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# Molecular Epidemiology and Clinical Characteristics of Hand, Foot and Mouth Disease in North Sichuan Region, China, 2018-2023: A Descriptive Study

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## Abstract

**Background**: Hand, foot and mouth disease (HFMD) remains an important public health problem in China. Many studies on the epidemiological characteristics of HFMD have been reported, but studies in North Sichuan region have been neglected. **Methods**: HFMD-related enterovirus infected cases were clinically confirmed and underwent real-time RT-PCR (rRT-PCR) from May 2018 to October 2023 in Guangyuan Central Hospital. **Results**: During 2018-2023, other EV (437 cases, 81.08%) was the most predominant serotype followed by CV-A16 (94 cases, 17.44%), EV-A71 (8 cases, 1.48%) was the least predominant serotype. Peak infections occurred in July and October. There were no significant differences in gender, age and serotypes. HFMD was concentrated in children under 47 months of age, with the highest incidence in children aged 12 - 23 months and the highest proportion of other EV infections in the whole age group. COVID-19 did not cause significant changes in gender, age and serotype. Overall, there was a significant increase in the proportion of children aged 12 - 23 months infected with CV-A16, and an increase in the proportion of children aged over 36 months infected with other EVs. **Conclusions**: The incidence of HFMD caused by EV-A71 has decreased significantly, and other EVs have become the main pathogens of HFMD in North Sichuan region in recent years. In the prevention and control of CV-A16, more attention should be paid to children aged 12 - 23 months and the dominant serotype should be closely monitored. Our study highlights the impor-

tance of developing of new diagnostic reagents and vaccines for the prevention and control of enterovirus infection. This study for the first time provides insights into district interventions to local conditions.

## Keywords

Enterovirus, HFMD, Epidemiology, Clinical Characteristics

## 1. Introduction

Hand, foot and mouth disease (HFMD) is a common infectious disease in children under 5 years of age, characterized by rash on the hands and feet, low-grade fever and oral herpes [1] [2]. It was first detected in Europe in 1957 and nowadays appears in many parts of the world [3]. HFMD is caused by a variety of human enteroviruses (EVs) and spreads primarily by fecal-oral transmission, respiratory droplets, and skin-to-skin contact [4]. Although most HFMD are mild and self-limiting, a small percentage of cases can rapidly progress to neurological and systemic complications, in which the condition worsens and even leads to death [5] [6].

There are more than 20 types of HFMD-associated EVs who are non-envelope positive single stranded RNA viruses and belong to the small RNA virus family [7]. Meanwhile, the types of HFMD-associated viruses are increasing. In 2022, a tomato influenza virus with HFMD symptoms was first detected in India, which is suspected to be a new enterovirus [8]. Based on a survey of all HFMD-associated EVs in China from 2008 to 2012, 90% of the viruses were EV-A71 and CVA16, as well as echovirus (Echo). Among them, EV-A71 infection resulted in a high proportion of severe cases [9] [10].

It is reported that vaccination of EV-A71 could reduce the proportion of severe illness and death in HFMD, thereby reducing the disease burden of patients [11]. In China, the first inactivated EV-A71 vaccine for prevention of severe HFMD was approved in 2016. Subsequently, significant preventive effects on EV-A71 associated HFMD were observed by clinical trials [12]. However, the monovalent vaccine of EV-A71 has limited protection to against other EVs [13], which resulted in a changing of causative spectrum of HFMD and a increasing of the incidence rate of HFMD such as CV-A16 in recent years [14]. Therefore, it is necessary to develop a broad-spectrum HFMD vaccine. Recent studies have demonstrated that combination vaccination against HFMD is more effective and safe [15], and the polyvalent HFMD vaccines against multiple enteroviruses are under continuous development [16] [17].

In addition, the time of incidence peak and the epidemiological cycle of HFMD varies considerably according to the latitude of the region, but the onset of the disease is mainly concentrated in warmer climates. Seasonal differences are related to local precipitation, sunshine duration, temperature and air pressure, a single climatic factor is not sufficient to explain the seasonal complexity

of HFMD incidence across the country. Geographic variability in incidence based on seasonal factors was not significantly correlated with climatic and demographic factors. Therefore, understanding the regional epidemiological characteristics of HFMD incidence is important for diagnosis, treatment and prevention [18].

This study provides a comprehensive analysis of the molecular epidemiology and clinical characteristics of HFMD-associated EVs infections in north Sichuan region from 2018-2023. We examined the changes in the incidence of different types of HFMD, and analyzed the differences caused by the gender of patients and their regions. These results provide valuable information for monitoring the prevalence spectrum and epidemiologic trends of HFMD-associated enteroviruses in this area. Furthermore, this study analyzed the distribution of HFMD in northern Sichuan area and documented the epidemiological dynamics of HFMD in this region, which facilitates the optimization of HFMD interventions and also provides a reference for the development of effective vaccines against the pathogenic enteroviruses.

