A Case-Control Study to Identify Environmental Risk Factors for Hand, Foot, and Mouth Disease Outbreaks in Beijing

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SUMMARY: A matched case-control study was conducted in Beijing to identify the relative importance of major environmental risk factors for outbreaks of hand, foot, and mouth disease (HFMD). A case was defined as a kindergarten class with at least 1 HFMD outbreak. As a control, a kindergarten class that did not experience an HFMD outbreak was used. To identify potential transmission factors, the control group was divided into 2 subgroups: a sporadic group and an HFMD-negative group. We collected data for 8 environmental factors and basic information of each class. The correlations between the suspected environmental factors and HFMD outbreaks were analyzed. Thirty outbreak classes, 19 sporadic classes, and 30 HFMD-negative classes were enrolled. Class grade, indoor solar radiation, and ultraviolet (UV) radiation revealed significant differences among the 3 groups. After controlling for other factors, UV radiation (adjusted \( \beta = -0.42 \)) and class grade (adjusted \( \beta = -0.46 \)) as protective factors and temperature (adjusted \( \beta = 0.31 \)) as a risk factor were significantly associated with the attack rate by multiple linear regression analysis. Logistic regression analysis showed that the probability of an HFMD outbreak in the lower grade was 6-fold greater than that in the higher grade (\( P = 0.0380 \), odds ratio = 0.157, 95% confidence interval = 0.027–0.903). We identified UV radiation and class grade as protective factors that were associated with the epidemic intensity in Beijing. However, more data is needed to evaluate the relationship between these factors and HFMD outbreaks.

INTRODUCTION

In China, hand, foot, and mouth disease (HFMD) is a notifiable disease, which is caused by several members of the genus Enterovirus, including enterovirus 71 (EV71) and coxsackievirus A16 (CVA16). HFMD primarily affects infants and children and is characterized by fever, skin eruptions on the hands and feet, and oral vesicles. Since the first documented case in 1957 (1), outbreaks and epidemics of HFMD have been reported worldwide (2–5). HFMD has become a notifiable infectious disease in China since 2008, with a high transmission capacity. In China, surveillance of HFMD was established in May 2008 and since then, the average number of reported HFMD cases in Beijing has reached approximately 30,000 annually, resulting from 36 to 81 outbreaks. In 2012, for example, there were 38,558 diagnosed cases of HFMD, of which 355 were considered severe. At least 2% of HFMD cases are directly caused by initial outbreaks, whereas the number of secondary infections is much greater (6–8). Therefore, the development of prevention and control methods is the key to control the spread of HFMD. Surveillance data collected in Beijing from 2008 to 2011 has shown that the prevalence of HFMD is obviously seasonal (the peaks appear in July), and suburban and preschool clustering is evident (6–8). On the basis of previous correlational studies (9–13), we speculated that environmental factors, including humidity, temperature, and atmospheric pressure, may be relative to incidence of HFMD outbreaks. However, only correlational studies, exploring the relationship between environmental factors and the HFMD incidence rate, have been reported. Here we conducted a case-control study to identify risk or protective environmental factors by comparing the characteristics of HFMD outbreaks among kindergarten-aged children in Beijing from January to July 2011.

METHODS

Study design: A matched case-control study was performed among kindergarten classes in Beijing at a ratio of 1:1:1. A case was defined as a kindergarten class with at least 1 HFMD outbreak between January and July 2011. Both registered kindergartens and non-registered kindergartens were included in this study. An HFMD outbreak was defined in accordance with the following criteria of the 2010 HFMD Surveillance Program in Beijing: (i) 3 or more clinical HFMD cases in 1 class within 1 day and (ii) 5 or more clinical HFMD cases within 3 successive days in the same class. A class that met any 1 of the above-described conditions was considered as experiencing an outbreak. A kindergarten class with no reported incidence of an HFMD outbreak was used as a control. To identify potential factors responsi-
ble for the spread of HFMD, the control group was divided into 2 subgroups: a sporadic group and an HFMD-negative group. The sporadic group included kindergarten classes that had sporadic cases of HFMD, but did not meet the outbreak criteria. The HFMD-negative groups consisted of kindergarten classes without HFMD patients from January to July 2011.

