus cases will occur when the major circulating serotype is EV71. The surveillance systems, both clinical and laboratory-based, will continue to serve public health workers and enable the earlier implementation of control and prevention measures to contain or limit the spread of EV71, reducing the numbers of severe and fatal enterovirus cases.

4. Routes and Rates of EV71 Transmission

Humans are the only known natural hosts of enteroviruses. Enteroviruses can infect humans through gastrointestinal cells, and through the respiratory tract. Enteroviruses are usually transmitted through fecal-oral contact, and sometimes through droplet/aerosol transmission. Furthermore, enteroviruses can
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live for over 24 hours in stainless steel containers, and such contaminated objects may be a means of viral transmission. These viruses can live in low pH conditions (pH 3), and are resistant to 70% ethanol and ether. They can remain active at room temperature for several days.

We found that the isolation rate of EV71 from throat swabs (93%) was significantly higher than that from rectal swabs or feces (30%) in EV71-infected cases with acute illnesses, suggesting that the oral-oral route or droplet transmission might be more important routes of transmission than the fecal-oral route during acute EV71 illness.

How high is the rate of enterovirus transmission? The secondary household transmission rates of enteroviruses vary, as shown for poliovirus, enterovirus 70 and Coxsackievirus A24. EV71 seropositivity was found to be concordant among siblings in our previous seroepidemiological study, suggesting that household transmission plays an important role in the spread of EV71. We therefore performed a prospective family cohort study of EV71 household transmission. We studied 433 family members from 94 families in which EV71 had been isolated from at least one family member. The overall EV71 transmission rate between household contacts was 52% (176/339): 84% (70/83) siblings, 83% (19/23) cousins, 41% (72/175) parents, 28% (10/36) grandparents and 26% (5/19) uncles/aunts. EV71 household transmission rates were high for children, medium for parents and low for other adults. Being male and less than 6 years old were factors associated with increased risks of EV71 infection. The EV71 isolation rate from child household contacts was 39% (41/106), which was significantly higher than the 4.3% (10/233) EV71 isolation rate from adult household contacts (p<0.001). The isolation rates from adult household contacts were possibly lower due to the presence of protective antibodies from previous exposures, or due to lower concentration of the virus in secretions. These figures suggest that infected children are more able to transmit the virus than are infected adults.

Why is the household transmission rate for children so high? Long periods of viral shedding may account for widespread transmission of enteroviral diseases, as is the case for polio and Coxsackievirus infections. In a previous study, we found that EV71 was present in the stools of infected patients for up to 5 weeks. Close household contact, higher viral load, and long viral shedding periods may therefore account for the high household transmission rate among children.

Most secondary cases in the family develop disease 3-4 days after the index cases. Case-to-case intervals are shown in Figure 1. The incubation period is usually 3-4 days, but can be as long as 10 days or more. Some household members may develop the disease on the same day, or 1 day

### Table 1: Number of cases of severe and fatal enterovirus infections in Taiwan from 1998 to 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of severe cases</th>
<th>Number of fatal cases (severe case-fatality rate)</th>
<th>EV71 number (%) among the fatal cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>405</td>
<td>78 (19%)</td>
<td>34 (44%)</td>
</tr>
<tr>
<td>1999</td>
<td>35</td>
<td>9 (26%)</td>
<td>1 (11%)</td>
</tr>
<tr>
<td>2000</td>
<td>291</td>
<td>41 (14%)</td>
<td>25 (61%)</td>
</tr>
<tr>
<td>2001</td>
<td>393</td>
<td>58 (15%)</td>
<td>27 (47%)</td>
</tr>
<tr>
<td>2002</td>
<td>162</td>
<td>30 (19%)</td>
<td>8 (27%)</td>
</tr>
<tr>
<td>2003</td>
<td>70</td>
<td>8 (11%)</td>
<td>4 (50%)</td>
</tr>
<tr>
<td>2004</td>
<td>50</td>
<td>5 (10%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>2005</td>
<td>142</td>
<td>16 (11%)</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>2006</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1559</td>
<td>245 (15.7%)</td>
<td>111 (45.3%)</td>
</tr>
</tbody>
</table>

Data from CDC, Taiwan.

