Seroepidemiology of Enterovirus 71 infection prior to the 2011 season in children in Shanghai

Mei Zeng, Névine Fahmy El Khatib, Shuyang Tu, Peijun Ren, Qianqian Zhu, Xiaowei Mo, Dongbo Pu, Xiaohong Wang, Ralf Altmeyer

Department of Infectious Diseases, Children’s Hospital of Fudan University, 399 Wuyuan Road, Shanghai 200102, China
Institut Pasteur Shanghai, Chinese Academy of Sciences, 225 South Chongqing Road, Shanghai 200025, China

Abstract

Background: In 2010, China experienced the largest outbreak on record of Enterovirus 71 (EV71)-associated Hand Foot and Mouth Disease (HFMD) with more than 1.7 million cases, 27,000 patients with severe neurological complications and 905 deaths. Understanding of the seroprevalence of neutralizing antibodies (NAb) against EV71 and their protective role against HFMD in children is crucial for the implementation of future therapeutic and prophylactic intervention.

Objectives: To correlate the prevalence of NAb against EV71 genotype C4a in children prior to the 2011 epidemic season with severe EV71-associated HFMD disease during the subsequent 2011 epidemic season.

Study design: 614 sera samples were collected from children without HFMD. EV71 NAb were tested by a quantitative PCR assay. Samples with NAb ≥ 1:8 were scored as positive.

Results: 122 (19.9%) of 614 sera were EV71-seropositive. The NAb seroprevalence was highest in infants 0–5 months of age (28.6%) and lowest in children 1–1.9 years of age (13.4%). 64.1% of severe EV71-associated HFMD occurred in children 1–2.9 years.

Conclusions: Despite the large 2010 outbreak, the overall seroprevalence of EV71 in children is relatively low. The seropositive rate of EV71 NAb prior to the 2011 season was inversely correlated with the number of EV71-infected severe cases in 2011. Loss of maternal antibodies in infants and lack of acquired anti-EV71 immunity are responsible for increased proportion of severe HFMD in the 1–2 years age group. Our data suggest that future vaccination campaigns should be initiated as early as 6 months.

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1. Background

Enterovirus 71 (EV71) is a member of the Picornaviridae family, genus Enterovirus, species A.1 EV71 was first isolated from cases of neurological diseases in California in 1969.2 Since then, EV71-associated cases with severe neurological disease have been reported in many outbreaks throughout the world with particular prevalence in Asian countries.3–5 EV71 caused a series of outbreaks of Hand Foot and Mouth Disease (HFMD) and fatal outcomes since 1997 in Malaysia, Singapore, Taiwan, Vietnam, Mainland China,6–10 In China, sporadic EV71-associated HFMD cases were reported in the southeast coastal area as well as inland regions from 1998 to 2004.11–13 In 2007, an outbreak of EV71-associated HFMD occurred in Shandong province,14,15 followed by a large outbreak in 2008 in Anhui province with significant mortality.10,16 By the end of 2010 a total of 3,419,149 cases and 1384 fatal cases attributable to HFMD were officially reported in China.17 The vast majority of severe and lethal HFMD were due to EV71 infection (China CDC, unpublished data). An unprecedented outbreak of HFMD occurred in mainland China in 2010 and in Shanghai 41,080 children were diagnosed with HFMD, a 2-fold increase compared to 2009, a 1.7-fold increase compared to 2008 and 4-fold increase compared to 2007. Approximately 90% of HFMD cases are below 5 years of age.18 EV71 caused over 50% of pediatric admissions for HFMD, about 90% of neurological complications and almost all HFMD-associated deaths.18

In this current study we describe that seroprevalence of NAb against EV71 in children after the 2010 outbreak in Shanghai is inversely correlated with the susceptibility to severe disease during the 2011 HFMD season. Our findings have implications for the definition of therapeutic and prophylactic intervention strategies.

