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Circulating trends of hand, foot, and mouth disease in Hubei Province, China: Impact from the COVID-19 pandemic

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ABSTRACT

Objectives: This study was performed to investigate the effect of non-pharmaceutical interventions on hand, foot, and mouth disease in Hubei Province China during the coronavirus disease 2019 pandemic.
Methods: Data and samples were collected from the hand, foot, and mouth disease surveillance laboratory network in Hubei Province between 2018 and 2022. The samples were identified as Enterovirus A71, Coxsackievirus A6or Coxsackievirus A16 via real-time polymerase chain reaction. Representative Coxsackievirus A6 and Coxsackievirus A16 samples were sequenced and subjected to phylogenetic analyses.
Results: A noticeable 3-fold reduction in the number of hand, foot, and mouth disease cases was observed from 2019 to 2020. The age and sex distributions of patients with hand, foot, and mouth disease were approximately the same from 2018 to 2022. The proportion of Coxsackievirus A6 accounted for 86 % in 2020 and 75 % in 2021 for hand, foot, and mouth disease compared with 48 % in 2018, 53 % in 2019, and 29 % in 2022. The proportions of Coxsackievirus A16 in 2020 and 2021 were 2 % and 17 %, respectively, showing a sharp decline in 2018 (37.8 %) and 2019 (35 %). In 2022, Coxsackievirus A16 was the dominant serotype (46 %). Only slight differences were found in the VP1 sequences across the different years.
Conclusions: Our study confirmed that a series of non-pharmaceutical interventions during the coronavirus disease 2019 period reduced the transmission of enteroviruses and that long-term restrictions could significantly change the prevalence of enterovirus serotypes causing hand, foot, and mouth disease.

1. Introduction

Coronavirus disease 2019 (COVID-19) began in December 2019 in Wuhan, Hubei Province, China, and rapidly spread worldwide. China succeeded in containing COVID-19 with a lockdown in its cities. Many strategies for non-pharmaceutical interventions (NPIs) were implemented, such as the use of masks, hand hygiene, social distancing, and the closure of schools to stop the epidemic from rebounding. Hubei Province was the center of the COVID-19 pandemic in China. The city lockdown lasted for three months. The most restrictive strategy of NPIs was implemented through the all of 2020 and lasted until 2022. NPIs implemented worldwide during the

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Abbreviations

COVID-19	Coronavirus disease 2019
NPIs	non-pharmaceutical interventions
HFMD	hand, foot, and mouth disease
UTR	untranslated region
ORF	open reading frame
RT-PCR	reverse transcript polymerase chain reaction
EV-A71	Enterovirus A71
CVA6	Coxsackievirus A6
CVA16	Coxsackievirus A16
UEV	unspecified enteroviruses

COVID-19 pandemic not only efficiently contained COVID-19 but also reduced the incidence of other respiratory and gastrointestinal infectious diseases [1–3].

Hand, foot, and mouth disease (HFMD) is a common disease caused by various enteroviruses in children under six years of age. Enteroviruses in the family Picornaviridae are classified into 15 species: enteroviruses A–K and rhinoviruses A–C. Among them, 116 serotypes mainly infect humans, and at least 23 serotypes are currently known to cause HFMD [4]. Enteroviruses are small, non-enveloped, single-stranded positive-sense RNA viruses [5]. Enteroviruses genomes are about 7500 nucleotides long and contain a 5′-untranslated region (UTR), a long open reading frame (ORF), and a 3′-UTR. The ORF encodes a polyprotein that is processed into three polyprotein precursors (P1, P2, and P3). The P1 precursor protein is cleaved into four structural proteins (VP4, VP2, VP3, and VP1). The P2 and P3 nonstructural protein regions encode seven nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D) [5]. Although HFMD manifests as a self-limiting disease, characterized by acute fever accompanied by erythema and vesicles on the hands, feet, and mouth, rapid progression to neurological complications can be lethal in some patients [6,7]. HFMD can be excreted in the respiratory tract and transmitted from person to person through direct or indirect contact, similar to the transmission route of COVID-19 [8,9]. Strict compliance with implementation during the COVID-19 pandemic, such as the use of masks, hand hygiene, and school closures, may also be effective against HFMD.

We observed a noticeable decrease in the number of HFMD cases during the COVID-19 pandemic, and almost no cases were reported between May and August 2020, the main epidemic period for HFMD. However, after schools reopened in September, the number of HFMD cases increased rapidly, and the species of enteroviruses-caused HFMD changed greatly compared with previous years. We hypothesized that this might be affected by COVID-19 NPIs strategies. Therefore, this retrospective study aimed to investigate the prevalence of HFMD using a surveillance laboratory network.

