

Original Article

Epidemiological characteristics and pathogens attributable to hand, foot, and mouth disease in Shanghai, 2008–2013

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Abstract

Introduction: Hand, foot, and mouth disease (HFMD) is a common childhood illness caused by enteroviruses. A passive surveillance system has been implemented in Shanghai Pudong since 2008 and etiology surveillance since 2009. We characterized the epidemiology and the etiology of HFMD in Pudong from 2008–2013.

Methodology: Health care providers were required to report any clinically diagnosed HFMD to Pudong District Center for Disease Control and Prevention. For all severe cases and randomly selected mild HFMD cases, throat or rectal swabs or feces were collected for enterovirus detection by real time reverse transcription polymerase chain reaction.

Results: A total of 50,149 cases were reported, with average 8,508 per year (range: 3,577–13,202) and average incidence of 167.5/100,000 persons (range: 81.4–254.1/100,000 persons). HFMD was more likely to occur in children under five years of age (85.6%), while severe cases were more likely to happen in children under three years of age (63.9%). Every year in May or June, HFMD peaked in the region; two peaks were observed from 2011 to 2013. The most common etiologic agents are EV71 and CA16. Different types of enterovirus circulate in different years. EV71 was the predominant pathogen in severe cases. The proportions of EV71 in severe cases was higher than in mild cases at the children's medical center ($p < 0.001$).

Conclusions: HFMD remains an important public health issue in Shanghai. HFMD pathogen surveillance is required for more types of enteroviruses besides EV71 and CA16, which would give a better picture of the etiology of HFMD.

Key words: hand, foot, and mouth disease (HFMD); enterovirus; epidemiology.

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Introduction

Hand, foot, and mouth disease (HFMD) is a common childhood viral infection, which is typically mild and self-limiting. It is characterized by fever, oral ulcers, and vesicular exanthema on the hands, feet, and buttocks. However, some patients rapidly develop neurological and systemic complications such as aseptic meningitis, encephalitis, pulmonary edema, and cardiopulmonary hemorrhage [1,2]. HFMD can be caused by numerous members of the enterovirus genus of the *Picornaviridae* family, but severe cases and deaths have been mainly caused by enterovirus 71 (EV71) and coxsackie virus A16 (CA16) [3]. Transmission occurs from person to person through direct contact with saliva, feces, vesicular fluid, or respiratory droplets of an infected person, and indirectly by contaminated articles [4]. Over the last decade, many large outbreaks of HFMD have been reported in countries of the Western Pacific Region, including

Japan, Malaysia, Taiwan, Singapore, Vietnam, mainland China, and Cambodia [5-11]. HFMD is a major public health problem in China, resulting in 7.2 million clinical cases and 2,457 deaths from 2008 to 2012 [12]. Prevention of HFMD has been declared a national priority in China. Several candidates for EV71 vaccines are under development, and an inactivated EV71 vaccine evaluated in phase 1, 2, and 3 clinical trials in China had good immunogenicity and acceptable safety in healthy children and infants, with a 98.5% seroconversion rate in the participants with seronegative baseline after two doses of the vaccine [13,14]. The vaccine efficacy against EV71-associated HFMD was 94.8% to 97.4%. Vaccine efficacies against EV71-associated hospitalization and HFMD with neurologic complications were both 100% [15,16].

Shanghai Pudong is the largest and most developed district in Shanghai with a highly dense population of 5.5 million in 2013. Around 45% of the Shanghai

Pudong population is made up of migrant workers and their families. The aim of this study was to describe epidemiology characteristic and pathogens attributable to HFMD in Shanghai Pudong and to examine the baseline disease burden before the vaccine era.

