The epidemiological and clinical characteristics of hand, foot and mouth disease in Hangzhou, China, 2016-2018

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Abstract

Background Hand, foot and mouth disease (HFMD) is most frequently caused by several serotypes of human enterovirus (EV) including Enterovirus 71 (EV71), Coxsackievirus A16 (CV-A16) or other types of EV. Children under 5 years old are the most susceptible to HFMD. The aim of this study was to determine the epidemiological characteristics and the relationship between severe and mild HFMD. Methods We collected 4760 probable HFMD cases with skin papular or vesicular rashes on the hands, feet, mouth or buttocks in Hangzhou from 2016 to 2018. Specimens of these cases were collected and the pathogen of EV71, CV-A16, CV-A6, CV-A10, CV-A2 and CV-A5 was classified by RT-PCR. Then the pathogen distribution and clinical status of children with HFMD were described. Results From Jan. 1, 2016 to Dec. 31, 2018, the incidence and prevalence of HFMD were seasonal each year. Among the 4760 probable HFMD cases, 3559 cases were confirmed (74.8%, 3559/4760), including 426 cases of EV71 infections (8.9%, 426/4760, 249 cases of CV-A16 infections (5.2%, 249/4760) and 2884 cases of other enteroviruses infections (60.6%, 2884/4760). The percentage of EV positive HFMD cases with non-EV71 and non-CV-A16 was more than 80% (2884/3559), which increased year by year. The percentage of EV71 decreased year by year in the last three years especially in 2018. Among the 1297 cases randomly selected of other EV infections in 2017 and 2018, there were 835 (64.4%) cases of CV-A6 infections, 177 (13.6%) cases of CV-A10 infections, 100 (7.7%) cases of CV-A2 infections, 40 (3.1%) cases of CV-A5 infections, 3 (0.02%) cases of mixed infections and 11.0% untyped enteroviruses infections. Preschool children were still the primary population susceptible to HFMD, and the age of the children infected with other enteroviruses tended to be younger. In severe cases, EV71 infection was the main cause. Conclusions The other EV especially CV-A6 increased obviously and EV71 decreased obviously in the three years. Characterizing the epidemiology and the relationship between severe and common cases of HFMD would provide relevant evidences for the prevention and treatment of HFMD.

Background

Hand, foot and mouth disease (HFMD), a common childhood illness, is increasingly recognized as a significant health issue worldwide. Clinical manifestations of HFMD are diverse and complex. Main symptoms include vesicular rashes on palms, soles, mouth and/or buttocks with or without fever and central nervous system (CNS) involvement in severe cases[1]. HFMD is caused by human enteroviruses (EV) . Enterovirus A71 (EV71) and coxsackievirus A16 (CV-A16) are the most common pathogens causing HFMD in children[2-5]. EV71 induced HFMD is often benign and self-limiting, but it also can be associated with neurologic complications. These complications include encephalitis, aseptic meningitis, transverse myelitis, cerebellar ataxia and Guillain–Barre syndrome[6-10]. Coxsackievirus A6 (CV-A6) and coxsackievirus A10 (CV-A10) are the most common serotypes for mild HFMD worldwide and in China since 2010[11-18]. We found that the number of HFMD cases has increased very much since the nationwide HFMD outbreak in China in 2008, and the number of HFMD cases has been the head of the lethal infectious diseases in our country for five consecutive years since 2013, according to the report of National Health Commission of the People's Republic of China.

In this study, we collected specimens from 4760 patients clinically diagnosed with HFMD in Hangzhou Children's Hospital from Jan. 2016 to Dec. 2018, including 1384 stool specimens, 3315 throat swab specimens and 61 cerebrospinal fluid specimens. And we aims to describe the epidemiological characteristics and the relationship between severe and mild HFMD which would provide relevant evidences for the prevention and treatment of HFMD.

Methods

Study participants

A total of 4760 specimens (1384 stool specimens; 3315 throat swab specimens; 61 cerebrospinal fluid specimens) were collected from children clinically diagnosed with HFMD in Hangzhou Children's Hospital from 2016 to 2018. HFMD cases were clinically diagnosed according to the Ministry of Health diagnostic criteria (2018 edition)[19]. Children who displayed vesicular rashes on the hands, feet, oral mucosa or buttock in epidemic seasons were clinically diagnosed with HFMD. In this study, 783 Children with serious complications, including central nervous system complications (such as encephalitis, meningitis and brain stem encephalitis) and/or cardiorespiratory failure were considered having severe HFMD. The main clinical manifestations of CNS complications are headache, vomiting, irritability, lethargy, nuchal rigidity and myoclonus. The main clinical manifestations of cardiorespiratory failure are pulmonary oedema or pulmonary haemorrhage. Other children without theserious complications mentioned above were classified as having mild HFMD. This study and the use of clinical samples were approved by the medical ethics committee of Hangzhou Children's Hospital and all experiments were performed in accordance with relevant guidelines and regulations.