2. Objectives

This study aimed to investigate the seroprevalence of EV71 NAb in children below 5 years after the 2010 outbreak in Shanghai and
Table 1
HFMD cases, EV71-associated HFMD and severe EV71 cases in 2011.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number (%) of HFMD outpatients (number = 8020)</th>
<th>Number (%) of EV71-infected cases (number = 704)</th>
<th>Number (%) of severe EV71-infected cases (number = 298)</th>
<th>p value</th>
<th>Percentage of severe EV71-infected cases in HFMD outpatients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5</td>
<td>24 (0.3%)</td>
<td>5 (0.7%)</td>
<td>2 (0.7%)</td>
<td>0.103</td>
<td>8.3%</td>
</tr>
<tr>
<td>0.5–0.9</td>
<td>564 (70.0%)</td>
<td>47 (6.7%)</td>
<td>15 (5.0%)</td>
<td>0.397</td>
<td>2.7%</td>
</tr>
<tr>
<td>1–1.9</td>
<td>2480 (30.9%)</td>
<td>272 (38.6%)</td>
<td>116 (38.9%)</td>
<td>0.000</td>
<td>4.7%</td>
</tr>
<tr>
<td>2–2.9</td>
<td>1719 (21.4%)</td>
<td>152 (21.6%)</td>
<td>75 (25.2%)</td>
<td>0.306</td>
<td>4.4%</td>
</tr>
<tr>
<td>3–3.9</td>
<td>1455 (18.1%)</td>
<td>113 (16.1%)</td>
<td>42 (14.1%)</td>
<td>0.105</td>
<td>2.9%</td>
</tr>
<tr>
<td>4–4.9</td>
<td>968 (12.1%)</td>
<td>66 (9.4%)</td>
<td>28 (9.4%)</td>
<td>0.045</td>
<td>2.9%</td>
</tr>
<tr>
<td>≥5</td>
<td>810 (10.1%)</td>
<td>49 (7.0%)</td>
<td>20 (6.7%)</td>
<td>0.005</td>
<td>2.5%</td>
</tr>
</tbody>
</table>

8020 HFMD outpatients were reported at CHFU, 1174 children were admitted to the wards for treatment and 343 were confirmed with severe complications. EV71 was detected in 704 (63.0%) of 1117 inpatients and 298 (89.8%) of 332 of severe cases.

to correlate the level of immune protection with severe disease during the 2011 HFMD season.

3. Study design

3.1. Serum samples collection

Samples used in this study were taken from children between November 2010 and early April 2011 during health checks requiring blood puncture upon parents’ request or physicians’ advice to test for allergy, trace elements, jaundice, HBV infection status or liver function at Children’s Hospital of Fudan University (CHFU). Inclusion criteria were as follows: children were <5 years, children and their mothers were local residents, parents consented to receive telephone interview of HFMD-related investigation. The Ethics Committee of CHFU approved this study.

614 samples were collected and the sample size in each age group was matched to the distribution of 440,057 children below the age of 5 in Shanghai in 2010 (<1 year: 23%, 1 year: 20%, 2 years: 22%, 3 years: 18% and 4 years: 17%).

3.2. EV71 neutralization

EV71 NAb titers were determined by a real time RT-PCR-based micro-neutralization assay as follows. Briefly, the sera were stored at −20 °C, thawed and inactivated at 56 °C for 30 min prior to the assay. Sera were serially diluted in minimal essential medium without FBS, starting from a two-fold dilution, and then incubated with 100 TCID50 (tissue culture infection dose) of EV71 (strain Fuyang573, genotype C4a) for 1 h at 37 °C. Then, the serum/virus mixtures were added to 0.1 ml of RD cell suspension (50,000 cells/0.1 ml) and incubated for 1 h at 37 °C 5% CO2 incubator before being washed and reincubated with minimal essential medium with 2% FBS. Viral replication was evaluated by detecting EV71 RNA in culture supernatant after 48 h incubation. 200 μl of culture supernatant was used for RNA extraction. Real-Time RT-PCR was performed to quantify EV71 copy using an ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, US), TIANamp RNA Kit for virus detection and Quant One Step qRT-PCR (Probe) Kit (Tiangen Biotech Beijing Co., Ltd.) were used throughout the study. Primers and probe were synthesized as described previously. Sera were scored positive if 50% neutralization was achieved at a serum dilution of ≥1/8.

The RT-PCR method was used to conduct the NAb study with the small serum volume available. Comparison of a selection of serum samples in both the RT-PCR and a traditional microneutralization assay shows that both assays yield comparable results as shown in Supplementary Table 1.

3.3. Clinical data of HFMD and EV71 infection

Clinical EV71-associated HFMD was defined by EV71-positive stool samples using a commercial real-time PCR assay (Da An Gene Co. Ltd.). Clinical data were collected from EV71-associated HFMD inpatients from January to September 2011 at CHFU. Severe HFMD was characterized by HFMD complicated with CNS involvement or cardiopulmonary failure.

3.4. Statistical analysis

Statistical analysis was performed by SPSS 15.0. NAb titers were log-transformed to geometric mean titers (GMT) for analysis using Kruskal–Wallis test. The seropositive rates and proportions expressed in Tables 1 and 2 for each age group were compared by Chi-square test.

4. Results

4.1. Demographic characteristics of children with EV71-associated HFMD

At least 90% of pediatric HFMD inpatients in Shanghai were admitted to CHFU. Age-specific distribution of HFMD and

Table 2
Age-related EV71 seroprevalence.