Methodology

Case definitions

A clinical case of HFMD was defined as a patient with maculopapular or vesicular rash on hands, feet, mouth, or buttocks, with or without fever, as listed in the guidelines of the China Ministry of Health [4]. A severe case was defined as an HFMD case with at least one of the following complications: aseptic meningitis, encephalitis, acute flaccid paralysis, pulmonary edema, hemorrhage, or cardiopulmonary collapse. A laboratory-confirmed case was defined as a clinically diagnosed case with laboratory evidence of enterovirus infection (including EV71, CA16, or other enterovirus) detected by real-time RT-PCR. A cluster was defined as two to nine cases occurring in a classroom within one week or five to nine cases occurring at an institution within one week. An outbreak was defined as ten or more cases occurring within one week at an institution.

Study site and data collection

On 2 May 2008, HFMD became a notifiable disease in China. Local health providers were required to report any HFMD cases to the local Center for Disease Control and Prevention (CDC) within 24 hours via an internet-based surveillance system. A standardized form including basic demographic information (name, gender, age, date of birth), address, residency (local resident or migrant), disease onset date, date of diagnosis, date of hospitalization (if applicable), case classification (clinical or laboratory confirmed), and outcomes (death or recovery) was updated continuously.

Daycare centers, kindergartens, and schools were required to notify the local CDC by telephone about any HFMD outbreak in their institution. The local CDC also actively monitored the HFMD outbreaks in any school on the national reporting system.

Specimen collection and laboratory testing

Stool/rectal swabs or throat swabs were collected from ten reported HFMD cases per month from outpatients at Shanghai Children's Medical Center. When a severe case was clinically diagnosed, a stool specimen and throat swab was collected by clinicians with a nylon swab for lab testing. A stool/rectal swab or throat swab was collected in a cluster/outbreak with five

or more cases. Two cases were sampled if there was a cluster with five or more cases, and five cases were sampled if there was an outbreak with ten or more cases. The swabs were sent in 3 mL of viral transport medium (Beijing Yocon Biotechnology, Beijing, China) to the Pudong District CDC within 48 hours of collection and stored at -70°C. All samples were tested in Shanghai CDC until 1 January 2010 and at the local CDC thereafter with one-step real-time RT-PCR assay for nucleic acid detection targeted for pan-enterovirus, EV71, and CA16. Any positivity in EV71 or CA16 would be defined as positive for that specific strain of virus. If none of these two strains of virus was positive and pan-enterovirus positive, the testing outcome would be defined as enterovirus positive with unknown genotype.

RNA extraction and real-time RT-PCR

Nucleic acid was extracted by the NucliSense easyMAG platform with the Nucli Sens magnetic extraction reagents (BioMerieux, Zaltbommel, The Netherlands) according to the manufacturer's instructions using a sample volume of 250 µL. The elution volume of nucleic acid was 50 µL. Real-time RT-PCR was performed with commercial kits (Enterovirus (EV) Real Time RT-PCR Kit; Human enterovirus 71 (EV71) Real Time RT-PCR Kit; Coxsackie virus A16 (CA16) Real Time RT-PCR Kit, Shanghai, China) according to the manufacturer's protocols. Testing was done in biosafety level two facilities. Test results were classified into the following categories: enterovirus negative, EV71 positive, CA16 positive, or positive for another enterovirus without further genotype identification.

Statistical analysis

The denominators for incidence calculation were the estimated mid-year population of each year from the Public Security Bureau of Pudong District. There was no age- and gender-specific demographic data available for the migrant population; therefore, only overall incidence was calculated among them. Pearson's Chi-square test and student's *t*-test or analysis of variance (ANOVA) was used for categorical data or continuous data wherever appropriate. Poisson regression was used to compare incidence rates. All statistical tests were two sided, and *p* values < 0.05 were considered statistically significant.