RNA extraction

Clinical specimens (stool specimens, throat swab specimens and cerebrospinal fluid specimens) were collected and stored at -80℃ for extraction of genomic RNA. RNA was extracted by nucleic acid automatic extraction instrument (NP968Tian-long company, Xian, China). Operate strictly according to the operating instructions of the nucleic acid extraction kit. The detection of EV/EV71/CVA16 was performed in ABI 7500 system via commercial one-step real-time RT-PCR assay kit (Shuo-shi company, Jiangsu, China). The real time RT-PCR was conducted under these conditions: 30 min at 45℃, 5 min at 94℃, and then followed by 40 cycles of 10 sec at 94℃ and 40 sec at 55℃.

To download multiple gene sequences including CV-A6, CV-A10, CV-A2 and CV-A5 from the National Center for Biotechnology Information (NCBI) database, the specific primers and probes of CV-A6, CV-A10, CV-A2 and CV-A5 subtypes for fluorescent quantitative RT-PCR assay were designed with Primer Express 3.0 software, and the primers and the probes' sequences are validated by Blast. PCR primers were synthesized by Shanghai ShengGong Gene Co, Ltd.

The RT-PCR mixture for each tube consisted of 5 μl viral RNA, 12.5 μl One step RT-PCR Buffer, 0.5 μl Takara EX Taq HS, 0.5 μl RT enzyme 1.6 μl specific primer mixture and 4.9 μl nuclease-free water, up to a

final volume of 25 μl. The PCR was conducted under these conditions: 30 min at 50℃, 5 min at 95℃, and then followed by 40 cycles of 15 sec at 94℃ and 45 sec at 55℃.

Statistical analysis

All statistical analyses were performed using SPSS version 19.0, and X^2 test was used to compare the distribution of EV71, CV-A16 and other enteroviruses positive rates for the groups categorized by age and gender. Count data was compared in percentage. All statistical tests were two sided, and P-value < 0.05 was considered statistically significant.

Results

Epidemiological dates of HFMD patients in Hangzhou

From 2016 to 2018, a total of 4760 (1854 in 2016; 1174 in 2017; 1732 in 2018) specimens of patients clinically diagnosed with HFMD were collected. Of these patients, 3977 (83.6%) presented mild symptoms, whereas 783 (16.4%) were diagnosed with severe HFMD with neurological or cardiopulmonary complications. A total of 3559 specimens were tested positive for EV by quantitative polymerase chain reaction (PCR), which were comprised of 426 cases positive for EV71 infections, 249 cases positive for CV-A16 infections and 2884 cases of other EV infections. These 3559 EV positive cases consisted of 2940 (82.6%) mild and 619 (17.4%) severe HFMD. For convenience, we defined EV-positive but both EV71 and CV-A16 negative cases as “other EV infections cases”.

Figure 1 shows that although HFMD cases occurred throughout the year, we observed the number of cases decreased between January and March, and obviously increased between May and July in the past three years. Two seasonal peaks were observed in the total HFMD cases in 2016 and 2017. The first and larger peak was between May and July reflecting the summer HFMD epidemic. The second peak was between October and November. Interestingly, the second peak was not obvious and the time was advanced to September in 2018,which is different from the previous two years.

Characteristics of the clinical data from HFMD patients

The distribution of HFMD causative pathogens in mild cases showed that other EV infections cases predominated from 2016 to 2018, accounting for 88.2% (2594 /2940).However, EV71 positive cases predominated in severe cases from 2016 to 2018, accounting for 47.7% (295 /619) which was a little higher than the proportion of other enteroviruses positive cases (Fig.2). Further analysis showed that the severe cases with EV71 positive accounted for 69.2% of the total EV71 positive cases (295/426). EV71 was more significantly associated with severe HFMD compared to CV-A16 (13.7%, 34 / 249) and other EV infections cases (10.1%, 290 / 2884) (P < 0.05) (Fig. 3).