### Table 2: Five most common enterovirus serotypes in Taiwan from 1998 to 2006

<table>
<thead>
<tr>
<th>Year</th>
<th>Rank</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>EV71</td>
<td>CA2</td>
<td>CA16</td>
<td>CA4</td>
<td>CA6</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>CB1</td>
<td>Echo4/30</td>
<td>CB3</td>
<td>CA16</td>
<td>CA10</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>EV71</td>
<td>CA16</td>
<td>Echo9</td>
<td>CB3</td>
<td>CA6</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>EV71</td>
<td>Echo4/30</td>
<td>Echo6</td>
<td>CB4</td>
<td>CA24</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>CA16</td>
<td>Echo6</td>
<td>CB5</td>
<td>EV71</td>
<td>CA24</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>CA16</td>
<td>EV71</td>
<td>Echo9</td>
<td>Echo11</td>
<td>CA6</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>CA4</td>
<td>CB4</td>
<td>CA10</td>
<td>EV71</td>
<td>CB3</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>CB3</td>
<td>CA16</td>
<td>EV71</td>
<td>CA6</td>
<td>CA5</td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>CA4</td>
<td>CA2</td>
<td>Echo18</td>
<td>CA5</td>
<td>CB2</td>
<td></td>
</tr>
</tbody>
</table>

Data from CDC, Taiwan, and modified from Reference 13. CB = Coxsackievirus B; CA = Coxsackievirus A; Echo = echovirus; Echo4/30 = indistinguishable between echovirus 4 and echovirus 30.
5. Clinical Spectra and Stages of EV71 Infection

The EV71 household study showed that only 6% of infected children, compared with 53% of infected adults, were asymptomatic. Interestingly, in the seroepidemiologic study, only 29% of the preschool children infected with EV71 developed HFMD/herpangina, suggesting that about 70% of children with community-acquired infections might be asymptomatic.

According to our clinical studies, symptomatic EV71 infection can progress through four stages: HFMD/herpangina (Stage 1), CNS involvement (Stage 2), cardiopulmonary failure (Stage 3), and convalescence (Stage 4). Most EV71 cases in these studies remained at Stage 1, though some progressed to Stage 2, and a few advanced to the most severe condition—Stage 3.

5.1. Stage 1: Uncomplicated EV71 illness

Uncomplicated EV71 illnesses include HFMD (about 80%), herpangina (about 10%), pharyngitis, nonspecific febrile illness (about 10%), generalized viral exanthema, and enteritis. The patients may have suffered from fever for 1–3 days, with temperatures sometimes reaching >39°C. HFMD patients develop oral ulcers on the tongue and buccal mucosa, and a vesicular rash or small erythematous maculopapular rash on their hands, feet, knees, and/or buttocks. The EV71-induced vesicular or maculopapular rash over the extremities is sometimes so inconspicuous that it can be overlooked by parents and even by doctors. Herpangina includes oral ulceration on anterior tonsillar pillars, the soft palate, buccal mucosa and/or the uvula. Oral ulceration causes pain while eating or drinking, and patients may need intravenous fluid supplementation if dehydration occurs. About 10% of the EV71 cases have febrile illness or pharyngitis without the usual HFMD/herpangina.

5.2. Stage 2: Complicated EV71 illness with CNS involvement

EV71 patients may develop complications 1–5 days after the onset of the illness. After the initial HFMD, herpangina or febrile illness and an intermittent fever that usually lasts 3–7 days, some patients may experience CNS involvement including meningitis, encephalitis, polio-like syndrome, or encephalomyelitis with or without pulmonary edema. Even when the clinical manifestations, cerebrospinal fluid (CSF) pleocytosis and image studies all suggest CNS involvement, EV71 is seldom isolated from the CSF. Huang et al. described three EV71-related neurologic syndromes in the Taiwan outbreak: aseptic meningitis, acute flaccid paralysis, and rhombencephalitis (brain stem encephalitis). EV71 cases with aseptic meningitis usually suffer from myoclonic jerks during sleep, vomiting, headache and/or irritable crying. They have mild, if any, neck stiffness and they usually recover within 3–7 days after hospitalization. The most common initial symptoms of EV71 encephalitis are myoclonic jerks during sleep, and these are followed by other symptoms or signs of encephalitis. Patients may also show other signs of changes in consciousness such as lethargy,
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sleepiness or coma, seizure attacks, ataxia, and cranial nerve palsies, such as abducens palsy, facial palsy, dysphagia, upward gaze and nystagmus. Subtle symptoms of increased sympathetic tone, such as insomnia, profuse sweating, paralytic ileus, neurogenic bladder, panic or increased startle reflex have also been recorded. If cases of encephalitis do not advance to Stage 3, they usually recover without sequelae 5–10 days later. EV71 cases with poliomyelitis-like syndrome have asymmetric acute limb weakness and decreased reflexes, with no disturbance of limb sensation. Children suddenly become unable to walk or raise their arms, or they easily fall 3–7 days after developing HFMD or herpangina. About half of the EV71 polio-like cases experience long-term sequelae, including limb weakness and atrophy. Patients with encephalomyelitis exhibit symptoms of both encephalitis and poliomyelitis-like syndrome.

