An Eight-Year Study of Epidemiologic Features of Enterovirus 71 Infection In Taiwan

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Abstract. In 1998, an epidemic of enterovirus 71 (EV 71) infection occurred in Taiwan. The purpose of this study was to assess the epidemiology of EV 71 infection in Taiwan. Between March 1998 and December 2005, a total of 1,548 severe cases of hand-foot-mouth disease and herpangina (HFMD/HA) was reported to the Center for Disease Control in Taiwan. A seasonal variation in number of severe cases was observed, with the annual peak in second quarter. Deaths from severe HFMD/HA varied from year to year ($\chi^2$ for trend = 6.781, $P = 0.009$). Most (92%) cases occurred in children $\leq 4$ years of age. Children infected with EV 71 had higher risk of pulmonary edema/hemorrhage and encephalitis than those not infected. Infection with EV 71 has emerged as an important infectious disease causing serious clinical illness and deaths of young children. Vaccine development is recommended to prevent future EV 71 infections.

INTRODUCTION

Enterovirus 71 (EV 71) has been associated with outbreaks in the United States, Europe, Australia, Japan, Brazil, and Malaysia. Infection with EV 71 can result in aseptic meningitis, encephalitis, myocarditis, or poliomyelitis-like paralysis. Since the first case of EV 71 infection in California in 1969, worldwide reports of outbreaks have followed. In early outbreaks during 1969–1974, serious central nervous system complications were uncommon. Although mild diseases are the predominant clinical features of EV 71 infection, neurologic involvement is the most serious complication. Rapid clinical deterioration and death have occurred in the previous outbreaks.

From March to December 1998, 405 severe cases of hand-foot-mouth disease and herpangina (HFMD/HA) and 78 deaths were reported to the Center for Disease Control in Taiwan. Enterovirus 71 has circulated in Taiwan for at least 18 years prior to 1998. In addition, sequences of some EV 71 isolates in 1998 showed a high degree (92%) of identity in the VP-1 genomic region with that of the EV 71 strain isolated in 1986. However, the factors underlying the widespread increase in the scale of EV 71 infection in 1998 remain unknown. Therefore, we analyzed the data reported by passive surveillance systems in Taiwan to assess the epidemiology of EV 71 infection in Taiwan.

SUBJECTS AND METHODS

Surveillance. Taiwan has a population of approximately 22.7 million, an area of 36,188 km², and a population density of 627/km². Most (95%) of the population live in the western part of Taiwan, which we divided into northern, central, and southern regions. Only 5% live in eastern Taiwan, where medical care and socioeconomic status are classified as underprivileged. A report system designed for monitoring severe cases of HFMD/HA was established on May 29, 1998. Patients who were hospitalized for HFMD/HA were reported to the Center for Disease Control, Department of Health, by Taiwan’s 23 academic medical centers, 80 regional hospitals, and 435 district hospitals, classified by the hospital size, care, and teaching capacity. The study was reviewed and approved by the Institutional Review Board of the Taiwan Centers for Disease Control.

Clinical definitions. A case was defined as severe by the presence of the symptoms/signs of HFMD/HA in addition to more than one of the following complications: encephalitis, aseptic meningitis, or acute flaccid paralysis, pulmonary edema, pulmonary hemorrhage, or myocarditis. Encephalitis was characterized by a disturbance in the level of consciousness, such as lethargy, drowsiness, or coma. Aseptic meningitis was characterized by headache, meningeal signs, mononuclear pleocytosis ($> 5 \times 10^6$ leukocytes/L if the patient was greater than one month of age or $> 25 \times 10^6$ leukocytes/L if the patient was a newborn), and negative bacterial culture. Pulmonary edema/hemorrhage was characterized by respiratory distress, tachypnea, tachycardia, pink frothy sputum, and rapidly progressing, patchy, diffuse pulmonary infiltrates and congestion as seen on a chest radiograph. Acute flaccid paralysis was defined as the acute onset of paresis or paralysis of one or more skeletal muscle groups, usually of one or more limbs. Myocarditis was characterized by evidence if decreased contractility from echocardiography, arrhythmia, an enlarged heart, and elevations in cardiac enzyme levels that are markers for cardiac damage.

Isolation and identification of enteroviruses. Viral laboratories were located in 11 hospitals and in the Taiwan Center for Disease Control. Specimens consisted of throat swabs, stool, cerebrospinal fluid, and in rare cases, blood samples. They were collected from inpatients suspected of having an enteroviral infection. Most of the patients had HFMD/HA with complications.