## 2. Materials and Methods

### 2.1. Definitions

The diagnosis of HFMD was established based on current epidemiology, clinical manifestation and virological test as described in the Chinese guidelines (2018 edition) [19]. HFMD cases were defined with EV positive which was detected by real time reverse-transcription polymerase chain reaction (rRT-PCR).

### 2.2. Study Setting

All cases at Guangyuan Central Hospital from 2018 to 2023 were first screened for HFMD on the basis of clinical symptoms according to the guidelines as described above. And then, HFMD nucleic acid testing was performed on patients with clinical suspicion or clinical diagnosis of HFMD. Cases without HFMD nucleic acid test results will be excluded from the study. A retrospective analysis of patient records of all labor confirmed enterovirus infection cases were performed. The etiological data such as age, gender, and serotypes of virus were collected. The study protocol was approved by the Clinical Research Ethics Committee of Guangyuan central hospital.

### 2.3. Data Collection

Throat swabs collected from all patients with clinical suspicion or clinical diagnosis of HFMD were sent to the laboratory in sterile sample tubes at 0 - 4˚C to perform the rRT-PCR test immediately. Those samples which couldn't be detected timely were stored at −20˚C for no more than 24 hours. The RNA samples were extracted using RNA nucleic acid Extraction Reagent (Tianlong Xian, China) according to the manufacturer's instruction. All samples were tested for EV-A71 and CV-A16 serotypes using a commercial rRT-PCR Kit (Mole BioS-

cience Co, Ltd, Jiangsu, China) following the protocol of the manufacturer's instruction.

### 2.4. Statistical Analysis

The numeric variables were tested for normal distribution using the Shapiro-Wilk normality test firstly, and nonnormally distributed variables were presented using the median (25th percentile (P25), 75th percentile (P75)). Comparisons between the two groups were made using the Manny-Whitney U test and Comparisons between the three or more groups were made using Kruskal-Wallis rank sum test. Categorical variables were presented as counts (n) and percentages (%) and the statistical analysis were performed using Chi-square test or Fisher's precision probability test. A p value of 0.05 or less was considered statistically significant. All the data were analyzed using SPSS 25.0.

## 3. Results

### 3.1. Basic Characteristics of the Patients

A series of analysis were conducted to study the overall distribution of enteroviruses in North Sichuan region, focusing on clinical-confirmed enterovirus infections caused by CV-A16, EV-A71, and other EVs from 2018 to 2023. A total of 599 cases (348 males and 251 females) were enrolled in the analysis, of whom 539 cases showed positive results for the enterovirus nucleic acid test. Of 539 positive cases, 102 cases suffered from single enterovirus, such as EV-A71 (8, 1.48%) or CV-A16 (94, 17.44%). Other EVs (437, 81.08%) were the predominant serotype. The relevant results were displayed in the flowchart (**Figure 1**), the median age (P25-P75) for the different serotypes were listed in table (**Table 1**), respectively.

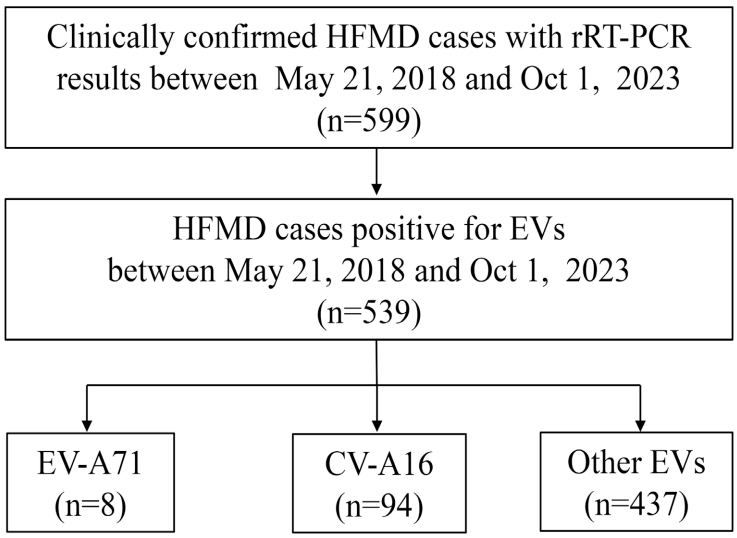


**Figure 1.** Flowchart illustrating the enrolled HFMD cases during the study period (from 21 May 2018 to 1 October 2023).

**Table 1.** Serotype distribution among single EV-infected HFMD cases in different gender groups.

| Serotypes | Total (n = 539) | Male (n = 311) | Sex Female (n = 228) | MFR | M (IQR) |
|---|---|---|---|---|---|
| EV-A71 | 8 (1.48) | 4 (1.29) | 4 (1.75) | 1 | 26.5 (15.25 - 41.5) |
| CV-A16 | 94 (17.44) | 57 (18.33) | 37 (16.23) | 1.54 | 35 (14.25 - 45.5) |
| Other EVs | 437 (81.08) | 250 (80.38) | 187 (82.02) | 1.34 | 17.5 11.28 - 21.75) |
| P-Value | 0.643a | | | | 0.048b |

Notes: EV, enterovirus; CV, Coxsackievirus; MFR: Male to Female ratio, a Fisher's precision probability test; b Kruskal-Wallis rank sum test.