Of the imaging methods used to investigate CNS involvement, brain or spinal computed tomography (CT) usually produce negative findings in cases of EV71 CNS infection, and CT is therefore not the imaging method of choice. Magnetic resonance imaging (MRI) is more suitable, and MRI studies usually show hyperintensity in the CNS lesions on T2-weighted images. The major CNS lesions in brainstem encephalitis and cerebellitis occur in the medulla oblongata,pons, midbrain, and the dentate nuclei of the cerebellum. In polio-like syndrome, lesions have been found in the anterior horn of the spinal cord (Figure 2). Some patients may present with normal MRI results, while patients with encephalomyelitis may have lesions of both the brainstem and the spinal cord (Figure 3). In follow-up MRI examinations of patients with sequelae, lesions have been shown to persist for 1–3 years after initial acute illness.

Figure 2 MRI of an EV71 polio-like case. Hyperintense lesions (arrows) are seen in the anterior horn of the spinal cord on T2-weighted image.

Figure 3 MRI of a case of EV71 encephalomyelitis plus pulmonary edema. (A) Sagittal view of T2-weighted image show hyperintense lesions in the posterior portion of the pons and the medulla and also in the anterior portion of the C-spinal cord. (B) Coronal view show hyperintense lesions in the posterior portion of the pons-medulla junction.

5.3. Stage 3: Cardiopulmonary failure or pulmonary edema

Several hours to 2 days after the onset of CNS involvement, some patients may advance to Stage 3A, when they develop sudden signs of tachycardia (135–250 beats per minute), tachypnea, and cyanosis. Patients are usually alert, except for mild lethargy, and have sometimes been found to have transient hypertension, lasting for several hours to 2 days. Laboratory findings include hyperglycemia and leukocytosis. Chest X-ray films show alveolar
density and no cardiomegaly, and in most cases examined during the 1998 epidemic, became completely white within the lungs within 12 hours. Electrocardiographic examination shows sinus tachycardia and no arrhythmia. Ejection fractions on cardiac echography range from about 40%–80%. Once intubated, children may produce a white frothy secretion, followed by a pink frothy fluid, and sometimes fresh blood, from the endotracheal tube. Patients frequently suffer from persistent fever and profuse sweating during critical points of this stage.

Progressive hypotension or shock, oliguria or anuria, tachycardia and decreased levels of consciousness have been found if the disease progresses to Stage 3B. Nearly 80% of children who reached this stage died within 12 hours of intubation, during the 1998 epidemic, but the fatality rate decreased to 30%–40% in 2000–2002, after the introduction of stage-based management. This significantly lower mortality may have been due to earlier and better intensive care, while earlier intubation, earlier usage of inotropic agents, and intensive care also reduced the rate and severity of pulmonary edema/hemorrhage after the year 2000.

Pathological studies of cases with pulmonary edema have revealed extensive inflammation in the CNS, with predominant lesions in the brain stem and spinal cord. Marked pulmonary edema with focal hemorrhage, with no evidence of myocarditis, has also been reported. EV71 has been isolated from CNS tissues but not from other tissues. Autopsy findings in Taiwan have been similar to those reported in Malaysia. EV71 was usually isolated from the brain, medulla, and cervical, thoracic and lumbar spinal cord, as well as from throat and rectal swabs, and sometimes from the blood. MRI studies of cases with cardiopulmonary failure have usually shown hyperintensity of the posterior aspect of the medulla (Figure 3B), with or without involvement of the cervical spinal cord on T2-weighted images.