The gender distribution ratio of male to female cases was 1.66:1 in 3559 EV positive cases .There are 2223 males consisted of 276 EV71,169 CV-A16 and 1778 other EV infections cases and there are 1336

females consisted of 150 EV71, 80 CV-A16 and 1106 other EV infections cases. There is no gender difference in the rate of infection of different enteroviruses.（X^2=4.901，p=0.086).Age distribution showed that children under 5 years old accounted for more than 90%. In total cases, the children between 1 year old and 2 years old had the highest incidence of HFMD. The average ages for EV71, CV-A16 and other EV infections were 2.5, 2.3 and 1.8 years, respectively. The mean age of patients with other EV infections was younger than that of those with EV71 or CV-A16 infections .The average ages for EV71 infections were2.3, 2.5and 4.1 years in 2016,2017 and 2018 respectively.The mean age of patients with EV71 infections was tends to be older year by year.

Novel epidemic patterns in HFMD etiology

As per our laboratory surveillance system, the annual proportions of other enteroviruses with both non-EV71 and non-CV-A16 were 69.5%, 80.4% and 92.9% from 2016 to 2018, which increased year by year. However, the annual proportions of EV71 decreased year by year in the last three years, and especially in 2018 only 13 cases were detected (Table 1). Other enteroviruses replaced EV71 and CV-A16 as the major serotype during 2016 to 2018, accounting for over 80% of EV positive HFMD cases. Regular fluctuations in EV71 infection proportions were observed with one large EV71 infection peak each in 2016 and 2017 lasting from May to July, while the other EV infection peak occurred in autumn and winter accounting for over 80% of these cases in these two years. Interestingly, there was no EV71 infection peak in 2018. CV-A16 distributed evenly throughout the year in the three years (Fig. 1).

In order to better understand the serotypes of the other EV infections, we randomly selected 1297 cases of other EV infections in 2017 and 2018. Among the 1297 samples with HFMD, there were 835 (64.4%) cases of CV-A6 infections, 177 (13.6%) cases of CV-A10 infections, 100 (7.7%) cases of CV-A2 infections, 40 (3.1%) cases of CV-A5 infections, 3 (0.02 %) cases of mixed infections and 142(11.0%) other untyped enteroviruses infections (Fig. 4)

Discussion

In the last few decades, HFMD has contributed to several large outbreaks and thousands of HFMD fatal cases worldwide, which has been a growing public health problem[20-22]. After the first report of HFMD in Shanghai in 1981, alternating epidemic of EV71 and CV-A16 occurred in the following years. HFMD poses a significant threat to public health in China and was classified as a C-class notifiable communicable disease in China in 2008. Our previous research revealed that EV71 and CV-A16 represented the two major epidemic enterovirus serotypes in Hangzhou between 2012 and 2013. However, the predominant types of enteroviruses have changed since 2013, and the positive rates of the EV positive HFMD cases that were both non-EV71 and non-CV-A16 have grown rapidly. The other EVs replaced EV71 and CV-A16 as the major serotype in Hangzhou between 2014 and 2015. This study continues to analyze the characteristics of HFMD epidemic that occurred in Hangzhou in 2016, 2017 and 2018.

