Enterovirus Coinfection
During an Outbreak of Hand,
Foot, and Mouth Disease in
Shandong, China

To the Editor—Hand, foot, and mouth disease (HFMD) is caused by human enteroviruses, most frequently human enterovirus 71 (HEV-71) and coxsackievirus A16 (CV-A16). Other viruses (CV-A4 to A7, A9, A10, A24, and B2 to B5; echoviruses 1, 4, 11, and 18; and HEV-18) may also be associated with HFMD outbreaks or sporadic cases. HFMD is generally a benign febrile exanthematous childhood disease, excluding a small proportion of HEV-71 infections associated with severe complications, including encephalitis, aseptic meningitis, pulmonary edema or hemorrhage, and acute flaccid paralysis. CV-B5 has been reported to cause more serious neurologic symptoms such as encephalitis [1–3]. Currently, HEV-71 and CV-A16 can be routinely detected from throat swab or stool samples in hospitalized cases in China, allowing physicians to make a diagnosis and predict disease progression and prognosis.

In China, a rapid expansion of HFMD outbreaks has occurred since 2004 [4]. From April to May 2009, a total of 110 HFMD children were brought to Linyi Hospital in Shandong, China; all patients were identified according to Ministry of Health diagnostic criteria (http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohyzs/s3586/201004/46884.htm). We collected clinical throat swabs and serum from patients and found that 97/110 (88.2%) swab samples were positive for HEV by semi-nested reverse-transcription polymerase chain reaction (RT-PCR) with general enterovirus primers. HEV-71 constituted 60/97 (61.9%) of positive cases, and CV-A16 constituted 16/97 (16.5%) of typed strains. We simultaneously detected four other HEVs: CV-B5 (14.4%), CV-A6 (3.1%), CV-A10 (1%), and CV-A12 (3.1%).

Following further cloning and sequencing of RT-PCR products of the CV-A16–positive swabs, 4 cases (Table 1) also carried another 1–2 types of enterovirus (CV-B5 and -A6). Although

### Table 1. Symptoms and Laboratory Analysis of the Four HFMD Cases

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Days after onset</th>
<th>Clinical symptoms</th>
<th>Virus detected by RT-PCR and sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>Fever, seizure, flaccid limb weakness, coarse breath sounds in lungs</td>
<td>A16, B5, A6</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Fever, seizure, vomiting, limb cool/damp/weakness/paralysis, coarse breath sounds in lungs</td>
<td>A16, B5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Fever, vomiting, arm rashes</td>
<td>A16, B5</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>Fever, extremity rashes, skin mottling, oral ulcer</td>
<td>A16</td>
</tr>
</tbody>
</table>

**NOTE.** HFMD indicates hand, foot, and mouth disease; RT-PCR, reverse-transcription polymerase chain reaction.

* With mechanical ventilation for supportive therapy.
CV-A16 occurrence alone is thought to rarely result in severe cases. 2/4 co-detected cases (Patients 1 and 2 in Table 1) exhibited severe clinical syndromes with rapidly progressive neurological and cardiopulmonary complications. Another two cases (Patients 3 and 4) displayed benign febrile exanthematous diseases. Serum samples were also assayed for enterovirus presence via the same procedure, revealing that the 2 severe cases were coinfected with CV-A16 and -B5, and the 2 benign cases carried CV-A16 only (Table 1).

In our study, 2 out of 4 (50%) CV-A16/-B5 codetected cases developed severe clinical symptoms (Table 1). These patients were diagnosed with CV-A16 infections, highlighting a very important point: In these cases, the CV-B5 infections were overshadowed by the CV-A16-positive results, and the risks of developing more severe illness, complications, or fatalities were underestimated. Therefore, the limitations of the enterovirus test make it difficult to identify multiple genotypes on the first pass, possibly leading to misdiagnosis and delayed treatment. We are uncertain whether enterovirus coinfection can lead to synergies in the pathogenic mechanism. However, given the frequency of enterovirus intra- and intertypic genetic recombination, monitoring of coinfection in the densely populated China epicenter is also important for the detection of emerging pathogens and epidemics [5]. Thus, this study underscores the need for enhancement of molecular biological tests for HFMD diagnosis and more detailed molecular-typing surveillance of enteroviruses in China.

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