Immunopathogenesis studies found that EV71 cases with both encephalitis and cardiopulmonary failure had much higher white blood cell counts, and higher levels of blood glucose, systemic pro-inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor-α, interleukin-1β, γ-interferon, IL-10, and IL-13 than those patients with EV71 encephalitis alone. Levels of CSF IL-6 in EV71 study patients were consistently higher during the initial 2 days of CNS involvement, regardless of the presence of pulmonary edema. Patients with pulmonary edema had lower circulating CD4+ T-cells, CD8+ T-cells, and natural killer cells. The CD40-ligand expression on T-cells was significantly reduced in children with meningoencephalitis. However, EV71-neutralizing antibody titers were detectable in children with pulmonary edema, with meningoencephalitis alone, and with uncomplicated illness, but there were no differences in EV71 neutralizing titers. We also measured the lymphocyte proliferation response and EV71-stimulated cellular response of Th1/Th2 cytokines and chemokines, and found that EV71 cases involving brain-stem encephalitis plus cardiopulmonary failure had significantly lower phytohemagglutinin stimulation indices and significantly lower cellular γ-interferon (p=0.04), IL-1β (p=0.04), IL-6 (p=0.04), and tumor necrosis factor-α responses (p=0.04), and lower cellular macrophage inflammatory protein-1α (p=0.04) responses, compared with other cases (p=0.04). These results suggest that a lower EV71-specific cellular response, rather than humoral immunity, may be associated with the immunopathogenesis of EV71-related cardiopulmonary failure.

5.4. Stage 4: Convalescence and long-term sequelae

We have assessed the neurological development and cognitive function of children in Taiwan who survived EV71 infection with CNS involvement between 1998 and 2003. The prospective study included 142 children with clinically confirmed EV71 infection who experienced mild CNS involvement including meningitis (n=61), severe CNS involvement including encephalitis, encephalomyelitis or polio-like syndrome (n=53), or cardiopulmonary failure following CNS infection (n=28). Nine of the 16 polio-like cases (56%) and one of the five encephalomyelitis cases (20%) experienced sequelae involving limb weakness/atrophy. Eighteen of the 28 cases with cardiopulmonary failure (64%) after CNS involvement experienced limb weakness and atrophy, 17 (61%) required tube feeding, and 16 (57%) required ventilator support. Delayed neurodevelopment was found in only one case (5%) with severe EV71 CNS involvement and in 21 cases (75%) with cardiopulmonary failure following CNS infection (p<0.001). The age of the children at the time of disease onset in the most clinically severe group was significantly lower than that in the other groups (p<0.001). The effects of EV71 infection on neurological development and cognitive function appear to be strongly related to the severity of CNS involvement and the patient’s age at disease onset.

6. Treatment Strategy: Stage-based Management

Nearly 90% of patients with cardiopulmonary failure in Taiwan’s 1998 epidemic died. In 2000, with the approval of Taiwan’s Enterovirus Ad Hoc Committee and the Taiwan CDC, we developed a disease
management program that varied according to the different stages of EV71 infection, with the aim of improving survival rates (Table 3).21

The clinical stages have been described above. Patients with Stage 1 disease (uncomplicated illness) require symptomatic treatment only. Patients identified as having Stage 2 disease (complicated illness with CNS involvement) are hospitalized, and are treated with fluid restriction and the administration of osmotic diuretics for those with signs of increased intracranial pressure, and furosemide for those suspected of having fluid overload. Intravenous immunoglobulin, usually 1g/kg, is administered, and heart rate, blood pressure, oximeter, coma scale and blood sugar are closely monitored. Patients are managed in the intensive care unit if they have tachycardia, tachypnea/apnea, hyper tension/hypotension, or signs of increased intracranial pressure, or hyperglycemia.

