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ORIGINAL ARTICLE

# Predicting severe enterovirus 71 infection: Age, comorbidity, and parental behavior matter



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Received 4 September 2014; received in revised form 7 November 2014; accepted 17 November 2014  
Available online 22 November 2014

## KEYWORDS

developmental delay;  
enterovirus 71;  
hand–foot–mouth  
disease;  
hand hygiene;  
herpangina;  
risk factors

**Objective:** Enterovirus 71 (EV71) is one of the major pathogens that cause severe enteroviral infections. Our aim was to study the behavioral and household risk factors for its serious complications.

**Methods:** Between May 2011 and November 2012, we enrolled children who had symptoms of EV71 infection from six hospitals in Taiwan. The caregivers of each patient were interviewed to determine their hand hygiene habits in relation to EV71 infection. The severity of EV71 infection was classified as follows: Stage 1, hand–foot–mouth disease or herpangina; Stage 2, meningitis or myoclonic jerk; Stage 3A, encephalitis; Stage 3B, cardiopulmonary failure. Stages 2 to 3B were defined as severe EV71 infection. Children with Stages 3A and 3B infection were designated as the critical group.

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**Results:** A total of 399 patients had laboratory-confirmed EV71 infection. Three risks factors were associated with the different degrees of severity in EV71 infection. Children <2 years old had much greater risks for severe EV71 infection [odds ratio (OR) 1.8; 95% confidence interval (CI), 1.2–2.8], delayed medical evaluation for critical infection (OR 9.4; 95% CI, 3.6–24.1), and developmental retardation for cardiopulmonary failure (OR 8.3; 95% CI, 2.0–33.7). Among all the habits and household factors, caregivers in the critical group had a significantly lower rate in terms of cleaning the faucet after washing their hands (OR 2.63; 95% CI, 1.14–6.08).

**Conclusions:** Children <2 years old, developmental retardation, and delayed medical intervention were associated with severe EV71 infection. Cleaning water faucets after hand washing was a protective habit that reduced the risk of complications.

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## Introduction

Enterovirus 71 (EV71), which belongs to the Picornaviridae family, induces both hand–foot–mouth disease (HFMD) and herpangina.<sup>1</sup> Compared with other enteroviruses, EV71 is notorious for its severe neurological and systemic complications, specifically in young children.<sup>2–5</sup> Unfortunately, little is known about the predisposing factors for these critical complications, which makes it more difficult to prevent this easily transmitted infection. Although most patients may fully recover from HFMD, the high mortality rate exacts a dreadful toll, especially when an unfavorable outcome occurs.<sup>2,6</sup> Early recognition of the critical symptoms and improving supportive management are the main strategies adopted to lower the morbidity and fatality rates among the infected patients.<sup>3,7–10</sup>

Several risk factors have been reported in enterovirus (EV) epidemiological studies. For instance, children younger than 3 years and those who attended kindergarten were significantly predisposed to EV71 infection.<sup>11–13</sup> However, these results only suggested susceptible hosts to enteroviral infection, and failed to point out the risks of serious complications. Hand hygiene was proposed to reduce the chances of contracting HFMD and herpangina, but its effectiveness in preventing severe complications is still unknown.<sup>1,14</sup> Moreover, the daily activities of young children are heavily dependent on their caregivers, which indicates that the adults' behavior may greatly affect the children's health. Thus, we conducted a multi-institutional study in three different areas in Taiwan between 2011 and 2012. By interviewing the caregivers, we analyzed the factors associated with the patients' clinical outcomes, such as awareness of severe symptoms, basic knowledge of EV infection, and personal hygiene. We hope that the results will encourage the public to modify their behavior or living environment in order to decrease the risks of EV71 complications.