In this study, we reported that other EVs had replaced EV71 as the primary viral serotype responsible for HFMD onset in Hangzhou between 2016 and 2018. Concurrently, the same trend was observed in other cities near Hangzhou in China[23,24]. The cases of EV71 decreased year by year in the past three years. And compared to 2016 and 2017, only 13 cases of EV71 were detected in 2018, which meant a significant decrease in the proportion. Therefore, 1297 cases randomly selected of the other EVs were tested in 2017 and 2018. There were 835 (64.4%) cases of CV-A6 infections and 177 (13.6%) cases of CV-A10 infections in 2017 and 2018. Less mixed infections was detected among different types in this study. The cases of CV-A6 infections also increased dramatically and CV-A6 became one of the main causative agents of HFMD in Hangzhou. CV-A10 also overtook CV-A16 to become the second most common viral serotype. Hangzhou is the capital city of Zhejiang province, and its high population mobility made it a HFMD-prone area and likely more conducive to virus faster evolution. Some epidemiologists suggest three primary factors were involved in the regionally increased CV-A6 infection rates and decreased EV71 infection rates. Firstly, after a long period of the EV71 epidemic, elevated antibody and thus immunity levels against EV71 in the population might impart a selection pressure for other EV serotypes to arise, because antibodies against EV71 and CV-A16 cannot protect the susceptible population from the other serotypes[25]. Secondly, the popularity of EV71 vaccination may play a role in this change in recent years. Mass EV71 vaccination is not expected to substantially reduce the total number of HFMD cases because the vast majority of HFMD cases are mild and more than half of the mild cases were due to CV-A16 and other EV serotypes infections. However, most of severe and death HFMD cases could be prevented by EV-A71 vaccination[26]. Thirdly, CV-A6 might have transformed into a more virulent strain to escape human immune protection. Yoshitomi H's study showed that the phylogenetic analysis of the entire VP1 gene revealed the diversity of the prevalent CV-A6 between 2013 and 2017 in Fukuoka. The CV-A6 strains were classified into seven genetic clades (A–G) and subgroups of clade A (subclades A1–A4) based on the entire VP1 sequences.The phylogenetic analysis revealed that all the CV-A6 strains detected in Fukuoka between 2013 and 2017 were classified into clade A□A3 and A4□,which were different from Hong Kong(D4 and D5)[27,28]. The emergence of CV-A6 strains with high genetic diversity was believed to be a factor associated with the epidemics. More studies including whole genome sequencing of the epidemic strains are warranted to determine whether these variations affect the epidemic incidence of CV-A6. We think more attention should also be paid to the other EV serotypes, such as CV-A6 and CV-A10, in the future surveillance and control of HFMD in Hangzhou and even in Zhejiang Province. We speculate that the year of 2018 may become a turn point in the EV serotypes causing HFMD, and we should pay more attention to this phenomenon.

Additionally, Hangzhou shared similar seasonal epidemic patterns of EV-predominant serotypes with other southern cities in China. Our data showed two seasonal peaks in 2016 and 2017 in the total HFMD cases. Similar finding was reported in Shenzhen, Wuxi and Hong Kong[28-30]. In these three districts, the season alternations from spring to summer and from autumn to winter are relatively mild, which might be in favor of the spread of certain EV viral serotypes. This is consistent with the results of some studies that found the number of patients with HFMD is affected by environmental factors such as temperature, humidity, sunshine time and so on[31]. In this study, we reported that other EV infection peaks mainly

occurred during October to December in 2016 and 2017. Biao Di's study also found CV-A6 infection mainly occurred during the autumn and winter, and the outbreak of CV-A6 infection might contribute to the second peak of the HFMD[32]. In 2018, the main infection was other EV infection in Hangzhou, and there was no EV71 infection peak, which might also be the reason why the second peak was not obvious in 2018. CV-A16 displayed a low detection rate throughout the year.

In our study, we investigated whether the etiological spectrum of mild and severe HFMD changed. EV71 was still the leading serotype responsible for severe HFMD cases, while the other EV infection accounted for a increase in severe cases. Between 2016 and 2018, other EV infection cases accounted for 46.8% of EV-positive specimens in severe HFMD cases in Hangzhou. This proportion was much higher than the result of our study in the previous several years. This may be due to the change in predominant types of the prevalent EV serotype distributions throughout the year. In our study, the severe case rate of EV71 infections (69.2% of the total EV71-positive cases) was significantly higher than that of CV-A16 infections (13.7%, 34 of 249) and other EV infections (10.1%, 290 of 2884), which suggested that EV71 infected patients had still a greater chance of developing severe cases of HFMD than other EV serotypes infected patients. However, the pathogenic mechanism of EV71 severity and fatality remained unclear. Ya-Ping Li et al's report suggested that the different genetic background of host innate immunity may lead to different clinical outcomes. They found that RIG-1 rs3739674 and RIG-1 rs9695310 polymorphisms are associated with EV71 HFMD risk. RIG-1 rs3739674 and TLR3 rs5743305 polymorphisms are associated with disease severity[33]. Previous reports showed that the levels of Serum interleukin-6, B-type natriuretic peptide, hyperglycemia and leukocytosis in children with severe neurological symptoms caused by EV71 infection significantly increased, causing a systemic inflammatory factor storm and leading to heart and lung failure, which might indicate a strong relationship between proinflammatory cytokines and severity in EV71 infection[34,35].