### 3.2. Overall Distribution of Enteroviruses in 2018-2023

Among the 599 HFMD cases, 60 (10.02%) were negative for enteroviruses nucleic acid test. As shown in **Figure 2(A)**, the annual statistical chart of enterovirus infections indicated that from 2018 to 2019, the total number of cases of HFMD increased from 67 cases to 126 cases. Then number of cases stabilized at 127 cases in 2020. Following this, the number of HFMD cases decreased sharply from 2021 to 2022, with 55 cases in 2021 and 38 cases in 2022. However, the number of cases of HFMD increased rapidly in 2023, reaching a peak of 186 cases. Among all enteroviruses detected from 2018 to 2023, the number of CV-A16 (94 cases) and other EVs (437 cases) shared a similar trend to the total number of cases, while the number of EV-A71 (8 cases) decreased year by year, even with no EV-A71 cases detected in 2023.

**Figure 2(B)** and **Figure 2(D)** showed that other EVs accounted for the majority of the total number of cases, with annual proportions of 86.54%, 77.68%, 74.78%, 85.71%, 97.22%, and 81.14%, respectively. During 2018 to 2023, the proportion of cases infected with EV-A71 decreased from more than 5% in 2018 to less than 1% in 2020, then it increased to 2% in 2021, no EV-A71 cases were detected between 2022 and 2023. While the proportion of cases infected with CV-A16 continued to increase from 2018 to 2020, with a peak of 24.35% in 2020, following this, it declined in 2021 and 2022, falling to a minimum of 3% in 2022, and then, it recovered to 18.86% in 2023 and this was similarity to 19.64% in 2019. It is noteworthy that the serotype in April 2019 and February 2021 were only CV-A16.

To further analyze the monthly characteristics of enterovirus infection from 2018 to 2023, as shown in **Figure 2(C)**, three main peaks were observed in 2019,2020 and 2023: from May to August, from September to November and from July to September, respectively. HFMD occurred mainly in July and October (**Figure 3(B)**). Due to the impact of the COVID-19 pandemic, few cases were recorded from January to July in 2020 and from November to December in 2022 (**Figure 2(C)**, **Figure 2(D)**). In addition, no cases of CV-A16 infection were detected from August to November 2018 and from July to December 2021 and 2022 (**Figure 2(D)**).