## Material and methods

### Setting and case definition

We enrolled children in six hospitals, which are located in three major metropolitan areas (Taipei, Taichung, and

Kaohsiung) in Taiwan. These facilities included three tertiary hospitals and three regional hospitals, representing 600 pediatric beds in total. This study was approved by the institutional review boards in all six hospitals. Between May 2011 and November 2012, children exhibiting symptoms of EV infection, either HFMD or herpangina, were enrolled. Signed consent forms were obtained from the patients' guardians. Patients were included for further analysis if the pathogens were confirmed to be EV71 by virological tests and when informed consent forms had been duly obtained. At the outset, we classified EV71 cases into four categories.<sup>2–5</sup> Uncomplicated children had mild symptoms (e.g., HFMD, herpangina, or fever) without any central nervous system (CNS) or systemic involvement (Stage 1). Children with severe EV71 infection, by contrast, presented neurological or systemic symptoms. These conditions included myoclonic jerk or meningitis (Stage 2), encephalitis (Stage 3A), and CNS involvement with cardiopulmonary failure (Stage 3B). Those having encephalitis or cardiopulmonary failure (Stages 3A and 3B) were defined as the critical group.

### Interview and records

We interviewed each participant's caregiver(s) during the enrollment stage of our study. The following aspects were investigated: patients' demographic data, socioeconomic status of the family, residential and childcare conditions, the caregivers' awareness regarding EV71 infection, and their hand hygiene habits.

### Laboratory methods

Two throat swabs were collected from each patient for viral isolation and molecular typing (real-time reverse transcription polymerase chain reaction). If the child has symptoms lasting for more than 4 days prior to enrollment, two additional rectal swabs would be collected for identification of viral etiology. A detailed description of the methods and materials has been delineated in our previous studies.<sup>15,16</sup> EV71 infection was confirmed on the basis of positive results from viral isolation with or without positive EV71 VP1 typing.

## Statistical analysis

We analyzed the clinical outcomes of all participants and the associations among disease severity and the possible predisposing factors. The Student *t* test and analysis of variance were used for continuous variables with normal distributions, and a chi-square test was performed to compare the categorical variables. Multiple logistic regression was applied for multivariate analysis. A *p* value less than 0.05 was considered statistically significant. The statistical analysis was processed using SPSS Ver. 20 (IBM, New York, USA) and SAS Statistical Package, version 9.4 (SAS Institute, Cary, NC, USA).

## Results

### Demography and outcomes

Overall, 399 participants with laboratory-confirmed EV71 infection were enrolled in our study. The monthly distribution of patient enrollment is demonstrated in Fig. 1. Most of the children were younger than 5 years (mean age,  $3.3 \pm 2.6$  years). Although boys outnumbered girls in this cohort by 1.5 to 1 (239/160), sex ratios were similar among the four groups. We compared the risks of severe EV71 infection between children younger and older than 2 years (Table 1). Children younger than 2 years had much greater chances of having severe EV71 infections [Stages 2–3B: 44%, 108/246, odds ratio (OR) 1.8, 95% confidence interval (CI) 1.2–2.8,  $p = 0.006$ ]. Among the prerequisite disorders, children with delayed development had increased risks of severe EV71 infection ( $p = 0.03$ ). They had significantly increased risks of cardiopulmonary failure, compared with children with normal development (OR 8.3; 95% CI, 2.0–33.7;  $p = 0.005$ ). Moreover, the majority of children (48/53, 91%) in the critical group (Stages 3A and 3B) did not receive medical attention within the first 2 days after their symptoms had emerged, compared with 51% (175/346) of those in noncritical groups (OR 9.4; 95% CI, 3.6–24.1).

As for the clinical outcomes, the majority of our participants completely recovered from the illness (392/399,

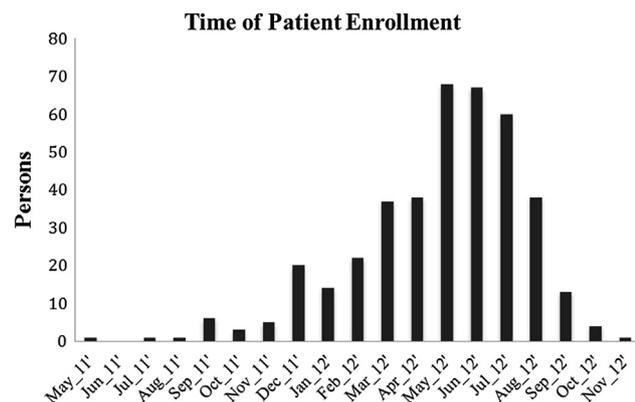
97.5%). However, two patients died (2/399, 0.5%) and five had neurological sequelae (5/399, 2%). More than half of the children (246/399, 62%) had complications, including meningitis, encephalitis, and cardiopulmonary failure. In particular, the children with CNS and cardiopulmonary failure had much greater risks of unfavorable outcomes, compared with those without systemic involvement ( $p < 0.001$ ).