Patients identified as having Stage 3 disease (cardiopulmonary failure or pulmonary edema) are put on ventilator support and administered inotropic agents. These patients can be subdivided into Stage 3A, having tachycardia, hypertension and/or pulmonary edema, and Stage 3B, having hypotension.22 Treatment of Stage 3A includes intensive care management with continuous fluid restriction, administration of milrinone (0.25–0.75 μg/kg/min) to control severe hypertension and to increase cardiac output, early intubation with positive pressure mechanical ventilation, and high frequency oscillatory ventilation if pulmonary edema/hemorrhage persists or severe hypoxemia develops. When a patient’s blood pressure drops below the normal range for his or her age, the disease is considered to have moved into stage 3B. In some cases, blood pressure is very unstable, requiring fine adjustments of inotropic agents such as dopamine (5–15 μg/kg/min) and epinephrine (0.05–0.4 μg/kg/min). Extracorporeal membrane oxygenation may be used if these strategies fail to maintain satisfactory blood pressure or tissue perfusion.

For Stage 4 (convalescence) patients, rehabilitation is provided for limb weakness/atrophy, dysphagia, diaphragm dysfunction, apnea or central hypoventilation. Sufficient chest care is necessary to avoid recurrent pneumonia, and some cases may need long-term tracheostomies, and may need to be transferred to respiratory care centers.

This stage-based management strategy has been associated with a reduction in the fatality rate of EV71-related cardiopulmonary failure, compared with the situation in 1998, but 75% of the survivors of EV71-related cardiopulmonary failure still suffer from severe sequelae.31 These data are based on historical comparisons rather than on prospective case-controlled studies, and we therefore need to be cautious in interpreting the results concerning stage-based management. It seems clear however that better preventive and treatment strategies for EV71 are still sorely needed.

### 7. Antiviral Agents

Since EV71 can cause severe diseases resulting in serious sequelae or death, EV71-specific antiviral therapy may help to improve the clinical outcome. The replication cycle of enteroviruses involves several
steps, including viral attachment, uncoating, viral RNA replication, and viral protein synthesis and processing, all of which provide potential targets for the development of antiviral drugs. Capsid-binding agents, which prevent viral attachment and receptor-mediated uncoating, are among the most promising antiviral agents.32

The VP1 hydrophobic pocket generally contains unidentified natural molecules (pocket factor), which may stabilize the integrity of the virus and modulate the uncoating of its receptors. Pleconaril is a novel agent for the treatment of picornavirus infections, including enteroviruses and rhinoviruses. Absorption of the drug through the gastrointestinal tract is good, and concentrations in serum and CSF exceeding 0.1 mg/mL are easily attained following oral administration of a standard dose of the liquid formulation. Pleconaril demonstrates excellent \textit{in vitro} antiviral activity against most enteroviruses. Furthermore, Groarke and Pevear reported antiviral activity of oral pleconaril in three animal models of lethal enterovirus infection: Coxsackievirus serotype A9 infection in suckling mice, Coxsackievirus serotype A21 strain Kenny infection in weanling mice, and Coxsackievirus serotype B3 strain M infection in adult mice.33 Treatment with pleconaril increased the survival rate in all three models using both prophylactic and therapeutic dosing regimens.33 This drug is currently undergoing late-stage clinical trials for the treatment of viral meningitis and viral respiratory infections.

However, pleconaril has failed to neutralize the cytopathic effects of EV71 isolates from the Taiwan 1998 outbreak on cultured cells.34 By using the skeleton of pleconaril and its related molecules, so-called WIN compounds, as a template, rational design, synthesis, and structure-activity relationship studies have led to the development of a novel class of imidazolidinones with significant antiviral activity.34 These synthetic compounds were evaluated for anti-EV71 activity, and some analogs were found that inhibited all EV71 genotypes, and possessed antiviral activity against Coxsackievirus B3 (50% inhibition concentration, IC$_{50} = 0.06-0.089 \mu$M) and moderate activity against EV71 (IC$_{50} = 0.32-0.65 \mu$M), with no apparent cytotoxic effect on rhabdomyosarcoma cell lines (50% cytotoxic concentration, CC$_{50} > 25 \mu$M).36 These promising anti-EV71 drugs are being tested in animal studies in Taiwan and may enter clinical trials if the animal studies show positive results.

8. Prevention and Vaccine Development

Hand washing precautions have been in practice since the 1998 epidemic in Taiwan. Hand washing is the standard preventive measure targeting the fecal-oral transmission route, but has limited efficacy in preventing droplet transmission. Furthermore, younger infected children have higher virus isolation rates as well as higher transmission abilities, and these young children are not usually able to consistently follow adequate hand washing routines.