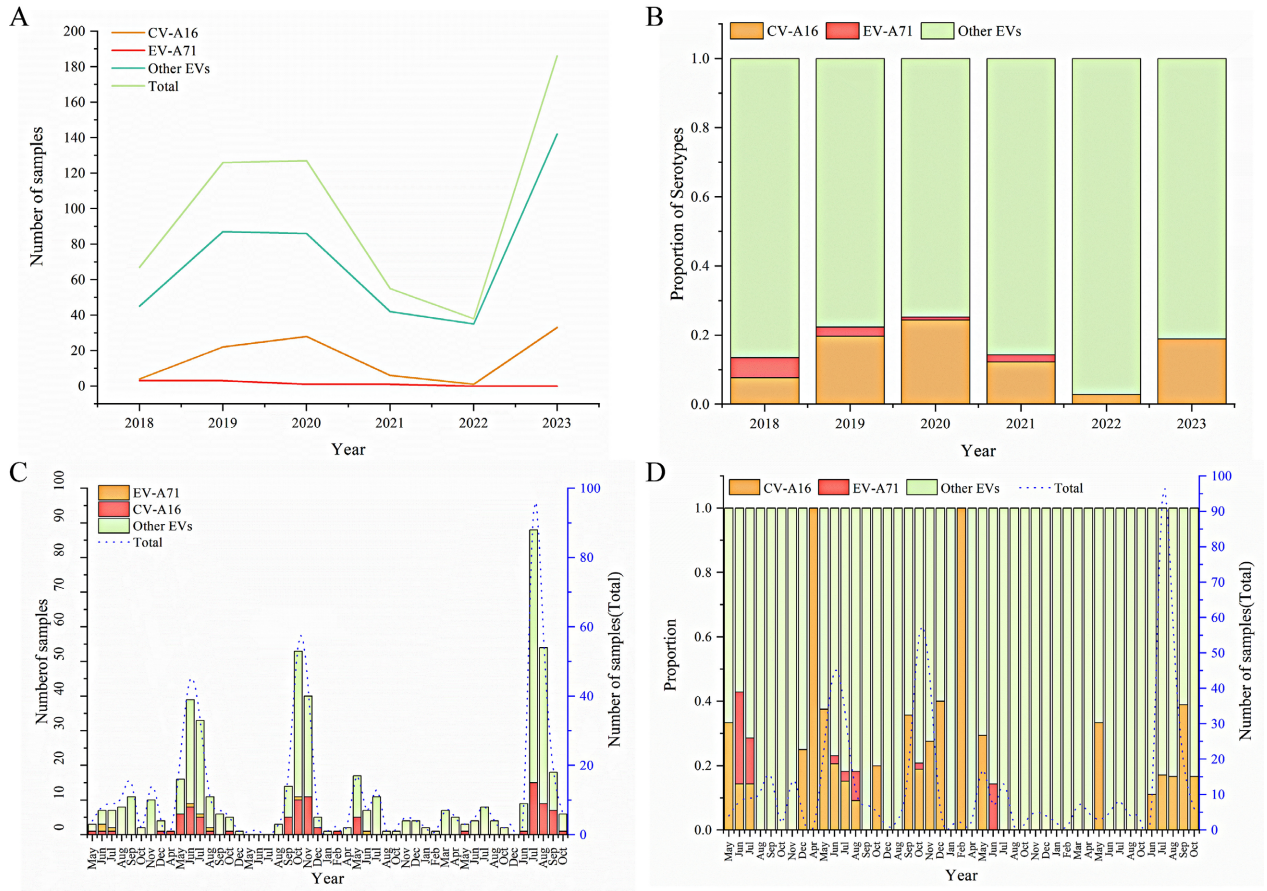


**Figure 2.** Overall distribution of enteroviruses. A: The yearly distribution of all HFMD cases in 2018-2023. B: The yearly proportion of all HFMD cases in 2018-2023. C: The monthly distribution of all HFMD cases in 2018-2023. D: The monthly proportion of all HFMD cases in 2018-2023. EV-A71: Enterovirus 71; CV-A16: Coxsackievirus A16; Other EVs: Not including EV-A71 and CV-A16.

The bar chart in **Figure 3(B)** showed that the EV-A71 predominantly distributed in June and July. Additionally, the proportion of CV-A16 infections was low compared to the overall total, accounting for approximately 15% of all positive cases.

### 3.3. Gender and Age Characteristics of Patients with Different Serotypes of Enterovirus Infection

To gain insights into clinical characteristics of cases infected with different serotypes, the relative analyses were conducted. Our study showed that the median age of CV-A16-infected patients (35 months) was older than EV-A71-infected patients (26.5 months) and other EVs-infected patients (17.5 months), and with no significant difference (P = 0.048) in age composition (**Table 1**). The ratio of male to female for EV-A71, CV-A16 and other EVs were 1, 1.54 and 1.34, respectively. For the gender, there were more males (311 cases) than females (228 cases) among the total of positive HFMD cases. Among the different serotypes, there were more males (57 for CV-A16 and 250 for other EVs) than females (37 for CV-A16 and 187 for other EVs), except for EV-A71, where the number of

males and females was the same, with 4 cases (Figure 3(A)). Whereas no significant difference was observed (P = 0.648) (Table 1). As shown in Table 2, the number of HFMD cases in different age groups dropped from 194 cases (12 - 23 months), 110 cases (24 - 35 months), 96 cases (36 - 47 months), 78 cases (<12 months) to 61cases (>48 months). Of all the positive cases, other EVs account the highest proportions in different age groups, with proportions of 88.46% (<12 months), 83.5% (12 - 23 months), 76.36% (24 - 35 months), 71.88% (36 - 47 months) and 86.88% (>48 months), respectively. Unlike other EVs and CV-A16, EV-A71 had the lowest proportions throughout whole age groups, decreasing from 1.82% (<12 months) to 1.04% (36 - 47 months), and there were very few cases of EV-A71 infections under 60 months of age (8 cases). The proportion of CV-A16 was 10 or even 20 times higher than that of EV-A71. The age distribution of CV-A16-infected cases were similar, ranging from 12 - 23 months, 24 - 35 months and 36 - 47 months, respectively (Figure 3(C), Figure 3(D)). No significant difference (P = 0.074) between age groups and serotypes was found (Table 2).