In China, HFMD is clinically diagnosed according to the 2009 edition to the HFMD Diagnosis and Treatment Guidelines (14), which specify that (i) during the epidemic season, HFMD is common in infants and young children, and (ii) the primary clinical syndromes are rashes of the hands, feet, mouth, and buttocks with fever. Some cases may not experience fever. A list of HFMD cases and outbreak classes can be acquired from the China Information System for Disease Control and Prevention, in which all cases of notifiable diseases are reported in accordance with the Chinese law (http://1.202.129.170). Once a kindergarten class with an outbreak was recruited, 1 matched sporadic class and 1 HFMD-negative class was randomly selected by the following method: if more than 1 outbreak, sporadic, or HFMD-negative kindergarten class was eligible, we selected only 1 using a random number table.

To calculate the sample size, we assumed a case-control ratio of 1:1:1 and 8 environmental factors. A total of 80 case-control sets were recruited to detect correlations between the incidence of HFMD and environmental factors using multiple linear regression analysis or logistic regression analysis (15), which indicated that each of the 8 environmental factors required 10 samples for the multiple analyses.

Data collection: We retrospectively surveyed 8 previously suspected indoor environmental factors for each group (9, 10, 12, 13, 16), including (i) indoor temperature and humidity, which were measured using a tape measure; (ii) effective ventilation area, for which the areas of all operable doors and windows were measured using a tape measure (if the doors or windows were inoperable, the corresponding area was excluded); (v) inhaled particulate matter (PM10), which was measured using the LD-5C microcomputer Laser Dust Monitor device; (vi) the ratio of window to floor area (Ac/Ad), where the total area of all windows and the floor was measured using a tape measure and the inoperable areas were subtracted; (vii) indoor solar radiation using the Model ST-85 Autoranging illuminometer device; and (viii) indoor ultraviolet (UV) radiation using the UV-B-254 UV illuminometer device.

The methods used to measure indoor temperature, indoor relative humidity, PM10, indoor solar radiation, and indoor UV radiation were similar and performed using 3 measuring points, of which 2 were in the opposing corners of the room and third in the center of the diagonal of the room, from which each point was measured for at least 2 min. Besides the baseline data, such as the male/female ratio of the surveyed classes, other data regarding tuition, number of kindergarten workers, the attack rate in a week, and grade of surveyed classes (most kindergartens have 3 grades: the lowest consists of 2- to 3-year-old children, the middle of 4-year-olds, and the highest of 5-year-olds) was collected from the person in charge of the kindergarten class. Information on outbreaks, such as the number of cases in each outbreak, throat swab test results, and primary symptoms, were collected from the China Information System for Disease Control and Prevention and outbreak reports. Well-trained staff from the Beijing Center of Disease Prevention and Control collected and recorded the data using previously designed structured survey tables.

Data management and statistical analysis: All data was entered into an Excel spreadsheet and analyzed using SAS 9.13 (SAS; Peking University, Beijing, China). Two researchers independently extracted data using a self-developed data extraction form. All input was verified by referencing the raw survey data tables. We calculated means and standard deviations to describe normally distributed continuous variables and medians and quartile ranges to summarize non-normally distributed continuous variables. Normally distributed data was analyzed using 2-way analysis of variance, in which kindergarten served as block factor to compare differences among the 3 groups for each factor. Otherwise, the Friedman M test was performed for analysis. In addition, we established a multiple linear regression model to screen relative factors and identify possible correlations between the attack rate and environmental factors. β and adjusted β were used to describe the degree of correlation. When possible, we attempted to use conditional logistic regression to control extraneous variables and access independent predictors. The odds ratio (OR) and 95% confidence interval (CI) were used to describe measurements among the 3 groups.