### Caregivers' knowledge and behaviors on hand hygiene

Among the 399 EV71-infected patients, 397 (99.5%) had complete interviews. We noticed that the parents' education levels and other household and childcare factors were not associated with severe EV71 infection (Table 2). Among the questions aiming to evaluate the caregivers' knowledge on EV infection, two topics showed marked variation among the different groups. First, we asked the caregivers whether adults of the general population can be infected by EV. The caregivers in the critical group had a much lower correction rate compared with that in the noncritical group (56% vs. 72%,  $p = 0.01$ ). Second, the understanding of the incubation period of EV infection was diverse among the four groups, and the correction rates were significantly lower in the two critical groups ( $p = 0.02$ ).

Hand hygiene has been considered an effective measure to prevent EV infection.<sup>3,7–10,14,17</sup> We further investigated whether severe EV71 infection was associated with the caregivers' hand washing habits (Table 3). First, we asked whether the interviewees understood the details in maintaining hand hygiene. In the following section, we wanted to know if the caregivers implemented these preventive measures as part of their daily routine. Most of the interviewees were aware of the details of hand hygiene. However, the awareness about hand hygiene was much lower in critical groups, with a significant difference noted in acknowledging the impact of hand washing in preventing EV infection ( $p = 0.005$ ). When we looked into the disparity of caregivers' habits between critical and noncritical groups, the results were diverse on practicing the following activities: cleaning the faucet after hand washing (60% vs. 76%,  $p = 0.01$ ), washing hands before hugging the children (21% vs. 39%,  $p = 0.01$ ), and washing hands before dining (63% vs. 79%,  $p = 0.02$ ).

To examine the connection between the severity of EV71 infection and the caregivers' habits, we conducted multivariate analysis with the adjustment of the following elements: patient's age and sex, and parental factors (Table 4). The results showed that if caregivers did not routinely clean the faucets after washing their hands, by either flushing the faucet with clean water or wiping the faucet with towels, the OR of severe EV71 infections would be increased to 2.63 (95% CI, 1.14–6.08).



**Figure 1.** Monthly distribution for the recruitment of enterovirus 71 (EV71) patients. Most patients were enrolled during the summer season, when enterovirus actively circulated.

## Discussion

This study examined the risk factors associated with severe EV71 infection in multiple medical institutes in Taiwan. Our trajectory was that the caregivers' habits and their awareness might play a decisive role on the children's