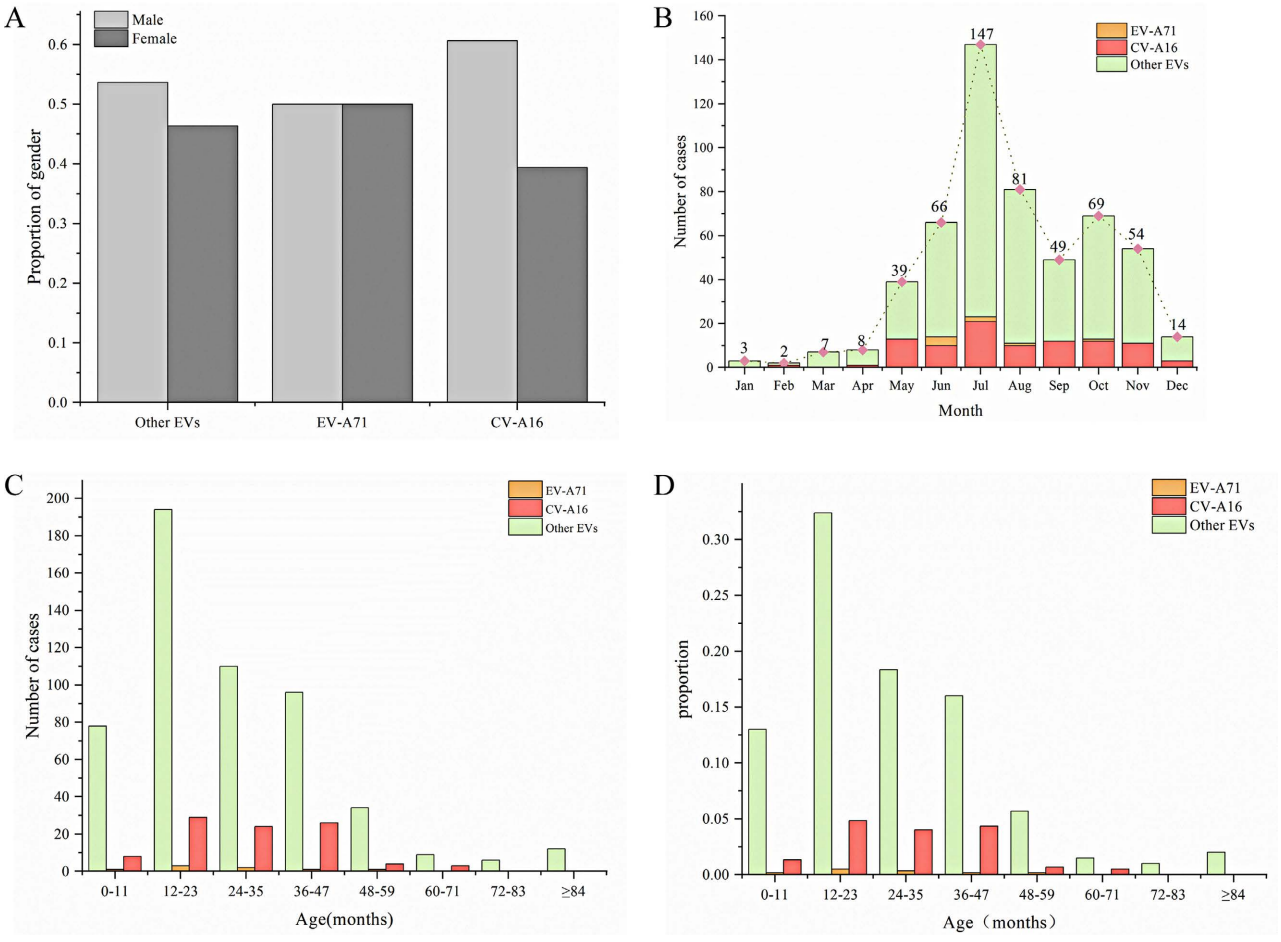


**Figure 3.** Clinical characteristics of HFMD cases in 2018-2023. A: The sex distribution of other EVs EV-A71 CV-A16, respectively. B: The monthly serotype distribution of enteroviruses positive HFMD cases. C: The age distribution of other EVs, EV-A71, CV-A16, respectively. D: The age proportion of other EVs, EV-A71, CV-A16, respectively.

**Table 2.** Serotype distribution among EV-infected HFMD cases in different age groups.

| Serotypes | Age <12 (n = 78) | Group 12 - 23 (n = 194) | Months 24 - 35 (n = 110) | 36 - 47 (n = 96) | ≥48 (n = 61) | P-Value |
|---|---|---|---|---|---|---|
| EV-A71 | 1 (1.28) | 3 (1.55) | 2 (1.82) | 1 (1.04) | 1 (1.64) | 0.072 |
| CV-A16 | 8 (10.26) | 29 (14.95) | 24 (21.82) | 26 (27.08) | 7 (11.48) | |
| Other EVs | 69 (88.46) | 162 (83.50) | 84 (76.36) | 69 (71.88) | 53 (86.88) | |

Notes: Fisher's precision probability test; EV, Enterovirus; CV, Coxsackievirus; other EVs, No-EV-A71 and No-CV-A16 enterovirus.