<table>
<thead>
<tr>
<th>Age groups (years)</th>
<th>Number of samples</th>
<th>Number of positive EV71 NAb</th>
<th>Seropositive rate of EV71</th>
<th>Symptomatic EV71 infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5</td>
<td>70</td>
<td>20</td>
<td>28.6%</td>
<td>0</td>
</tr>
<tr>
<td>0.5–0.9</td>
<td>72</td>
<td>12</td>
<td>16.7%</td>
<td>0</td>
</tr>
<tr>
<td>1–1.9</td>
<td>127</td>
<td>17</td>
<td>13.4%</td>
<td>3</td>
</tr>
<tr>
<td>2–2.9</td>
<td>122</td>
<td>17</td>
<td>13.9%</td>
<td>3</td>
</tr>
<tr>
<td>3–3.9</td>
<td>112</td>
<td>27</td>
<td>24.1%</td>
<td>5</td>
</tr>
<tr>
<td>4–4.9</td>
<td>111</td>
<td>29</td>
<td>26.1%</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>614</td>
<td>122</td>
<td>19.9%</td>
<td>28</td>
</tr>
</tbody>
</table>

Symptomatic EV71 infection is defined as positive EV71 NAb plus previous HFMD. Percentage of symptomatic EV71 infection is number of EV71-seropositive children with previous HFMD divided by total number of EV71-seropositive children.
EV71-infected cases are shown in Table 1. The proportion of children aged 1–1.9 years was significantly higher in EV71-infected HFMD cases than in HFMD outpatients, suggesting that EV71 infection occurred more often in inpatients than in outpatients among this age group. By contrast, the proportion of children ≥4 years was significantly lower in EV71-infected HFMD inpatients than in HFMD outpatients, suggesting that EV71 infection is less common among children ≥4 years. More than 30% of EV71-infected cases were seen in children 1–1.9 years and the fewest EV71-infected cases were seen in infants 0–5 months.

4.2. Seroprevalence of EV71 NAb

Of 614 sera samples tested, 122 (19.9%) showed EV71 NAb titers ≥1:8. Seropositive rates of EV71 NAb in different age groups are shown in Table 2 and Fig. 1. Between the 0.5–0.9 and the 2–2.9 age groups, there was a statistical age-specific difference in seroprevalence of NAb ($\chi^2 = 13.838, p = 0.017$). The seroprevalence of EV71 NAb was the highest among infants 0–5 months, as high as 28.6%. Besides, the seropositive rate of antibodies peaked up to 66.7% in neonates, then declined during the first 5 months of life. EV71 seropositivity slightly increased from 6 to 8 months of age, then decreased again at age 9 months and were undetectable at age 10–11 months. Seroprevalence of EV71 NAb showed an ascending trend with age increase among children 1–4 years. Seropositive rate of antibodies was significantly higher in children 3–4.9 years than in children 1–2.9 years ($\chi^2 = 10.167, p = 0.017$).

We observed that the age-specific seropositive rate of EV71 NAb is inversely correlated with age-specific case numbers of EV71-infected HFMD (Fig. 2). In addition, out of 90 children aged 1–5 years with serologically confirmed EV71 infection (Table 2), only 12 (13.3%) developed HFMD previously, suggesting that subclinical EV71 infection had occurred in 78 (86.7%) children. We rule out presence of maternal antibodies in these 78 children, which do not persist beyond 1 year of age.

4.3. Titer distribution of EV71 NAb by age groups

Titer distribution pattern of EV71 NAb were significantly different in each age group ($\chi^2 = 3854.451, p = 0.000$, shown in Table 3). Our data show that GMT of EV71 NAb was significantly lower in infants despite the overall highest seropositivity rate in this age group. The GMT of EV71 NAb was lowest in infants 6–11 months, similar to that seen in infants 0–5 months ($\chi^2 = 0.854, p = 0.355$).

Table 3

<table>
<thead>
<tr>
<th>EV71 NAb</th>
<th>&lt;0.5 y</th>
<th>0.5–0.9 y</th>
<th>1–1.9 y</th>
<th>2–2.9 y</th>
<th>3–3.9 y</th>
<th>4–4.9 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>9.1–99.4</td>
<td>9.0–89.7</td>
<td>8.6–512</td>
<td>9.1–512</td>
<td>9.1–512</td>
<td>9.1–512</td>
</tr>
<tr>
<td>GMT</td>
<td>22.50</td>
<td>17.05</td>
<td>54.01</td>
<td>126.6</td>
<td>67.67</td>
<td>121.6</td>
</tr>
<tr>
<td>95% CI</td>
<td>16.2–31.3</td>
<td>10.46–27.8</td>
<td>21.1–138.6</td>
<td>59.5–289.1</td>
<td>36.9–124.0</td>
<td>67.3–219.8</td>
</tr>
</tbody>
</table>