**Table 1** Demographic factors and clinical outcomes of EV71-infected patients

| Demography/outcome                        | EV71 severity                      |  |                                  |   | <i>p</i> |
|---|------------------------------------|--|----------------------------------|---|----------|
|   | Uncomplicated<br>( <i>n</i> = 153) | Meningitis/myoclonic<br>jerk ( <i>n</i> = 193) | Encephalitis<br>( <i>n</i> = 35) | CNS involvement<br>with PE ( <i>n</i> = 18) |          |
| Mean age ± SD (y)                         | 3.29 ± 2.64                        | 3.32 ± 2.62                                    | 2.23 ± 3.87                      | 2.49 ± 0.77                                 | 0.25     |
| Sex                                       |                                    |  |                                  |   |          |
| Male                                      | 92 (60%)                           | 114 (59%)                                      | 21 (60%)                         | 12 (67%)                                    | 0.94     |
| Female                                    | 61 (40%)                           | 79 (41%)                                       | 14 (40%)                         | 6 (33%)                                     |          |
| Age                                       |                                    |  |                                  |   |          |
| >2 y                                      | 107 (70%)                          | 107 (55%)                                      | 21 (60%)                         | 10 (56%)                                    | 0.05     |
| ≤2 y                                      | 46 (30%)                           | 86 (45%)                                       | 14 (40%)                         | 8 (44%)                                     |          |
| Prerequisite illness                      |                                    |  |                                  |   |          |
| Preterm birth                             | 8 (5%)                             | 19 (10%)                                       | 4 (11%)                          | 2 (11%)                                     | 0.37     |
| Developmental delay                       | 3 (2%)                             | 6 (3%)   | 0 (0%)                           | 3 (17%)                                     | 0.004    |
| Previous admission due to EV              | 15 (10%)                           | 29 (15%)                                       | 2 (6%)                           | 0 (0%)                                      | 0.10     |
| Interval between onset and hospital visit |                                    |  |                                  |   |          |
| 0–2 d                                     | 69 (45%)                           | 102 (53%)                                      | 4 (11%)                          | 1 (6%)                                      | <0.001   |
| 3–8 d                                     | 84 (55%)                           | 91 (47%)                                       | 31 (89%)                         | 17 (94%)                                    |          |
| Clinical outcome                          |                                    |  |                                  |   |          |
| Full recovery                             | 153 (100%)                         | 193 (100%)                                     | 34 (97%)                         | 12 (67%)                                    | <0.001   |
| Morbidity                                 | 0 (0%)                             | 0 (0%)   | 1 (3%)                           | 4 (22%)                                     |          |
| Fatality                                  | 0 (0%)                             | 0 (0%)   | 0 (0%)                           | 2 (11%)                                     |          |

CNS = central nervous system; EV = enterovirus; SD = standard deviation.

**Table 2** Demographic data and the caregivers' knowledge associated with EV71 Infection

| Caregivers' characteristics                           | EV71 severity                      |  |                                  |   | <i>p</i> |
|---|------------------------------------|--|----------------------------------|---|----------|
|   | Uncomplicated<br>( <i>n</i> = 153) | Meningitis/myoclonic<br>jerk ( <i>n</i> = 192) | Encephalitis<br>( <i>n</i> = 34) | CNS involvement<br>with PE ( <i>n</i> = 18) |          |
| <i>Interviewee</i>                                    |                                    |  |                                  |   |          |
| Relationship with patient                             |                                    |  |                                  |   |          |
| Father  | 30 (20%)                           | 38 (20%)                                       | 8 (23%)                          | 1 (6%)                                      | 0.60     |
| Mother  | 118 (77%)                          | 149 (78%)                                      | 24 (71%)                         | 17 (94%)                                    |          |
| Others  | 5 (3%)                             | 5 (3%)   | 2 (6%)                           | 0 (0%)                                      |          |
| Father's education                                    |                                    |  |                                  |   |          |
| Senior high school and below                          | 64 (42%)                           | 88 (46%)                                       | 17 (50%)                         | 11 (61%)                                    | 0.41     |
| College and above                                     | 89 (58%)                           | 104 (54%)                                      | 17 (50%)                         | 7 (39%)                                     |          |
| Mother's education                                    |                                    |  |                                  |   |          |
| Senior high school and below                          | 66 (43%)                           | 79 (41%)                                       | 15 (44%)                         | 8 (44%)                                     | 0.97     |
| College and above                                     | 87 (57%)                           | 113 (59%)                                      | 19 (56%)                         | 10 (56%)                                    |          |
| Household information                                 |                                    |  |                                  |   |          |
| Daycare at home                                       | 58 (38%)                           | 100 (52%)                                      | 16 (47%)                         | 7 (39%)                                     | 0.06     |
| Having siblings                                       | 109 (71%)                          | 136 (71%)                                      | 27 (79%)                         | 12 (67%)                                    | 0.73     |
| Living with other children                            | 15 (10%)                           | 27 (14%)                                       | 5 (15%)                          | 2 (11%)                                     | 0.65     |
| Patients have more than one caregiver                 | 109 (71%)                          | 138 (72%)                                      | 24 (71%)                         | 15 (83%)                                    | 0.75     |
| Family history of severe EV infection                 | 5 (3%)                             | 6 (3%)   | 0 (0%)                           | 1 (6%)                                      | 0.68     |
| Patient having HFMD history                           | 23 (15%)                           | 35 (18%)                                       | 5 (15%)                          | 0 (0%)                                      | 0.23     |
| Knowledge of EV infection                             |                                    |  |                                  |   |          |
| Clinical presentations of EV infection<br>in children | 142 (93%)                          | 181 (94%)                                      | 30 (88%)                         | 17 (94%)                                    | 0.63     |
| Clinical presentations of EV infection<br>in adult    | 102 (67%)                          | 148 (77%)                                      | 19 (56%)                         | 10 (56%)                                    | 0.02     |
| Transmission route of EV infection                    | 151 (99%)                          | 190 (99%)                                      | 34 (100%)                        | 18 (100%)                                   | 0.88     |
| Virulence of EV71 infection                           | 133 (87%)                          | 172 (90%)                                      | 29 (85%)                         | 15 (83%)                                    | 0.75     |
| Incubation period of EV infection                     | 152 (99%)                          | 179 (93%)                                      | 32 (94%)                         | 16 (89%)                                    | 0.02     |