### 3.4. Changes in Molecular Epidemiological and Clinical Characteristics of HFMD in Northern Sichuan Region in Different Periods of Time

COVID-19 caused a pandemic since the end year of 2019, to assess the effect of COVID-19 on HFMD, the period was divided into three subperiods: 2018-2019, 2020-2022, and 2023, and the results were presented in Table 3 and Table 4. Our study revealed that COVID-19 did not cause the significant changes in gender, age and serotypes. However, the number of HFMD cases varied slightly among all cases that tested positive for enterovirus nucleic acid, with 164 (2018-2019), 200 (2020-2022) and 175 (2023) cases. There was a slight decrease in the male to female ratio of 1.52, 1.27 and 1.33, respectively. The median age for the three subperiods was 24 months, 21 months and 28 months respectively. The proportion of EV-A71 peaked at 3.66% before COVID-19 (2020-2022), then dropped to 1% during the subperiod and was not even observed in 2023. In contrast, the proportion of CV-A16 increased slightly while the proportion of other EVs remained stable at around 80%. During the 2023 subperiod, there was an increase of about 5 % in cases older than 36 months compared with the subperiod (2020-2022). However, the proportion of children under 12 months of age decreased by 6 % compared with subperiod (2020-2022) (Table 3). To further analyze the effect of COVID-19 on the age distribution in the different serotypes. We analyzed the age variation of different serotypes in the subperiods; for EV-A71, there were 6 cases in the subperiod (2018-2019), only 2 cases in the subperiod (2020-2022), and 1 case each in 12-23 months and >48 months. For CV-A16, in contrast to the decrease in the proportion of children aged under 12 months decreased from 11.54% (2018-2019) to 2.5% (2020-2022), with no cases found in 2023, there was an increase in the proportion of children aged over 12 months by about 5% - 30%. For other EVs, the proportion of cases older than 36 months was trending upwards, at about 6%, except for cases aged 12 - 23 months, where there was an increase of about 10% in the comparison between 2023 and 2020-2022.

**Table 3.** Characteristic changes of HFMD cases in 2018-2019, 2020-2022, 2023 of North Sichuan region.

| Characteristics | 2018-2019 (n = 164) | 2020-2022 (n = 200) | 2023 (n = 175) | P1-Value | P2-Value |
|---|---|---|---|---|---|
| Gender n(%) | | | | 0.401a | 0.824a |
| Male | 99 (60.37) | 112 (56) | 100 (57.14) | | |
| Female | 65 (39.63) | 88 (44) | 75 (42.86) | | |
| MFR | 1.52 | 1.27 | 1.33 | | |
| Median Age (P25-P75) | 24 (16- 38) | 21 (13 - 35) | 28 (15 - 42) | 0.584b | 0.795b |
| Age group Month n (%) | | | | 0.388 a | 0.1 a |
| <12 | 21 (12.80) | 36 (18) | 21 (12) | | |
| 12 - 23 | 58 (35.37) | 79 (39.5) | 57 (32.57) | | |
| 24 - 35 | 40 (24.39) | 36 (18) | 34 (19.43) | | |
| 36 - 47 | 27 (16.46) | 31 (15.5) | 38 (21.71) | | |
| ≥48 | 18 (10.98) | 18 (9) | 25 (14.29) | | |
| Serotypes, n (%) | | | | 0.238c | 0.582c |
| EV-A71 | 6 (3.66) | 2 (1) | 0 (0) | | |
| CV-A16 | 26 (15.85) | 35 (17.5) | 33 (18.86) | | |
| OtherEVs | 132 (80.49) | 163 (81.5) | 142 (81.14) | | |

Other enteroviruses: No-EV-A71 andNo-CV-A16 enterovirus; P1 value: the data in 2018-2019 compared with 2020-2022; P2 value: the data in 2020-2022 compared with 2023; a, Chi-square test; b means Mann Whitney U test was used for statistical analysis. c, Fisher's precision probability test.

**Table 4.** The age distribution of different serotypes in 2018-2019, 2020-2022, 2023 of north Sichuan region.

| | 2018-2019 (n = 164) | 2020-2022 (n = 200) | 2023 (n = 175) | P1-Value | P2-Value |
|---|---|---|---|---|---|
| EV-A71 Age group Month n (%) | | | | 0.786[b] | - |
| <12 | 1(16.67) | 0(0) | - | | |
| 12 - 23 | 2(33.33) | 1(50) | - | | |
| 24 - 35 | 2(33.33) | 0(0) | - | | |
| 36 - 47 | 1(16.67) | 0(0) | - | | |
| ≥48 | 0(0) | 1(50) | - | | |
| CV-A16 Age group Month n (%) | | | | 0.542[b] | 0.246[b] |
| <12 | 3(11.54) | 5(2.5) | 0(0) | | |
| 12 - 23 | 6(23.08) | 11(5.5) | 12(36.36) | | |

**Continued**

| | | | | | |
|---|---|---|---|---|---|
| 24 - 35 | 6 (23.08) | 9 (4.5) | 9 (27.27) | | |
| 36 - 47 | 7 (26.92) | 9 (4.5) | 10 (30.30) | | |
| ≥48 | 4 (15.38) | 1 (0.5) | 2 (6.06) | | |
| Other EVs | | | | | |
| Age group Month n(%) | | | | 0.39[a] | 0.142[a] |
| <12 | 17 (12.88) | 31 (19.02) | 21 (14.79) | | |
| 12 - 23 | 50 (37.88) | 67 (41.10) | 45 (31.69) | | |
| 24 - 35 | 32 (24.24) | 27 (16.56) | 25 (17.61) | | |
| 36 - 47 | 19 (14.39) | 22 (13.50) | 28 (19.72) | | |
| ≥48 | 14 (10.61) | 16 (9.82) | 23 (16.20) | | |