RESULTS

We surveyed a total of 32 kindergartens in Beijing that experienced HFMD outbreaks from January to July 2011, representing 59.3% of the total of 54 HFMD outbreaks during this period. Of the 32 kindergartens, 22 were located in the Fengtai district, 5 in the Haidian district, and 5 in the Tongzhou district. Two kindergartens were excluded because both had only 1 class each. In addition, there were only 19 kindergartens with an HFMD sporadic class. Finally, a total of 30 outbreak classes, 19 sporadic classes, and 30 HFMD-negative classes were included in this study (Fig. 1). A total of 305 cases were diagnosed in these 30 outbreaks. Only 1 severe case was positive for enterovirus nucleic acids. Of the 305 cases, 68 presented with fever, 228 with skin eruptions on the hands, 159 with skin eruptions on the feet, and 189 with oral vesicles. Owing to refusal by parents/guardians, only 77 throat swab samples were collected, of which 57 were positive (74% positive rate). Of these 57 positive samples, 24 (42%) were positive for EV71 and 18 (31%) for CVA16. Only 4 outbreaks were caused by more than 1 infectious agent. As shown in Table 1, the baseline characteristics of all groups were similar in terms of tuition, class scale, and gender ratio, but there was a significant difference in proportion of infections in the lowest grade of the kindergarten class (2-3-year-olds), which was closely related to age. Therefore, we included class grade with other environmental factors.

The 8 environmental factors were analyzed using
Environmental Factors for HFMD in Beijing

Table 1. Baseline characteristics of surveyed classes in Beijing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Outbreak class (n = 30)</th>
<th>Sporadic class (n = 19)</th>
<th>HFMD-negative class (n = 30)</th>
<th>F/χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuition (RMB)</td>
<td>963.0 ± 389.1</td>
<td>990.7 ± 418.6</td>
<td>963.1 ± 396.9</td>
<td>0.028</td>
<td>0.972</td>
</tr>
<tr>
<td>Class scale (no./class)</td>
<td>28.1 ± 6.1</td>
<td>27.0 ± 4.1</td>
<td>28.1 ± 7.3</td>
<td>0.232</td>
<td>0.793</td>
</tr>
<tr>
<td>Proportion of low grade (%)</td>
<td>73.3</td>
<td>64.7</td>
<td>29.6</td>
<td>0.423*</td>
<td>0.007</td>
</tr>
<tr>
<td>Gender ratio (male/female)</td>
<td>1.3 ± 0.55</td>
<td>1.0 ± 0.36</td>
<td>1.3 ± 0.54</td>
<td>1.594</td>
<td>0.210</td>
</tr>
</tbody>
</table>

*: Use χ² analysis to test the difference among 3 groups about proportion of low grade.

Table 2. Selected environmental factors in each group, Beijing, 2011

<table>
<thead>
<tr>
<th>Variable</th>
<th>Outbreak class</th>
<th>Sporadic class</th>
<th>HFMD-negative class</th>
<th>F/χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>18.2 ± 2.97</td>
<td>17.6 ± 3.0</td>
<td>17.68 ± 2.38</td>
<td>1.103</td>
<td>0.5761</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>43.52 ± 1.26</td>
<td>45.35 ± 1.33</td>
<td>44.57 ± 1.29</td>
<td>0.25</td>
<td>0.7808</td>
</tr>
<tr>
<td>Average area (m²)</td>
<td>1.70 ± 1.64</td>
<td>1.79 ± 1.458</td>
<td>1.65 ± 1.49</td>
<td>0.02</td>
<td>0.9825</td>
</tr>
<tr>
<td>Ventilation area (m²)</td>
<td>3.01 ± 1.64</td>
<td>3.12 ± 1.80</td>
<td>3.05 ± 1.70</td>
<td>0.0752</td>
<td>0.9631</td>
</tr>
<tr>
<td>AC/AD (m²)</td>
<td>6.22 ± 2.00</td>
<td>6.06 ± 6.09</td>
<td>6.19 ± 1.72</td>
<td>3.6566</td>
<td>0.1607</td>
</tr>
<tr>
<td>PM10 (mg/m³)</td>
<td>0.12 ± 1.75</td>
<td>0.144 ± 1.58</td>
<td>0.12 ± 1.68</td>
<td>3.9291</td>
<td>0.1402</td>
</tr>
<tr>
<td>Ultraviolet (μw/cm²)</td>
<td>5.43 ± 4.02</td>
<td>7.19 ± 3.38</td>
<td>9.35 ± 3.39</td>
<td>10.27</td>
<td>0.0002</td>
</tr>
<tr>
<td>Solar radiation (10² × lx)</td>
<td>1.69 ± 4.07</td>
<td>1.82 ± 3.11</td>
<td>2.91 ± 3.26</td>
<td>8.68</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