CNS = central nervous system; EV = enterovirus; HFMD = hand, foot, and mouth disease; PE = pulmonary edema.

**Table 3** Caregivers' knowledge and behavior on hand hygiene associated with EV71 severity

| Knowledge and behavior  | EV71 severity                      |  |                                  |   | <i>p</i> |
|---|------------------------------------|--|----------------------------------|---|----------|
|   | Uncomplicated<br>( <i>n</i> = 153) | Meningitis/myoclonic<br>jerk ( <i>n</i> = 192) | Encephalitis<br>( <i>n</i> = 34) | CNS involvement<br>with PE ( <i>n</i> = 18) |          |
| <b>Knowledge on hand hygiene</b>                                |                                    |  |                                  |   |          |
| Using soap to maintain hand hygiene                             | 149 (97%)                          | 188 (98%)                                      | 31 (91%)                         | 17 (94%)                                    | 0.17     |
| Cleaning hands >20 s  | 137 (90%)                          | 175 (91%)                                      | 27 (79%)                         | 16 (89%)                                    | 0.24     |
| Flushing the faucet after hand cleansing                        | 151 (99%)                          | 191 (99%)                                      | 34 (100%)                        | 18 (100%)                                   | 0.76     |
| Drying hands to decrease pathogen survival after hand cleansing | 122 (80%)                          | 154 (80%)                                      | 24 (71%)                         | 12 (67%)                                    | 0.36     |
| Washing hand is effective to prevent EV infection               | 152 (99%)                          | 190 (99%)                                      | 33 (97%)                         | 16 (89%)                                    | 0.005    |
| <b>Behavior on hand hygiene</b>                                 |                                    |  |                                  |   |          |
| Using soap in hand cleansing                                    | 84 (55%)                           | 95 (49%)                                       | 14 (41%)                         | 8 (44%)                                     | 0.44     |
| Cleaning hands >20 s  | 63 (41%)                           | 87 (45%)                                       | 13 (38%)                         | 6 (33%)                                     | 0.66     |
| Cleaning the faucet after hand washing                          | 116 (76%)                          | 145 (76%)                                      | 19 (56%)                         | 12 (67%)                                    | 0.08     |
| Drying hands after hand cleansing                               | 113 (74%)                          | 136 (71%)                                      | 24 (71%)                         | 9 (50%)                                     | 0.22     |
| Washing hands after touching the nostrils                       | 101 (66%)                          | 121 (63%)                                      | 19 (56%)                         | 7 (39%)                                     | 0.12     |
| Washing hands before hugging the children                       | 58 (38%)                           | 76 (40%)                                       | 8 (24%)                          | 3 (17%)                                     | 0.09     |
| Washing hands before dining                                     | 125 (82%)                          | 146 (76%)                                      | 21 (62%)                         | 12 (67%)                                    | 0.06     |
| Washing hands right after arriving home                         | 105 (69%)                          | 133 (69%)                                      | 24 (71%)                         | 8 (44%)                                     | 0.18     |
| Changing clothes or bathing after work and arriving home        | 55 (36%)                           | 77 (40%)                                       | 8 (24%)                          | 5 (28%)                                     | 0.25     |