Previous research has demonstrated a higher rate of EV71 isolation from throat swabs than from rectal swabs or stools (90% vs. 32%, respectively),14 and very high household transmission among children.15 We speculate that respiratory transmission by droplets from the oral cavity may explain the high secondary infection rate within households and in kindergartens in Taiwan despite hand washing precautions. Isolation of infected patients within single rooms and masks for the patients and their close contacts may therefore be recommended for the prevention of respiratory droplet transmission of EV71. In Taiwan, home isolation of kindergarten or elementary school students was suggested for 1 or 2 weeks to prevent droplet transmission within kindergartens or schools. It was suggested that younger children (<3 years old) should refrain from attending kindergartens during the EV71 epidemic, in order to reduce the risk of exposure to EV71.

Enterovirus excretion through stools can persist for up to 11 weeks and hand washing and general hygiene precautions should therefore be advocated during convalescence. We found that subclinical enterovirus (including EV71) infection could occur,
and that live vaccine poliovirus did not interfere with the invasion of other non-polio enteroviruses.\textsuperscript{19}

Although the above preventive measures have been in practice for 10 years in Taiwan, dozens of fatal EV71 cases still occurred from 2000 to 2002. Furthermore, another EV71 outbreak occurred recently in Taiwan, with about 340 severe enterovirus cases involving the CNS, and 10 fatalities from January to August 12, 2008.\textsuperscript{37} Development of a vaccine might prove to be the only effective measure against EV71. Since poliovirus was eradicated by polio vaccination, EV71 has become the most important enterovirus affecting children, and is the top candidate for future vaccine development.

No EV71 receptor has yet been found, and no transgenic mouse model is available, but a newborn mouse model has been used.\textsuperscript{38} In that model, experimental infection with EV71 induced death in neonatal mice in an age- and dose-dependent manner. The mortality was 100% following intraperitoneal inoculation of 1-day-old ICR mice, and this gradually decreased with increasing age at the time of inoculation (60% in 3-day-old mice and no deaths in mice older than 6 days of age). Two studies in Taiwan have demonstrated that an inactivated EV71 virus vaccine can protect against lethal EV71 infection in newborn mice by passive immunization from immunized mother mice or adult mice.\textsuperscript{38, 39}

Yu et al found that protection against EV71 challenge in neonatal mice was seen following passive transfer of serum from actively immunized adult mice, 1 day after inoculation with the virus.\textsuperscript{38} Pups from hyperimmune dams were resistant to EV71 challenge. Additionally, maternal immunization with a formalin-inactivated whole-virus vaccine prolonged the survival of pups after EV71 lethal challenge.

In a study by Wu et al, the inactivated virus vaccine (10\textsuperscript{μ}g protein/mouse), and subunit vaccines—VP1 DNA vaccine (100\textsuperscript{μ}g/mouse) or recombinant VP1 protein (10\textsuperscript{μ}g/mouse)—were injected into female dams to elicit maternal antibodies and to provide protection against lethal infection by EV71 in suckling mice.\textsuperscript{39} With a challenge dose of 2300 50% lethal dose (LD\textsubscript{50}) virus/mouse, suckling mice born to dams immunized with inactivated virus showed 80% survival. The subunit vaccines provided protection only at the lower challenge dose of 230LD\textsubscript{50} per mouse, with 40% survival for the DNA vaccine and 80% survival for the VP1 protein. In addition, the inactivated virus elicited a much greater immune response than the subunit vaccines, including total IgG, all four IgG subtypes, and T-helper cell responses. This data suggests that an inactivated virus is the preferred vaccine preparation.

Animal studies with newborn mice are still in progress, and studies with monkeys or chimpanzees may be performed in the near future. It is hoped that an EV71 vaccine can be developed to control future epidemics in Asian-Pacific areas.

In conclusion, EV71 is one of the most important enteroviruses worldwide, and continued EV71 disease and laboratory surveillance in Taiwan is essential. The development of anti-EV71 drugs and vaccines may provide the most promise for the control of epidemics. Much research is currently being carried out in Taiwan aimed at developing effective control measures, including the development of antiviral drugs and EV71 vaccines.

References

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