*: The variables do not meet normal distribution, use median, quartile range and Friedman’s M test to analyze those variables.
3: The reciprocal of AC/AD was used to describe the area ratio of window to floor.
SD, standard deviation.

univariate analysis for blocked designs with “kindergarten” designated as the block. As shown in Table 2, indoor solar radiation and indoor UV radiation were identified as significant protective factors among the 3 groups. The mean indoor solar radiation and indoor UV radiation were ranked as the lowest factors in the outbreak group. For multiple comparisons using the least significant difference t test, the outbreak group had significantly lower solar radiation density than the HFMD-negative group and significantly lower UV radiation density compared with the other groups at α = 0.05.
Because of the imbalance in baseline class grades among the 3 experimental groups, we included this disparity as a factor for multiple linear regression analysis to identify correlations between suspected factors and the attack rate. Collectively, these 9 factors could explain 36% of the variation in the attack rate ($R^2 = 0.3619$, adjusted $R^2 = 0.2662$). After controlling for all other factors in the multivariate analysis, indoor UV density and class grade remained as significant protective factors (Table 3) and were significantly associated with HFMD outbreaks in the univariate analysis. However, after adjusting for other factors, we found that temperature was significantly associated with the attack rate. Class grade (adjusted $\beta = -0.46$) and UV (adjusted $\beta = -0.42$) were identified as protective factors and temperature (adjusted $\beta = 0.31$) was identified as a risk factor in the multivariate analysis. Collinearity diagnosis analysis revealed a mild collinearity between UV and indoor solar radiation (condition index $= 8.62$).

We established a conditional logistic regression model to detect correlations between the epidemic intensity and suspected protective and risk factors. Owing to the unavailability of established criteria to evaluate environmental factors of kindergarten classes, the suspected factors were transformed into dichotomous data with medians of each. The class grade was also transformed into dichotomous data, which consisted of grouping of middle and high grades in 1 part and low grade in the other part. We found that only class grade was a protective factor significantly associated with the epidemic intensity ($P = 0.0380; \text{OR} = 0.157; 95\% \text{ CI} = 0.027-0.903$). Furthermore, our results indicated that the probability of an HFMD outbreak in a low grade was 6-fold greater than that in a higher grade.

## DISCUSSION

HFMD is a notifiable infectious disease that primarily affects infants and children, and more than 1.6 million cases were reported in 2008 and 2009 throughout mainland China (17). Therefore, medical care required for HFMD treatment is demanding of healthcare resources. Based on current surveillance data and previous studies (5,7,11), the 3-dimensional distribution of people, time, and place showed obvious correlations to the factors of season, suburban, and childhood aggregation in China. Therefore, we speculated that the incidence of an HFMD outbreak may be associated with environmental factors. Hence, the aim of our study was to explore possible correlations between environmental factors and HFMD outbreaks.

Approximately 60% of the kindergarten classes in Beijing that experienced HFMD outbreaks in 2011 were surveyed in the present study. In China, because children attend kindergarten for approximately 8 h per day, the HFMD outbreaks were considered to have spread from within the classes. The proportions of EV71 (41%) and CVA16 (31%) detected in the surveyed outbreaks were similar to recorded epidemic trends in Beijing from 2007 to 2010 (45% for EV71 and 34% for CVA16) (18), whereas the positive rate of the included outbreaks in this study was higher than that reported in the 2007–2010 HFMD surveillance data (74% and 56%, respectively) (18). It appears that samples collected in HFMD outbreaks were more likely to contain enteroviruses, indicating that the samples from HFMD outbreaks had higher viral loads.