CNS = central nervous system; EV71 = enterovirus 71; PE = pulmonary edema.

illness. We interviewed the caregivers in the beginning of the medical evaluation to avoid any bias caused by the patient's clinical outcome and the health education received during the treatment period. Through a series of

inquiries, we observed that most of the caregivers were aware of the measures against EV infection. However, the practice rates of these behaviors were much lower. Under a stringent analysis by logistic regression, cleaning the faucet after hand washing proved to be the key behavior associated with the severity of the children's EV71 infection. To our knowledge, this is the first study to report on the impact of the caregivers' awareness and hand hygiene habits on the severity of EV71 infection in children.

Hand hygiene is considered a cost-effective protocol for infection control in hospitals and other healthcare facilities.<sup>11–13,18,19</sup> Maintaining hand hygiene has been demonstrated to halt the spread of numerous respiratory and gastroenteric viruses.<sup>20–23</sup> By using soap and water, this simple practice reduced the colonization of polio-1 virus by 2–4 logs.<sup>24</sup> Yet, little evidence concludes the impact of hand hygiene on preventing EV71 infection. In 2011, Ruan et al<sup>14</sup> reported that hand washing substantially reduced the rates of HFMD and herpangina. This study, nevertheless, did not further investigate the viral etiology of HFMD and herpangina, nor did it examine whether these methods could significantly reduce the severe complications. In our study, the act of cleaning faucets after hand washing significantly reduced the risk of EV71 complications. The other two habits—washing hands before dining or before hugging children—failed to show significance as examined by logistic regression. It might well be that manual faucets could be easily contaminated with microbes and viruses.<sup>25,26</sup> The habit of cleaning faucets might have also decreased the viral loads and thus limited the transmission rates. Moreover, cleaning faucets after hand washing was a more sophisticated habit in maintaining hand hygiene. Those who cleaned faucets after washing their hands might

**Table 4** Risk factors of EV71 severity associated with caregivers' knowledge and performance between critical and noncritical groups

|  | Unadjusted          |          | Adjusted*           |          |
|--|---------------------|----------|---------------------|----------|
|  | OR (95% CI)         | <i>p</i> | OR (95% CI)         | <i>p</i> |
| Not aware of the clinical presentations of EV infection in adult | 2.09<br>(1.15–3.79) | 0.01     | 1.69<br>(0.78–3.65) | 0.19     |
| Do not clean the faucet after washing hands                      | 2.14<br>(1.15–3.99) | 0.02     | 2.63<br>(1.14–6.08) | 0.02     |
| Do not wash hands before hugging children                        | 2.48<br>(1.20–5.13) | 0.01     | 1.93<br>(0.72–5.16) | 0.19     |
| Do not wash hands before dining                                  | 2.11<br>(1.13–3.92) | 0.02     | 1.41<br>(0.57–3.47) | 0.45     |

Cases of encephalitis (Stage 3A), and CNS involvement with pulmonary edema (Stage 3B) were defined to be the critical group and cases of uncomplicated HFMD (Stage 1) and myoclonic jerk or meningitis (Stage 2) were defined as noncritical group. CI = confidence interval; CNS = central nervous system; EV71 = enterovirus 71; HFMD = hand, foot, and mouth disease; OR = odds ratio; SD = standard deviation.

\* Adjusted factors include patient's age and sex, and parental factors.

be more concerned about preventing transmission of pathogens to children than those who did not.

Young age ( $\leq 3$  years old) and several clinical manifestations (i.e., fever of more than 3 days, lethargy, and leukocytosis) were associated with critical illness in EV71-infected children.<sup>7,11</sup> We noticed that children were more susceptible to severe EV71 infection if they were younger than 2 years, or if they received medical evaluation in hospitals more than 3 days after the initial symptoms appeared. It might result from the delayed recognition of illness or insufficient health education on the part of their caregivers. Furthermore, we found that children with delayed development were significantly associated with cardiopulmonary complications in EV71 infection (OR, 8.3). These young children might not be able to express their symptoms clearly at the initial stage of infection, which makes it more difficult for their caregivers to discover the illness. To our knowledge, no studies have reported that children with retarded growth are predisposed to severe EV71 infection, nor to other types of EV infection. This underlines the importance of providing information on the preliminary signs of severe EV71 infection not only to the general public, but also to families with children requiring special attention. It is also important to emphasize the need for children to receive proper medical evaluation when the symptoms have persisted for more than 3 days.