Statistical analysis of the gender distribution of all cases in the present study showed that more infection in males than in females in our study. Male predominance of HFMD was observed, possibly because of higher physical activity and less attention to hygiene of boys. However, there was no gender difference in the ratio of each different enterovirus infection. Statistical analysis of the age in the present study showed that children under age five were the most susceptible to HFMD, which is consistent with previous reports[36]. We found the age of patients with other EV infection was younger than those with EV71 or CV-A16 infection. With the popularity of other EV serotypes, the small-aged infants become the focus of the prevention and treatment of HFMD in Hangzhou.We also found that the average age of EV71 infection gradually increased from 2016 to 2018, especially in 2018, indicating that the protection of EV71 vaccine in low age groups has been obvious effective. In this study, enteroviruses were detected in throat swab and feces, and there was no significant difference in the detection rate of enteroviruses in throat swab and feces (X^2=0.127,P=0.722) .No enterovirus was detected in cerebrospinal fluid specimens in our study. Relevant reports in abroad also showed that the detection rate of enterovirus nucleic acid in cerebrospinal fluid was lower than in throat swab, feces or bleb fluid[37].

Conclusions

Other EV replaced EV71 and became the predominant causative agent of HFMD in Hangzhou, China from 2016 to 2018. Other untyped EV serotypes such as CV-A6 and CV-A10 have recently surfaced in China, and have become the prominent serotypes leading to HFMD in Hangzhou. Thus, monitoring the long-term serotypes responses to enterovirus strains such as CV-A6 and CV-A10 should be emphasized in the future, as the EV71 vaccine has been successfully applied in Hangzhou and other regions in China. Further research on effective multivalent vaccines is needed to prevent and control the outbreak and epidemic of HFMD.

Abbreviations

HFMD:Hand, foot and mouth disease; EV71:Enterovirus 71; CV-A16:Coxsackievirus A16;CV-A6:Coxsackievirus A6;CV-A10:Coxsackievirus A10;CV-A2:Coxsackievirus A2;CV-A5:Coxsackievirus A5

Declarations

Acknowledgments

Not applicable.

Authors' contributions

The study was designed by WYD. The manuscript was written by WJ. The laboratory evaluation was performed by XGL and ZSF . The data was collected by ZJ and WJ. The data analysis was performed by WJ and LB. CY made critical revisions. All authors have read and approved the manuscript.

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Availability of data and materials

Data analyzed during this study, such as the number of case , the pathogen distribution and clinical date are included in this published article and The datasets are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study and the use of clinical samples were approved by the medical ethics committee of Hangzhou Children's Hospital and the written informed consent was obtained from guardian for participants under 16 years old. All experiments were performed in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

We have no competing interests.

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Tables

Table 1 The pathogen distributions of HFMD cases in 2016-2018

Type	2016	2017	2018
Pan EV-positive	1366	833	1360
EV71（%）	274（20.1）	139（16.6）	13（1.0）
CV-A16（%）	142（10.4）	24（3.0）	83（6.1）
Other EVs（%）	950（69.5）	670（80.4）	1264（92.9）

