

## Enterovirus 71 Outbreak in the People's Republic of China in 2008<sup>▽</sup>

Since the discovery of enterovirus 71 (EV71) in 1969, numerous outbreaks have occurred worldwide in countries in Europe, America, and the West Pacific region (1). Since 1997, the prevalence of EV71 infection in the Asia-Pacific region, especially in southeast Asia, has greatly increased. Outbreaks have been recorded in Malaysia (subgenogroups B3, B4, B5, and C1), Singapore (subgenogroups B3, B4, and C1), Japan (subgenogroups B4, C1, and C4), and mainland China and Taiwan (subgenogroups B4, C2, and C4) (2). Hand, foot, and mouth disease (HFMD) cases with EV71-associated neurological syndrome continually increased in many countries during the past 10 years (3). EV71 infection has become an important issue in public health.

In the People's Republic of China, EV71 (subgenogroup C4) was first identified in Guangdong province in 1998 (4). Five to 10 years ago, EV71 epidemics usually occurred in only the south of China, such as Guangdong province. However, a rapid expansion of EV71 infections has occurred since 2004 (5). Several epidemics have been reported in the middle or north of China, such as in Anhui, Shandong, Shanghai, and Beijing. From April to June 2008, HFMD was epidemic in the Anhui province of China. At the same time, sporadic HFMD cases emerged in other provinces. A total of 488,955 HFMD cases were reported nationwide, including 126 fatal cases in 2008. EV71 was confirmed as the major pathogen, while CVA16 played only a subsidiary role (<http://www.moh.gov.cn/publicfiles/business/htmlfiles/mohbgt/s3582/200902/39079.htm>).

This study was conducted to understand the molecular epidemiology of EV71 in China in this epidemic. For the present study, a total of 20 strains were isolated from six different provinces during the spring and summer of 2008. The clinical samples were randomly collected from EV71-infected patients

with diverse clinical symptoms, ranging from uncomplicated HFMD to severe encephalitis (Table 1).

Complete VP1 gene sequencing is considered the most rigorous method for determining the molecular phylogeny of EV71 strains since it can support serotype-specific information (3). In this study, the complete VP1 gene sequences of 20 new isolates were amplified by reverse transcriptase PCR (RT-PCR) with specific primers (F, GGAGATAGGGTRGCAGATGTAAT; R, ATTTCCCAAGAGTAGTGATCGC). The PCR products were then sequenced using BigDye, version 3.0, and run on an ABI3730 automated sequencer (Applied Biosciences), and the nucleotide sequences were aligned (MEGA 4). A total of 49 EV71 strains, including 20 isolates from this study, 8 Chinese isolates available from GenBank which represented several Chinese EV71 outbreaks from 1998 to 2008, and 21 other EV71 reference strains, were investigated for their genetic diversity.

Our study showed that all 20 recently identified EV71 isolates belonged to subgenogroup C4 within the range of 94.4 to 100% identity. It is interesting that in the People's Republic of China (with the exception of one C3 strain isolated in the Heilongjiang province in 1997 [2]), only C4 is predominantly responsible for EV71 infections, while C4 and the remaining subgenogroups circulate in surrounding countries. It is noteworthy that so few other subgenogroups are detected in a 10-year period in the People's Republic of China.

The EV71 infections have become more widespread in China in recent years. The possible reasons might be the following: (i) Ever-increasing travel and migration spreads the disease further by moving infected people between population centers; (ii) in May 2008, China added HFMD to its category C of notifiable diseases, meaning that all diagnosed cases must be reported, and the recent apparent increase in EV71 infection might be due to higher reporting rates rather than an increase in disease prevalence; and (iii) genetic changes have occurred in the circulating EV71 strain. Before 2004, the predominant strain was called C4b; since then, a different strain, C4a, has been most common. C4a also caused the epidemic in 2008 (6).

An effective enterovirus infection surveillance system has not yet been established in mainland China, so current information on EV71 circulation is not available (6). Once this is in place, continued EV71 pathogen surveillance will be useful to allow a more detailed analysis of the molecular epidemiology of EV71 in China.

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All of the authors report no conflicts of interest.

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TABLE 1. EV71 isolates in this study (People's Republic of China, 2008)

| Strain <sup>a</sup> | Origin    | Accession no. |
|---------------------|-----------|---------------|
| 542-Anhui-08*       | Anhui     | FJ765416      |
| 549-Anhui-08*       | Anhui     | FJ765417      |
| 552-Anhui-08        | Anhui     | FJ765418      |
| 559-Anhui-08*       | Anhui     | FJ765419      |
| 562-Anhui-08*       | Anhui     | FJ765420      |
| 566-Anhui-08*       | Anhui     | FJ765421      |
| BJCDC01-08          | Beijing   | FJ765422      |
| BJCDC03_08          | Beijing   | FJ765423      |
| CC01_08             | Jilin     | FJ765424      |
| kunming24_08        | Yunnan    | FJ765425      |
| kunming29_08        | Yunnan    | FJ765426      |
| kunming41_08        | Yunnan    | FJ765427      |
| SZ-A-08             | Guangdong | FJ765428      |
| SZ-C-08             | Guangdong | FJ765429      |
| SZ-H-08             | Guangdong | FJ765430      |
| WH-2-08             | Hubei     | FJ765431      |
| WH-3-08             | Hubei     | FJ765432      |
| WH-4-08             | Hubei     | FJ765433      |
| WH-8-08             | Hubei     | FJ765434      |
| WH-9-08             | Hubei     | FJ765435      |

<sup>a</sup> Strains marked with an asterisk were isolated from patients with severe encephalitis; the other strains were all isolated from HFMD patients.

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