The methods used to identify enteroviruses differed in the various laboratories. Monolayers of Vero, rhabdomyosarcoma, and MRC-5 cells were most commonly used for viral isolation. An immunofluorescence assay was used for identification. Cultures that showed a cytopathic effect characteristic of enteroviruses were screened for enteroviruses with an enterovirus screening set (catalog no. 3365; Chemicon International, Temecula, CA), which included pan-enterovirus, coxsackievirus B virus, echovirus, and poliovirus mixtures. EV 71 was identified by monoclonal antibodies 3323 and
Monoclonal antibody 3323 is specific for enteroviruses, and monoclonal antibody 3324 is specific for EV 71. The identification of EV 71 was confirmed by a neutralization test with a polyclonal rabbit antiserum against EV 71, which had been prepared during a previous outbreak in 1986, or a rabbit anti-EV 71 serum. Final enterovirus typing was done by neutralization testing with the use of polyclonal antiserum (American Type Culture Collection, Manassas, VA). For quality control purpose, a sample of specimens was sent for confirmation of enterovirus to the Viral Gastroenteritis Section of the U.S. Centers for Disease Control and Prevention (Atlanta, GA). Similarly, a representative of specimens was sent to the Taiwan Center for Disease Control for confirmation of enteroviral test results. In both instances, there was good concordance of results between the primary and reference laboratories.

**Statistical analysis.** Annual mortality rates were calculated by dividing the number of deaths resulting from severe cases of HFMD/HA for children < 15 years of age by the number of severe cases in the same year as reported between 1998 and 2005. The annual mortality rates of severe cases were expressed as the number of deaths per 100 severe cases. All statistical analyses were performed using the STATA Statistical Software Release 8.0. We used the chi-square test with Yates' correction for analyzing categorical data and Student's \( t \)-test for analyzing continuous data. The accepted level of significance for all analyses was \( P < 0.05 \).

**RESULTS**

Between 1998 and 2005, a total of 1,548 severe cases of HFMD/HA were reported to the Center for Disease Control in Taiwan. The mean age of the patients was 2 years (range = 3 months to 14 years), and the male-to-female ratio was 1.5:1. Most (92%) of the severe cases of HFMD/HA occurred in children < 4 years of age, with 74% occurring in children who were \( \leq 2 \) years of age. Patients infected with EV 71 had higher mortality rates than those without infections (Table 1).

**Surveillance.** Figure 1 shows the number of severe cases of HFMD/HA reported by physicians from March 1998 through December 2005. During the eight-year study period, epidemic peaks occurred every year, with the highest number of cases during an epidemic in the second quarter. The peak was reached a week earlier in the central region and a week and half later in the southern region. The first wave encompassed all four regions of Taiwan. The second wave was largely limited to the southern region and lasted from the first week of September to the second week of December. It peaked during the first week of October. The number of cases reported varied year by year with the highest number of cases reported in 1998.

**Mortality rates.** Of the 1,548 patient-cases, 246 (16%) died. This is a crude mortality rate used for a comparison with data from other countries. Figure 2 shows the number of severe cases and deaths. The annual mortality rates changed significantly during the eight-year study period. The annual mortality rates were 19.3% (78 of 405) in 1998, 25.7% (9 of 35) in 1999, 14.1% (41 of 291) in 2000, 14.8% (58 of 393) in 2001, 18.5% (30 of 162) in 2002, 11.4% (8 of 70) in 2003, 10.0% (5 of 50) in 2004, and 11.3% (16 of 142) in 2005 (\( \chi^2 \) square for trend = 6.781, \( P = 0.009 \)).

The age-specific annual mortality pattern was the differ-

![Figure 1](image-url) Cases of severe hand-foot-mouth disease and herpangina in regions of Taiwan, by quarter, 1998–2005.

![Figure 2](image-url) Cases of severe hand-foot-mouth disease and herpangina and deaths caused by this disease in Taiwan, by year, 1998–2005.
ence within different age groups (Table 1). Overall, the mortality rate of HFMD/HA was higher among younger children than older children. Compared with age group ⩾ 5 years of age, children ⩾ 6 months of age and those < 1 year old of age had the highest mortality rates during the studied time period (odds ratio [OR] = 2.87, 95% confidence interval [CI] = 1.45–5.79, P < 0.001).

Clinical complications. Table 2 shows the clinical complications and EV 71 isolated from 1,548 patients with severe infections. Each complication or combination of complications shown in Table 2 are mutually exclusive. The overall frequency of EV 71 isolates in these patients was 38.4% (594 of 1,548). EV 71 was more frequently isolated from patients with encephalitis and pulmonary edema/hemorrhage (93%). Compared with the frequency of EV 71 isolated among patients with aseptic meningitis, there was a significant higher frequency of EV 71 isolated among patients with pulmonary edema/hemorrhage only (odds ratio = 3.61; 95% CI = 2.22–5.85; P < 0.0001) and encephalitis only (relative risk = 3.03, 95% CI = 2.06–4.48, P < 0.001).