Other enteroviruses: No-EV-A71 and No-CV-A16 enterovirus; P1 value: the data in 2018-2019 compared with 2020-2022; P2 value: the data during 2020-2022 compared with 2023; a means Chi-square test; b, Fisher's precision probability test, "-" indicates that no data of CV-A16 and EV-A71 were collected in 2023.

## 4. Discussion

This study described the epidemiologic features and clinical characteristics of HFMD from 2018 to 2023 in north Sichuan region, which centered on Guangyuan City, and involved the surrounding areas including south region of Gansu and Shaanxi provinces, as well as Bazhong city which located at the southeast to Guangyuan city. Guangyuan Central Hospital as the north Sichuan regional medical centre has a separate branch for women and children and receives the patients from above mentioned areas. In this study, clinically confirmed cases of HFMD in Guangyuan central hospital from 2018 to 2023 were analyzed, including age, gender, number of cases and laboratory results.

The total number of HFMD cases was first evaluated, which broadly showed a slowly increasing trend from 2018 to 2023. In agreed with previous study [20], the number of HFMD cases decreased sharply from 2020 to 2022, this may attributed to the stringent respiratory protection measures adopted in the country during the COVID-19 pandemic. The increasing trend in total number of HFMD cases was contrasted to some other studies [21] [22], in which they demonstrated a decreasing trend from 2017 to 2022. This discrepancy may be due to an incomplete data for several following reasons: first, no HFMD-related nucleic acid testing was performed at our hospital prior to 2018; second, relative nucleic acid testing was not performed on some of the HFMD cases; and finally, data were temporarily missing from published HFMD-related studies in 2023 [21] [22] [23]. Generally, our findings indicated an increased incidence of HFMD in north Sichuan region.

To reduce the incidence of HFMD, the domestic EV-A71 vaccine was launched in Sichuan province in August 2016. The number of cases of HFMD, the rate of severe illnesses and the mortality rate of patients subsequently decreased [23]

[24] [25], our data further confirmed the efficiency of vaccine and the EV-A71-associated cases were even not detected in our laboratory from 2022 to 2023. Additionally, consistent with other study [26], there was a trend of semi-annual outbreaks of HFMD in studied area. The peak incidence of HFMD in our study occurred in July and October, compared to from April to May and from November to December in study of Chengdu City, the central Sichuan region [27]. There are two possible reasons for this discrepancy: on one hand, seasonal trends in HFMD are most affected by latitude [28] [29], our studied area is located in the southern foothills of the Qinling Mountains, a transition zone between the north and the south climate, and at higher latitude 32.43˚ compared to latitude 30.67˚ of Chengdu city, which give this region distinct climatic characteristics; on the other hand, it is reported that meteorological factors, such as temperature, rainfall, relative humidity and hours of sunshine, are positively correlate with the incidence of HFMD [30], in our study, the peak of HFMD infection was in July with a maximum temperature 27.9˚C, when the maximum temperature in Chengdu region was 30.6˚C, which indicated that Chengdu reached a temperature suitable for virus transmission earlier. Furthermore, there were no diagnosed HFMD cases from January to May in 2023. This phenomenon might be due to the new policies to relax a series of stringent measures by Chinese government for the COVID-19 from November 2022, followed by endemic of respiratory syncytial virus and influenza virus from March to May in 2023, who dominated the endemic viruses at that time and were focused on in the strategy of prevention and control in our region.

Long-term investigation in our study indicated a largest proportion of unclassified serotypes in HFMD cases, more than 75 % compared with less than 25 % of total for EV-A71 and CV-A16 serotypes together. Especially in 2023, CV-A16 serotype constituted approximately 20% of total types, while the EV-A71 serotype was not detected. CV-A6 serotype was reported as the major epidemiologic agent of HFMD in recent years [18] [23]. According to our study, it further confirmed the altered dynamics of enteroviruses, other unclassified viruses that have become the current major epidemic viruses. It suggests that there is an urgent need for further molecular classification of enteroviruses and development of multivalent vaccines to provide a more accurate basis for HFMD prevention. A more detailed classification of enteroviruses could not only provide molecular medical evidence for the diagnosis of HFMD, but also provide a basis for epidemiological studies of HFMD and the development of vaccines. In addition, clearly typed enterovirus and its molecular epidemiological characteristics provide the basis for precise prevention and control of HFMD.