The Taiwan Centers for Disease Control has established a surveillance network for EV infection, particularly for cases with severe complications. The health authorities have also disseminated information on the numerous methods for preventing EV transmission.<sup>27</sup> In our study cohort, parental age and education status were not associated with critical illness in EV71-infected children, nor were other household factors. Most caregivers were familiar with the methods for EV prevention regardless of their socioeconomic status. Yet, the key factor was the practice of hand hygiene. This suggests that the authorities and medical personnel should develop new strategies to promote the habit of maintaining hand hygiene. We also observed that children's sex and their attendance at kindergarten were not associated with severe EV71 infection. These results were slightly different from those observed in 2002 in Taiwan. The preceding predictors for EV71 infection might not correspond to the present indicators for severe complications.<sup>12</sup> This may indicate that the current risks for severe EV71 infection are greatly reduced in those previously reported susceptible hosts, as hygiene and public health education have improved since then.

Since the implementation of stage-based management in treating patients with severe EV, mortality and morbidity rates have considerably improved in Taiwan. The overall complication rates in severe cases were substantially reduced in the past 10 years, especially when compared with the results in 2004.<sup>8</sup> In our cohort, the full recovery rate was 97% in those with encephalitis, compared with 82% in children with the same severity scale in 2004. Moreover, the morbidity rate in those presenting with pulmonary edema decreased from 43% to 22%. This suggests that the practice of stage-based management greatly enhanced the quality of life of this particular group of children.

In our study, we evaluated the caregivers' behaviors and their knowledge on EV prevention in order to clarify their

impact on the severity of EV71 infection among children. Although we interviewed the main caregivers, it was difficult to verify the time they actually spent with their sick children and their roles in nursing the children on the daily basis. To minimize potential bias from the interviewees, we recruited the largest cohort of patients with severe EV71 and their caregivers, compared with previous studies for EV71 behavioral risk factors. We also evaluated the associated factors, such as housing environment, childcare condition, and parents' socioeconomic status. The results indicated these factors did not contribute to the severity of EV71 infection in our cohort. However, the habit of maintaining optimal hand hygiene affected the risks of serious complications. Our findings not only promote the role of hand hygiene in reducing EV transmission but also suggest that more epidemiological studies need to be conducted to search for more behavioral risk factors that could be critical to severe EV infection.

In conclusion, our study examined the influence of caregivers' awareness and hand hygiene habits on the severity of children's EV71 infection. Cleaning faucets after hand washing, which was the most critical factor, greatly reduced the risks of critical EV71 infection. We also observed that children younger than 2 years and delay in receiving medical care in hospitals were significantly associated with severe complications. Special consideration should be given to children with developmental retardation owing to their significant risks for cardiopulmonary failure.

## Conflicts of interest

All the authors have no conflicts of interest to disclose.

## Acknowledgments

This study was supported by National Science Council grants, NSC 99-2321-B-002-025, NSC 100-2321-B-002-012, and NSC 101-2321-B-002-004.

Dr W.-C. Huang carried out the analysis, drafted the initial manuscript, and approved the final manuscript as submitted. Drs S.-C. Yang, T.-Y. Yen, Y.-C. Huang, J.-T. Lee, C.-C. Li, Y.-C. Hsieh, T.-Y. Lin enrolled the patients, supervised the data, and revised the manuscript. Dr W.-L. Shih performed the data analysis and revised the manuscript. Dr L.-M. Huang critically reviewed the manuscript and approved the final manuscript as submitted. Dr L.-Y. Chang conceptualized, designed, and coordinated the study; critically reviewed the manuscript; and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and have agreed to be accountable for all aspects of the work.

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