DISCUSSION

Because polioviruses have nearly been eradicated, nonpolio enteroviruses remain an important cause of illness in the absence of vaccine and effective antiviral therapy. These data show the epidemiologic features of severe EV 71 infection in Taiwan. It is characterized by neurotropism and may cause serious complication or even death. Male children and younger children (< 4 years of age) are at an apparently increased risk for infection.

Most patients infected with enteroviruses are young children (< 4 years of age), with the peak incidence occurring at one year of age. Consistent with previous reports, our study found that more than 92% of severe HFMD/HA cases were in children ≤ 4 years of age.

This study found that the mortality rate was much higher in young age groups and differed in other age groups. The mortality rate in the age group younger than 6 months of age was much lower than the children who were 0.5 to < 1 year (21% versus 25%). This may indicate that a preexisting neutralizing antibody to EV 71 acquired by infection in outbreak or transplacental transfer was protective against severe outcome of infection.

The factors underlying the pathogenesis of pulmonary edema/hemorrhage caused by EV 71 infection are unknown.

In conclusion, EV 71 infection has emerged as an important public problem causing serious neurologic manifestations and pulmonary edema/hemorrhage and death of young children. Vaccine development is recommended for prevention of EV 71 infection in the future.

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<table>
<thead>
<tr>
<th>Complications*</th>
<th>No. of patients n = 1,548</th>
<th>EV 71 (+) n = 594</th>
<th>EV 71 (-) n = 954</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aseptic meningitis only</td>
<td>236</td>
<td>42 (18)</td>
<td>194 (82)</td>
<td>Referent</td>
</tr>
<tr>
<td>Encephalitis only</td>
<td>588</td>
<td>233 (40)</td>
<td>355 (60)</td>
<td>3.03 (2.06–4.48)†</td>
</tr>
<tr>
<td>Pulmonary edema/hemorrhage only</td>
<td>155</td>
<td>68 (44)</td>
<td>87 (56)</td>
<td>3.61 (2.22–5.85)†</td>
</tr>
<tr>
<td>Myocarditis only</td>
<td>30</td>
<td>4 (13)</td>
<td>26 (87)</td>
<td>0.71 (0.20–2.30)</td>
</tr>
<tr>
<td>Acute flaccid paralysis only</td>
<td>28</td>
<td>3 (11)</td>
<td>25 (89)</td>
<td>0.55 (0.13–2.05)</td>
</tr>
<tr>
<td>Combined</td>
<td>406</td>
<td>214 (53)</td>
<td>192 (47)</td>
<td>5.15 (3.44–7.72)†</td>
</tr>
<tr>
<td>Encephalitis with pulmonary edema or hemorrhage</td>
<td>353</td>
<td>190</td>
<td>163</td>
<td></td>
</tr>
<tr>
<td>Encephalitis with myocarditis</td>
<td>31</td>
<td>15</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Encephalitis with acute flaccid paralysis</td>
<td>22</td>
<td>9</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>105 (7)</td>
<td>30 (29)</td>
<td>75 (71)</td>
<td>1.85 (1.04–3.28)‡</td>
</tr>
</tbody>
</table>

* EV 71 = enterovirus 71; OR = odds ratio; CI = confidence interval. The categories of complications are mutually exclusive.
† P < 0.001.
‡ P < 0.05.

The pattern of prevalence of non-polio enterovirus varies with time and location. In temperate climates, infections occur with high incidence throughout the year. Taiwan has a pattern of seasonal enteroviral infection similar to that found in other temperate regions.

On the basis of genotype analysis of EV 71 collected from isolates from Japan and Malaysia in 1997 and Taiwan in 1998, a group of genetically similar strains have, for the first time, been observed to be recently circulating in the western Pacific region, although not elsewhere. These strains are different from the EV 71 genotypes that caused previous outbreaks, such as the large epidemics in Bulgaria and Hungary in 1975 and 1978. Whether the Asian virus is a mutant variant of an enterovirus or a different enterovirus from another source is not known.

In conclusion, EV 71 infection has emerged as an important public problem causing serious neurologic manifestations and pulmonary edema/hemorrhage and death of young children. Vaccine development is recommended for prevention of EV 71 infection in the future.

CHEN AND OTHERS

TABLE 2

Clinical complications in 1,548 patients with severe hand-foot-mouth disease and herpangina in Taiwan, 1998–2005*
REFERENCES