Table 1. Sociodemographic characteristics of the reported HFMD cases and incidence rate in Pudong, Shanghai, 2008 to 2013

	Total (n = 51,049) n (%)	2008 (n = 3,577) n (%)	2009 (n = 5,178) n (%)	2010 (n = 9,198) n (%)	2011 (n = 9,823) n (%)	2012 (n = 13,202) n (%)	2013 (n = 10,071) n (%)
Incidence (/100,000 persons)							
Overall	NA	81.4	117.7	187.0	190.4	254.1	174.3
Local residents	NA	59.8	82.2	152.8	156.0	235.4	213.2
Migrant population	NA	111.6	174.4	230.1	230.3	276.0	137.1
Sex							
Male	31,473 (61.7)	2,334 (65.3)	3,199 (61.8)	5,677 (61.8)	6,014 (61.2)	8,075 (61.2)	6,174 (61.3)
Female	19,576 (38.3)	1,243 (34.7)	1,979 (38.2)	3,521 (38.2)	3,809 (38.8)	5,127 (38.8)	3,897 (38.7)
Age (years)							
< 1	3,384 (6.6)	243 (6.8)	413 (8.0)	578 (6.3)	527 (5.4)	751 (5.7)	872 (8.7)
1-4	40,331 (79.0)	2,782 (77.8)	4,253 (82.1)	7,291 (79.3)	7,848 (79.8)	10,381 (78.6)	7,776 (77.2)
5-9	6,529 (12.8)	461 (12.9)	436 (8.4)	1,185 (12.9)	1,313 (13.4)	1,872 (14.2)	1,262 (12.5)
≥ 10	805 (1.6)	91 (2.5)	76 (1.5)	144 (1.5)	135 (1.4)	198 (1.5)	161 (1.6)
Residents							
Local residents	24,935 (48.8)	1,595 (44.6)	2,222 (42.9)	4,186 (45.5)	4,323 (44.0)	6,588 (49.9)	6,021 (59.8)
Migrant population	26,114 (51.2)	1,982 (55.4)	2,956 (57.1)	5,012 (54.5)	5,500 (56.0)	6,614 (50.1)	4,050 (40.2)
Occupation							
Students (kindergartens, elementary schools)	23,335 (45.7)	1,573 (44.0)	2,051 (39.6)	4,308 (46.8)	4,820 (49.1)	6,207 (47.0)	4,376 (43.5)
Children at home	27,543 (54.0)	1,984 (55.5)	3,112 (60.1)	4,867 (52.9)	4,976 (50.7)	6,942 (52.6)	5,662 (56.2)
Other	171 (0.3)	20 (0.5)	15 (0.3)	23 (0.3)	27 (0.2)	53 (0.4)	33 (0.3)

Results

Epidemiology and seasonality of HFMD

A total of 51,049 clinical diagnosed HFMD cases were reported during 2008–2013. Of them 1,056 (2.1%) were laboratory confirmed, 153 (0.3%) were severe cases (Table 1), and there were seven HFMD-associated deaths. Of the cases, 85.6% were under five years of age. More males (61.7 %) were observed, with a median age of 3.0 years (range: 0–60 years). Children at home (54.0%) were the leading population, followed by students in kindergarten and elementary schools (45.7%). The proportion of migrant cases varied over the years, with an average of 51.2% (range: 40.2% in

2013 to 57.1% in 2009). The difference between the age-specific and gender incidence rates among local residents were statistically significant ($p < 0.05$, data not shown). Males had a significantly higher incidence rate of HFMD compared with females among local residents, with the relative risk ranging from 1.41 in 2009 to 1.69 in 2008 (data not shown).

There was only one single peak in May to June observed in 2008 to 2010, whereas two peaks were observed in early summer and late fall seasons in 2011 to 2013, with the first peak consistently higher than the second (Figure 1).