Figures

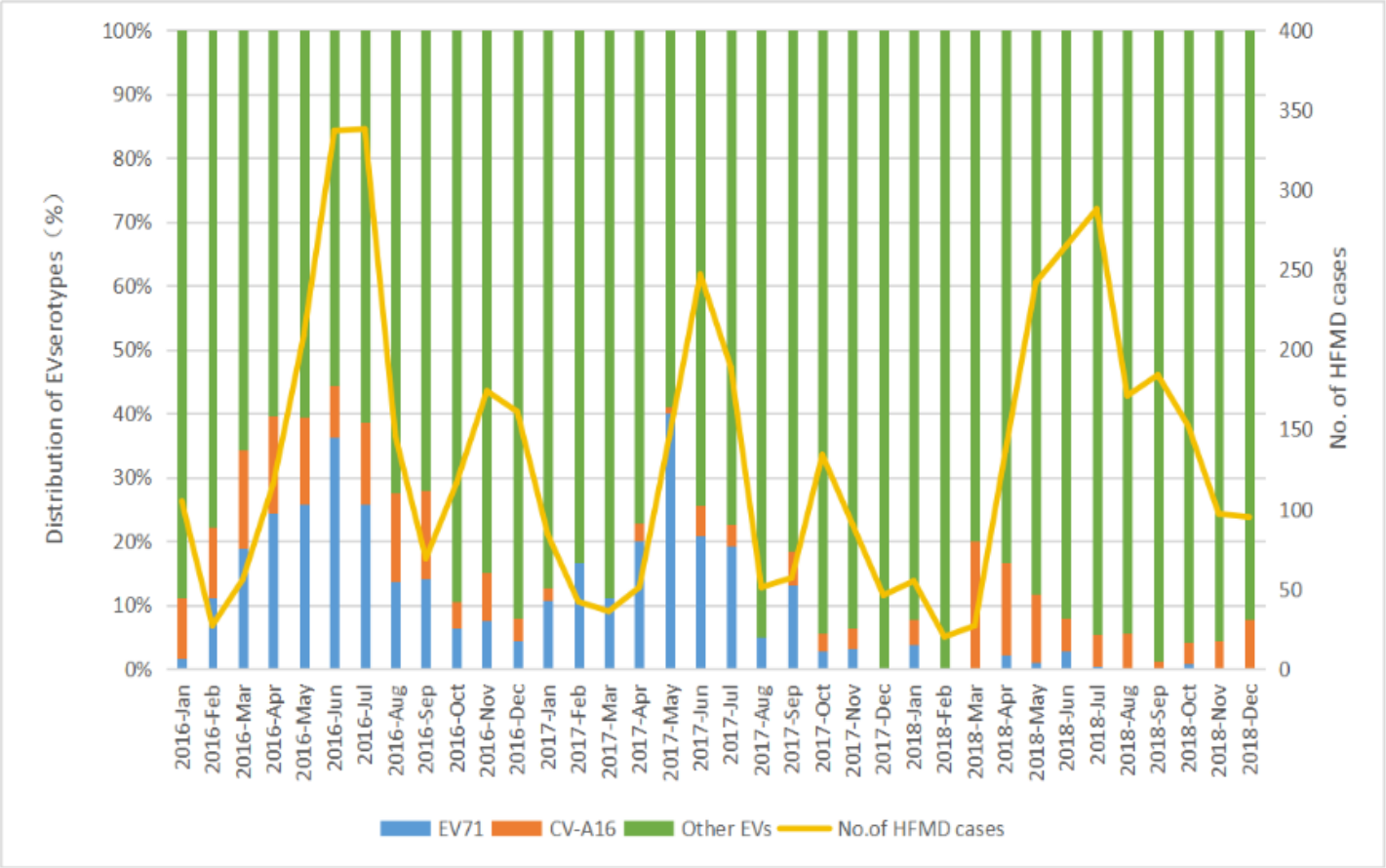
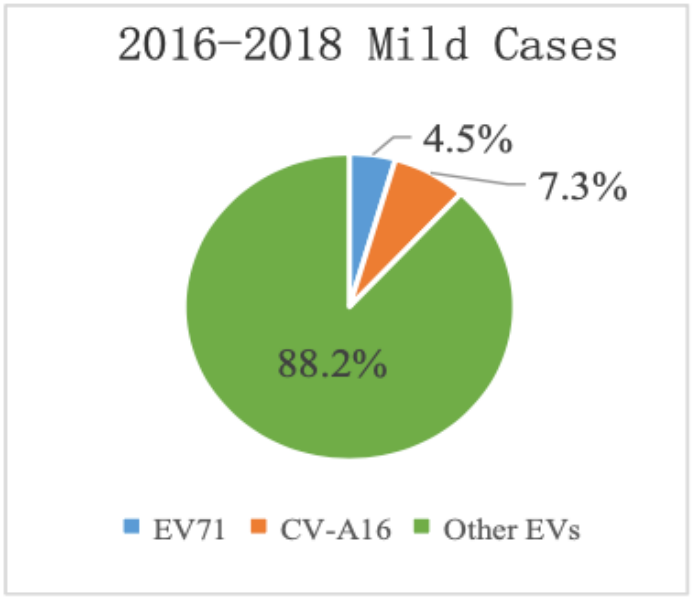


Figure 1

The time distribution of and samples in Clinical diagnosis HFMD casesand HEVs distribution in Hangzhou,China, from 2016 to 2018. Continuous lines represent the number of HFMD cases; the histogram shows the Monthly distribution of EV71, CV-A16 and other enterovirus isolated from EV-positive samples.