y indicates years of age.
5. Discussion

Epidemiological surveillance in Japan, Malaysia, and Taiwan showed that epidemics of EV71 follow a 2–3 years’ cyclical pattern.\textsuperscript{5,10–12} It is generally thought that this pattern is due to increased herd immunity among the susceptible children after a major outbreak. In this study we describe seroprevalence of EV71 in Shanghainese children after the large epidemic of EV71-associated HFMD in 2010. Our results show that the overall seroprevalence of EV71 NAb in Shanghainese children (19.9%) was slightly lower than the 24% observed in children in Taipei in 1999, after the 1998 outbreak.\textsuperscript{23} We noticed that more severe HFMD occurred in infants in Taiwan between 1998 and 2005 than in Shanghai between 2007 and 2010 (27.8% vs 5.9%).\textsuperscript{18,22} We found that the seropositive rate of EV71 NAb in children aged 6–11 months was much higher in Shanghai than in Taiwan (16.7% vs 4%),\textsuperscript{24} suggesting that Taiwanese children become susceptible to EV71 earlier, at infancy, while Shanghainese children become susceptible to EV71 from 1 year of age. This could be explained by the fact that most children in China live in one-child households and are less frequently exposed to other children before 1 year of age. Although we could not compare seroincidence of EV71 infection among susceptible children before and after the EV71 outbreak in Shanghai, it was striking to notice that seroprevalence of EV71 infection in young children was much lower in Shanghai after the 2010 outbreak than in other regions of China in 2005.\textsuperscript{25}

Previous studies showed that seroprevalence of EV71 NAb rises with age during early childhood and reaches a plateau in school children.\textsuperscript{23,24,26–28} The age-dependent ascending pattern of seroconversion rates of EV71 NAb among children aged 1–4 years in this study is consistent with previous studies. This study demonstrates that seroprevalence of EV71 NAb is inversely correlated with the age-specific HFMD case number. These results indicate that individual susceptibility to EV71 depends on the protective level of EV71 NAb.

We observed that the level of EV71 NAb steadily decreased during the first 5 months but remained at a plateau level of 14–25% until the age of 9 months and the age groups of 10 and 11 months showed no NAb. This differs to studies from Taiwan and Vietnam which showed that maternal-derived antibodies against EV71 decreased to almost undetectable level at 6 months.\textsuperscript{28,29} Although we are uncertain how long maternal-transferred EV71 antibodies persist during infancy, this serological survey suggests that maternal-derived EV71 NAb could persist up to 9 months of age. The study from Vietnam demonstrated that maternal-derived EV71 NAb could not be detected in any of infants 9 or 12 months of age.\textsuperscript{28}

Our study shows that 67% of neonates had EV71 NAb, indicating that most pregnant women living in Shanghai have EV71 NAb. This finding is similar to a recent study from Jiangsu Province, showing 79.7% of pregnant women seropositive for EV71 NAb during 2007–2009.\textsuperscript{10} As EV71 has been widely circulating in China since 2007, most of childbearing women should be naturally infected with EV71 and the levels of maternal protective antibodies are boosted through recurrent exposure. Seropositive rates of EV71 NAb reported outside mainland China were lower, 51% and 55% at birth in Taiwan and Vietnam, respectively.\textsuperscript{28,29}

Altogether, our data indicate that loss of maternal antibodies at late infancy and lack of acquired anti-EV71 immunity are responsible for the increased proportion of severe HFMD in the 1–2 years age group. Our data also suggest that future vaccination campaigns should be initiated as early as 6 months.

We believe that subclinically EV71-infected children serve as a source of continued spread of EV71 in the population. Inapparent EV71 infection ranged from 71% to 100% based on the serological evidences of EV71 infection in Taiwan and Vietnam.\textsuperscript{23,28} We observe that 86.7% of children age 1–4 years with serologically confirmed EV71 infection had never developed HFMD. Since the nationwide outbreak of HFMD in mainland China, it has been compulsory to keep sick children at home for 2 weeks. Indeed, we observed that the number of institutional outbreaks of HFMD in children declined in Shanghai. However, the outbreak among home-cared younger children is more difficult to control.\textsuperscript{19}

This study reflects that herd immunity against EV71 infection is at a lower level among young children in Shanghai even after the outbreak of EV71 infection. Hence, immunologic protective barrier cannot be built through natural infection. It is imperative to develop a safe and effective vaccine and a therapeutic agent against EV71 for children to control EV71 outbreak and reduce EV71-associated disease burden and fatality.

Funding

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Competing interests

None.

Ethical approval

The Ethics Committee of Children’s Hospital of Fudan University approved this study.

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Appendix A. Supplementary data


References