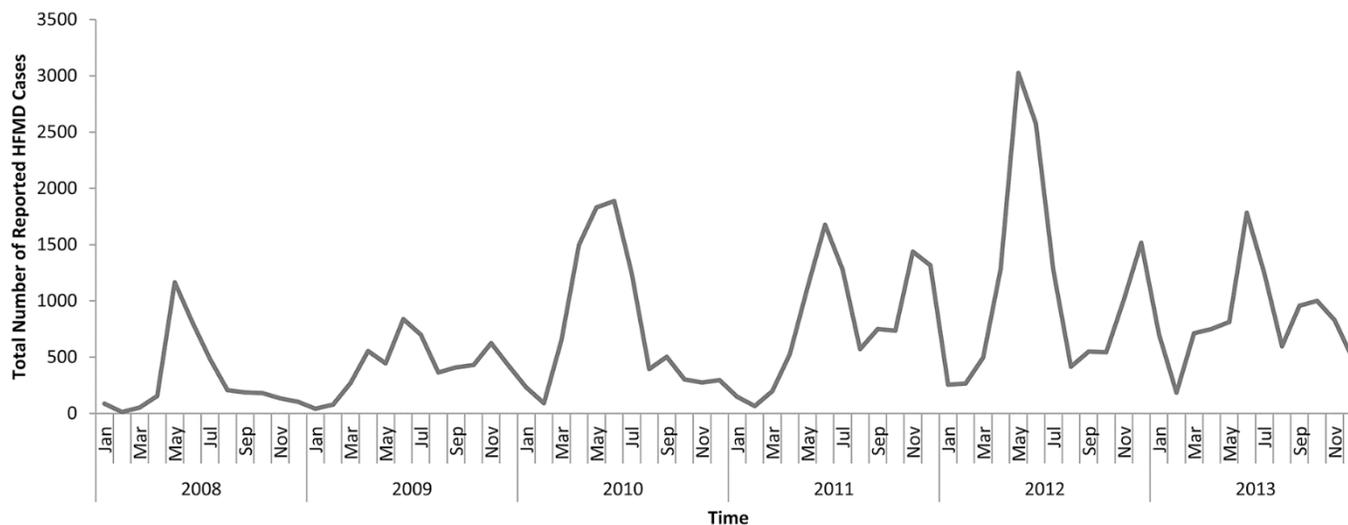
Figure 1. Monthly reported hand, foot, and mouth disease cases in Pudong, Shanghai, 2008 to 2013

Table 2. Viral distribution among mild cases collected at Shanghai Children’s Medical Center and severe cases

	2009		2010		2011		2012		2013	
	Mild	Severe	Mild	Severe	Mild	Severe	Mild	Severe	Mild	Severe
No. tested	169	45	100	47	120	40	120	17	120	4
No. positive	133	42	63	47	74	37	80	14	89	3
Virus genotyped	n (%)	n (%)								
EV71	32 (24.1)	33 (78.6)	40 (63.5)	44 (93.7)	50 (67.6)	35 (94.6)	14 (17.5)	13 (92.9)	13 (14.6)	2 (66.7)
CA16	65 (48.9)	5 (11.9)	21 (33.3)	1 (2.1)	12 (16.2)	1 (2.7)	38 (47.5)	1 (7.1)	10 (11.2)	0
Other	36 (27)	4 (9.5)	2 (3.2)	2 (4.2)	12 (16.2)	1 (2.7)	28 (35)	0	66 (74.4)	1 (33.3)
P value	0.001		0.001		0.001		0.001		0.001	

CA: coxsackie A virus; EV: enterovirus

During the study period, there were 153 severe cases, and seven HFMD-associated deaths were reported. Of the 153 patients with demographic data available, 70.6% were under 6 years of age. Of the 108 hospitalized children, the age ranged from 6 months to 5.9 years with a mean of 2.6±0.1years; 63.9% of the patients were under 3 years of age. Migrant children comprised 74.1% of the patients, and 70.4% of the cases were male. The case severity rate was statistically higher in boys than in girls, and in migrant children than in local children (p=0.001, and p<0.001, respectively). The mean hospitalization duration was 5.7±0.3days; the longest was 16 days. Of the cases, 75% had complications with aseptic meningitis; eight cases had more than two kinds of complications. One hundred and three cases recovered, and five died of pulmonary edema, hemorrhage, or cardiopulmonary collapse. The most common symptoms in the 108 hospitalized children were fever (100%), rashes on hand or foot, oral and buttock ulcers (99.1%), and vomiting (67.6%). Fifty-eight (53.7%) patients had fever greater than 39°C. Myoclonic jerk was noted in 50 patients (46.3%).