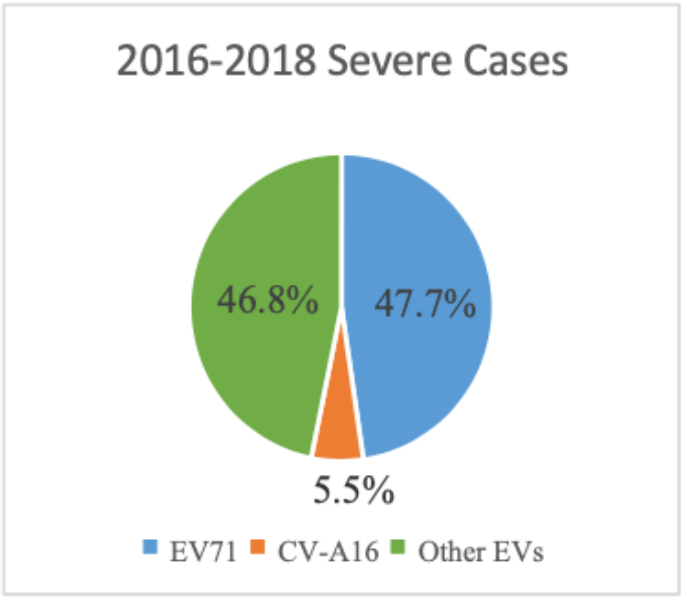


Figure 2

The pathogen distribution of mild and severe HFMD cases in 2016-2018

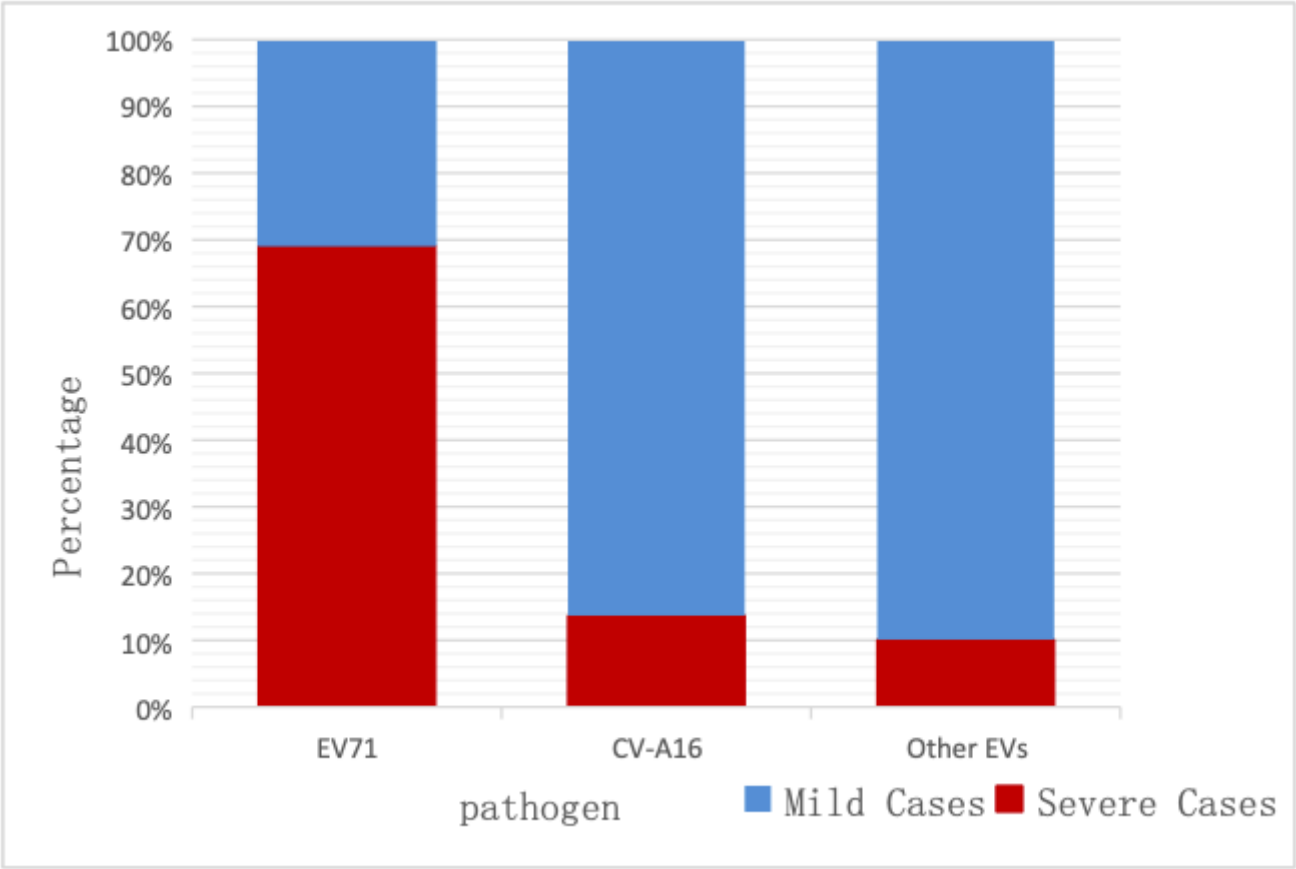


Figure 3