The univariate analysis showed that the risk of indoor solar radiation and indoor UV radiation were significantly different among the 3 groups. After controlling for other factors, indoor UV radiation still remained significant in multiple linear regression analysis. We are inclined to agree with the results of a previous ecological study that found that indoor UV radiation was a significant protective factor (6), which was also in accordance with another previous etiological study that reported the sensitivity of enteroviruses to UV light (19). The application of UV radiation presents a possible preventative measure to reduce the incidence of HFMD outbreaks by inactivating viruses contained in vesicle fluids, respiratory secretions, and other excreta, which may contaminate the air and common surfaces. Although indoor solar radiation was identified as a significant protective factor, after adjusting for other factors, it was not independently associated with the incidence of HFMD outbreaks. The mean value of UV radiation increased in the HFMD group compared with HFMD-negative group, and showed a dose-dependent relationship. The mean value of indoor solar radiation was evidently lower in the HFMD-negative group than in the sporadic group, but the difference was minimal. Besides, the mild collinearity between UV and indoor solar radiation may explain a potential contributing mechanism. Therefore, we presumed that the effect of outbreak prevention by indoor solar radiation could be accomplished with the use of UV light. Accordingly, increasing classroom area, extending the duration of outdoor activities, and reducing the occlusion area should present economical and simple measures to reduce the incidence of HFMD outbreaks. UV lamps are commonly used for sterilization in medical facilities; therefore, the use of such lamps in kindergarten classes present a useful complementary method to reduce the HFMD outbreaks. In a previous water disinfection study (20), enteroviruses were found to be the most sensitive infectious agents to UV light. However, the effect of UV disinfection is easily influenced by angle, position, duration, wavelength, and other factors. A randomized controlled trial study (comprising 14,854 patients) (21) on the effect of UV radiation exposure to postoperative wound infections reported that the overall wound infection rate was unaffected by UV radiation. Moreover, the safety of UV exposure to children should be considered. After consulting the Criteria for Disinfection and Sterilization in Healthcare Facilities in China, we recommend the prudent use of UV lamps for sterilization of kindergarten classrooms, which should be in-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>Standardized estimate</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class grade</td>
<td>-0.07688</td>
<td>0.01909</td>
<td>-0.45793</td>
<td>-4.04</td>
<td>0.0002</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.01618</td>
<td>0.00545</td>
<td>0.31716</td>
<td>2.97</td>
<td>0.0043</td>
</tr>
<tr>
<td>Ultraviolet</td>
<td>-0.00354</td>
<td>0.00156</td>
<td>-0.42356</td>
<td>-2.27</td>
<td>0.0269</td>
</tr>
</tbody>
</table>
stalled 1.8–2.2 m above the floor with a radiation intensity of at least 75 μW/cm² and an irradiation time of more than 30 min in an unoccupied room. In our investigation, the grade or children’s age in kindergarten played an important role in the frequency of HFMD outbreaks. Based on our results from the univariate analysis, linear regression analysis, and logistic regression analysis, class grade (age) was significantly associated with HFMD outbreaks. Previous surveillance data and studies (6–8) in Beijing also showed that 2–4-year-old children were more prone to acquire HFMD infections than 4–6-year-old children. Because of the lack of circulating antibodies, 0–4-year-old children are most susceptible to HFMD infections (22,23). Besides, 2–6-year-old children are eligible to attend kindergartens; therefore, children aged 2 to 4 years are at a risk of cross-infection owing to enterovirus exposure and susceptibility to enterovirus infections. Hence, more resources and energy should be allocated to prevent transmission of HFMD infections among children aged 2 to 4 years.

Multiple linear regression analysis identified temperature as a significant risk factor, which was consistent with the findings of several ecological studies (9,10,12,16) and epidemic trends in Beijing (6–8). Meanwhile, the univariate analysis showed that temperature was not a significant risk factor. However, more evidence is needed to arrive at definite conclusions. The other environmental factors were found to be insignificant in our analysis.

This study had some limitations that should be addressed. First, over-matched groupings existed in our study. Because a matching factor was kindergarten, classes sharing the same room had the same layout and similar facilities. The average area, PM10, and ventilation area among the 3 groups were similar, which may be an important reason for the relatively low determination coefficient ($R^2 = 0.36$). Second, there were 11 kindergarten classes without a sporadic HFMD case in our study. This condition was taken into account in a previous study that reported that this factor reduced the stability in the multivariate analysis. Third, established cut-off points of the environmental factors were not available; therefore, the logistic regression analysis results were limited, which supplied the OR values.

In summary, our results showed that indoor UV radiation and age as protective factors were associated with the epidemic intensity of HFMD among kindergarten-aged children in Beijing, which were consistent with those of previous studies and current surveillance data. However, because of the limitations of case-control studies, our findings should be interpreted with caution. In the future, a well-designed cohort study or a randomized control trial should be conducted to evaluate the correlations between the incidence of HFMD outbreaks and indoor UV radiation and age.

**Conflict of interest** None to declare.

### REFERENCES