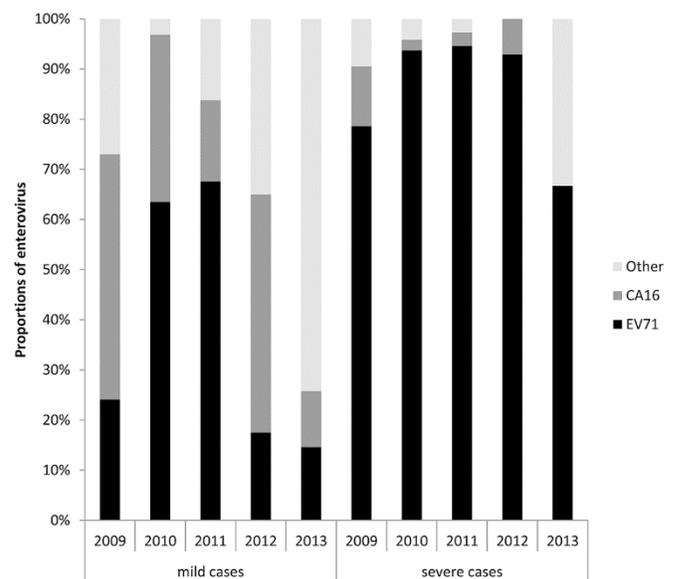
A total of 1,593 clusters and 146 outbreaks with 8,225 cases (19.5% of total reported cases) were reported from 2010 to 2013. Most (97.3%) of the reported clusters/outbreaks occurred in childcare centers and kindergartens, where the cases were between three and six years of age, and 2.5% of the reported clusters/outbreaks occurred in elementary schools.

Pathogen distribution

A total of 1,891 specimens were collected from 1,742 cases during 2009–2013. Throat, vesicle, stool, and rectal swabs accounted for 76.7%, 12.9%, 7.5%, and 2.9% of the samples, respectively (data not shown). Throat swabs and stools were collected from 136 severe cases simultaneously. The positive rate between throat swabs and stool were 91.5% and 94.6%, but this was not statistically significant (McNemar’s test, p>0.05). Of the samples, 62.6% were positive for enterovirus.

The majority of the enteroviruses detected from the HFMD cases were EV71 in 2010 and 2011, accounting for 60.8% and 71.6% of cases, respectively, while CA16 (41%) was predominant in 2012, but other enterovirus accounted for 74.4% of cases in 2013 (Table 2). Both CA16 and EV71 were almost equally distributed in 2009. EV71 predominated in laboratory-confirmed cases, accounting for 88.8% of severe cases. However, EV71 accounted for 38.3% of mild cases. The proportion of EV71 in severe cases was higher than in mild cases at Shanghai Children’s Medical Center, and this was statistically significant (p<0.001) (Table 2, Figure 2). A large percentage of non-EV71 and non-CA16 enterovirus was detected in 2009, 2012, and 2013, accounting for 21.8%, 31.1%, and 74.4% of cases, respectively, but further genotype was not identified.

Figure 2. Proportions of enterovirus types in mild cases and severe cases in Shanghai, 2009-2013



EV71: enterovirus 71; CA16: coxsackie A16

Discussion

Results from this study indicated that children with HFMD were younger and that more than 85% of the patients were under five years of age, with males being predominant. The seasonal patterns of HFMD had one peak in the spring in 2008–2010, but had two peaks during 2011–2013, in spring and autumn, in our region. The most common etiologic agents were EV71 and CA16, but other enterovirus have also been associated with HFMD, and enteroviruses of different genotypes have been circulating year by year. EV71 was the predominant pathogen in severe cases with HFMD.