The ratios of mild to severe HFMD cases for different pathogens

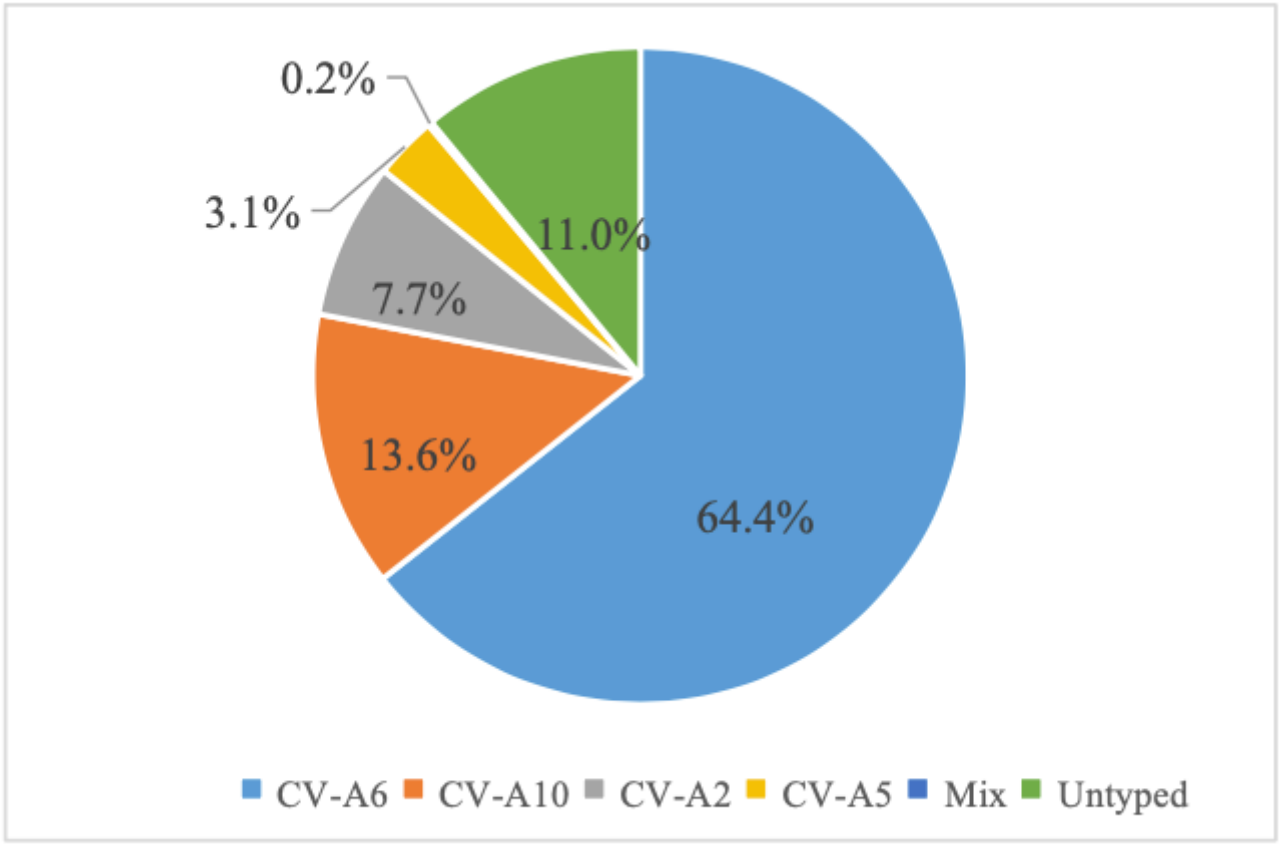


Figure 4

The proportion of different serotypes in the other enterovirus cases