The correlation between gender, age, number of cases and enterovirus serotypes were analyzed to investigate clinical characteristics of HFMD patients. Our study confirmed previous finding that there is no direct relationship between patient gender and infectious enterovirus serotypes [25]. Additionally, a higher transmissibility of HFMD in male than that in female was observed, which was in line with previous finding [31]. It is well known that children under the age of

60 months are the main victims of HFMD [27] [32]. In our study, HFMD mainly affected the children under age of 47 months, in particular, the HFMD cases over the age of 60 months were less than the HFMD cases of age between 48 and 59 months. These findings demonstrated that in our region, infection rates began to diminish after the age of 4 years, and it was more difficult to be infected after the age of 5 years, which was in agreement with the report of a weak transmissibility in patients aged over 5 years old [33]. Further, our study indicated that children between the ages of 12 and 23 months were the most susceptible to HFMD, followed by those aged 24 - 47 months and 0 - 11 months respectively, which supported previous findings [23] [33] and contrasted to that children aged 2 years were found to be the most susceptible to HFMD [34]. In addition, we found that children infected with CV-A16 were mainly concentrated in the age group of 1 - 3 years, whereas children infected with EV-A71 were mainly concentrated in the age group of 1 - 2 years. This difference may be due to climatic and socio-economic differences in studied area [28]. As reported before [31], the male-to-female ratio of EV-A71 was smaller than that of CV-A16 and other EVs. This may be due to the fact that the different serotypes are more transmissible in males than in females. Another possible explanation for this difference is that the EV-A71 vaccination effectively protected children from this virus regardless of gender, and the EV-A71 vaccination may change the epidemiological characteristics of HFMD [35]. And this difference may be also related to the fact that boys engage in more outdoor activities, which increases their risk of infection compared to girls.

There were similarities between our findings and those of other studies in that COVID-19 has little effect on the gender of HFMD cases and, in our study, no significant changes were observed between different age groups, serotypes, and subperiods [23]. Further analysis indicated a declining trend of the male-to-female ratio of HFMD cases, which was also observed in previous study [27]. Although the number of HFMD cases was higher in the 2020-2022 period than in the other subperiods, the average number of cases per year was actually lower in this period. The decrease in the number of HFMD cases may be due to the fact that non-pharmacologic interventions targeting COVID-19 (e.g., wearing masks, improving personal hand hygiene, and talking at arm's length) reduced the number of HFMD cases, as described in studies [36] [37] [38]. It is well known that HFMD is mainly transmitted through close contact, and schools are the main HFMD transmission sites. In the 2020-2022 period, Chinese government took a series of strict measures to restrict mobility of personnel to prevent the transmission of COVID-19 (e.g., close schools, work at home, and reduce the use of public transportation), which significantly inhibited the spread of HFMD from the transmission route and source. In addition, in post-epidemic period, Chinese government took routine preventive and control measures, including wearing masks, improving personal hand hygiene, and talking at social distancing, these were effective methods for not only preventing the spread of COVID-19 but also controlling the epidemic of HFMD.

The differences in the characteristics of HFMD between years were evaluated in our study. The proportion of children under 12 months of age with HFMD was higher in 2020-2022 than in 2018-2019 and 2023. Further, we found evidence that the EV-A71 vaccine was as effective as previously reported [39]. Although a clear increasing trend in CV-A16 prevalence was observed in children aged 12 - 47 months, and the highest CV-A16 prevalence was observed in children aged 12 - 23 months, no statistically significant differences were found. Nevertheless, children aged 12 - 23 months should receive more attention. The slightly higher prevalence of other enteroviral infections in children older than 36 months and younger than 12 months in our study further illustrated the importance of serologic typing of enteroviruses.

This study also has some limitations; first, the initial period of HFMD nucleic acid testing was not sufficiently widespread in the clinic, resulting in a low number of cases completing nucleic acid testing; second, we used only throat swabs as samples for HFMD nucleic acid testing, which carries the risk of false negatives; third, the strict preventive and control policies during the Epidemic of COVID-19 resulted in a significant decrease in the number of HFMD cases during some months, such as January-March 2023; fourth: only two serotypes, CV-A16 and EV-A71, were detected in this study, and the serotypes of enteroviruses that accounted for the predominant portion of the population were not further detected, so this untyped portion of enteroviruses may have an important role in HFMD prevention and treatment.

## 5. Conclusion

In summary, we for the first time analyzed the molecular epidemiology and clinical characteristics of HFMD cases from 2018 to 2023 in North Sichuan region. HFMD will continue to be an important public health problem in this area. Due to the EV-A71 vaccine, the predominant serotypes of HFMD in studied area have changed dynamically. Therefore, more detailed molecular epidemiologic analysis should be conducted to develop vaccination strategies. The optimal time for intervention needs to vary depending on the time of HFMD incidence peak in different regions. Our study not only contributes to the prevention and control of HFMD, but also to the subsequent application of HFMD surveillance systems in North Sichuan region.

## Conflicts of Interest

There were no conflicts of interest for all authors.

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