The incidence rate of HFMD was highest in preschool children under five years of age, who comprised 78.5% to 87.9% of the reported cases. This is consistent with the findings of other researchers [7,17,18]. A male predominance of HFMD was observed in our study, which is consistent with that of precious reports [7,19,20]. The reason for the difference observed in gender incidence rates is still unknown. Young migrant children play a central role in the transmission of HFMD in the community because they initiate the HFMD outbreak and subsequently sustain the spread of HFMD among children. Furthermore, migrant children are at significantly increased risk of developing severe HFMD. Mass internal migration in large cities poses a challenge to public health in China, and infectious diseases become the first concern with respect to migrant health [21]. Migrants usually have no access to urban medical insurance and local government-funded medical assistance programs. A seasonal peak of HFMD occurs in Mayor June of each year, and two peaks were observed in 2011–2013, which is similar to the results of other reports [12,19,22]. Previous studies have demonstrated that seasonal patterns of HFMD were associated with climatic factors, such as temperature, relative humidity, and precipitation [23–26].

We did not observe clear epidemic intervals for either EV71 or CA16 types of enterovirus. This was in contrast with findings observed in Singapore and Malaysia, where levels of EV71 and CA16 infection peaked every two to three years over the past decade [7,17]. The main enteroviruses causing epidemics of HFMD in Shanghai were EV71 and CA16 from 2009 to 2012. EV71, as the most predominant enterovirus, represented about 45% of EV-positive samples. A similar trend was also observed at a national level [18,21]. EV71 was the predominant pathogen in severe cases, as reported in other studies [1,2,12]. Studies in Taiwan showed that the level of seroprevalence against EV71 in different parts of the island and the age-

specific seroprevalence were correlated with the incidence of severe disease and mortality rates [27]. In the past decade, HFMD due to EV71 has become a major public health concern in the Asia-Pacific region, which appeared to be the epicenter for the generation of epidemic genotypes. In recent years, several candidates for EV71 vaccines are being developed, and an inactivated EV71 vaccine has been evaluated in phase 1, 2, and 3 clinical trials in China. It had good immunogenicity and acceptable safety in healthy children and infants, with a 98.5% seroconversion rate in the participants with a seronegative baseline after two doses of the vaccine [13,14]. The vaccine efficacy against EV71-associated HFMD was 94.8% to 97.4%. Vaccine efficacies against EV71-associated hospitalization and HFMD with neurologic complications were both 100% [15,16].

We observed a large percentage of non-EV71 and non-CA16 enteroviruses in 2009, 2012, and 2013. Samples positive for other enteroviruses were not further genotyped during the study period. One report about a CA6 outbreak in China had just been published, in which CA6 was identified as the predominant serotype associated with an HFMD epidemic from late 2012 to 2013 in Guangdong [28]. Although CA6, a rare viral pathogen in previous epidemics, has long been known to cause HFMD [7], it has not usually been considered to play a major role in this disease. Except in a few countries, CA6 has been infrequently detected until recent years. However, since 2008, this virus has caused major outbreaks of HFMD in some countries of eastern Asia and Europe and, more recently, in the United States [29–35]. Sporadic cases of CA6 infection had been reported in areas such as Guangdong, Shandong in China. HFMD pathogen surveillance is required for more types of enteroviruses besides of EV71 and CA16 in our region. The previously infrequently detected CA6 is becoming the predominant HFMD pathogen, thus highlighting the necessity for comprehensive surveillance of its circulation in HFMD epidemics in China as well as the rest of the Asia-Pacific region.

Our study had three main limitations. First, data collected during 2008 and 2009 are probably less reliable than those from more recent years. The data are insufficient for understanding the magnitude of HFMD in those two years. Second, the serotype of enteroviruses other than EV71 and CA16 could not be monitored. We could not obtain a better understanding of the evolution of the virus from one genotype to another and its epidemiological linkage with viruses isolated from other countries in the region. Third, the

detailed information (such as age and gender) of migrant populations was not available, so the description of age- and gender-specific incidence rates is insufficient.

Conclusions

To some extent, this study can represent the transmission and distribution of HFMD and viral infections in Shanghai, and it provided the baseline disease burden of HFMD. EV71 can cause severe complications and deaths in our region. HFMD is still an important public health problem in Shanghai. HFMD pathogen surveillance is required for more types of enteroviruses besides of EV71 and CA16, which would give a better picture of etiology with HFMD.

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