2. Methods

2.1. Data and specimen collection

Demographic data (age and sex) were collected from the HFMD surveillance laboratory network of Hubei Province, China, from January 2018 to December 2022. Clinical specimens were collected from patients with suspected cases of HFMD when they sought treatment at the hospital. Specimens were kept at −80 °C until examination for enterovirus by real-time reverse transcript polymerase chain reaction (RT-PCR). The main inclusion criteria were suspected cases of HFMD and input of case information into the HFMD surveillance laboratory network of Hubei Province. The key exclusion criterion was the failure of the nucleic acid test for the sample.

2.2. Lockdown during COVID-19 pandemic

Emergency response in China was categorized into four levels (1–4) based on the emergency plan for public emergencies in China. Level 1 emergency response, the highest level of response, initiates the most stringent public health intervention measures, such as regional lockdown and travel restrictions. The Hubei provincial government initiated a Level 1 emergency response from January 24, 2020, to May 1, 2020, to control the COVID-19 epidemic in 2020. The Level 2 response was implemented from May 2, 2020, to June 12, 2020. On June 13, 2020, a Level 3 response was implemented in Hubei. The nurseries and kindergartens were closed during the lockdown, and the start date was suspended on September 1, 2020. Throughout 2021 Hubei had been in a state of routine control. In 2022, an irregular lockdown occurred in many cities of Hubei Province, and nurseries and kindergartens were closed from September 1, 2022, to December 31, 2022.

2.3. Real-time RT-PCR detection

Viral RNA was extracted from clinical specimens using the Tianlong Viral RNA Kit (Xi'an, China), according to the manufacturer instructions and subjected to real-time RT-PCR. This real-time RT-PCR kit (Jin Hao Ltd., China) specifically detects enterovirus. Fluorescence data were analyzed using an Abi Q7 (Life Technologies, USA). Real-time data analysis was used to classify the viruses as

Enterovirus A71 (EV-A71), Coxsackievirus A6 (CVA6), Coxsackievirus A16 (CVA16) or unspecified enteroviruses (UEV).

2.4. Definition

Suspected cases were defined as patients with negative nucleic acids for enterovirus but matching the clinical features of HFMD or with an epidemiological history of HFMD. Laboratory-confirmed cases were defined as patients with enterovirus-positive nucleic acids.

Table 1
Strains used for molecular analysis of CVA6[a].

Accession number	Strain name	Serotype	Genotype
LC481403	C42-YN-CHN-2018	CVA6	D3
LC707409	79-QJ-YN–CHN–2021-CVA6	CVA6	D3
LC712974	26-BS-YN–CHN–2020	CVA6	D3
MK357081	CVA6/JN058/CHN/2018	CVA6	D3
MN233721	18B0521/PD/SH/CHN/2018	CVA6	D3
MN233761	18T0404/PD/SH/CHN/2018	CVA6	D3
MN233763	18T0204/PD/SH/CHN/2018	CVA6	D3
MN233767	17T1214/PD/SH/CHN/2017	CVA6	D3
MN233775	17T1006/PD/SH/CHN/2017	CVA6	D3
MN541023	CVA6/SWG55/SD/CHN/2018	CVA6	D3
MN845848	22/HLJ/CHN/2018	CVA6	D3
MN845879	75/TJ/CHN/2018	CVA6	D3
MN864925	HA149/2018/Beijing/Tongzhou	CVA6	D3
MN864935	HA125/2018/Beijing/Tongzhou	CVA6	D3
MN864938	HA95/2018/Beijing/Tongzhou	CVA6	D3
MN864942	HA81/2018/Beijing/Tongzhou	CVA6	D3
MT119385	CVA6/07/CHN/GZ/2018	CVA6	D3
MT569395	E201805/Huzhou/CHN/2018	CVA6	D3
MW178701	CD2019-260-CVA6	CVA6	D3
MW178716	MS2019-334-CVA6	CVA6	D3
OL688750	AS199/NC/CHN/2019	CVA6	D3
OL688752	AS363/NC/CHN/2019	CVA6	D3
OL688753	AS398/NC/CHN/2019	CVA6	D3
OL830028	CVA6/BCHV19218/BJ/CHN/2019	CVA6	D3
OL830029	CVA6/BCHV19037/BJ/CHN/2019	CVA6	D3
OL830036	CVA6/BCHV17919/BJ/CHN/2017	CVA6	D3
OL839938	HA308/YunN	CVA6	D3
OL839948	HF345/YunN	CVA6	D3
OL840679	CVA6/19622/BJ/CHN/2019	CVA6	D3
OL840682	CVA6/18731/BJ/CHN/2018	CVA6	D3
OL840693	CVA6/18705/BJ/CHN/2018	CVA6	D3
OL840721	CVA6/18286/BJ/CHN/2018	CVA6	D3
OL840732	CVA6/18218/BJ/CHN/2018	CVA6	D3
OL840737	CVA6/18173/BJ/CHN/2018	CVA6	D3
OL840745	CVA6/18013/BJ/CHN/2018	CVA6	D3
OL840746	CVA6/17919/BJ/CHN/2017	CVA6	D3
OP896719	CU3482 THA2019	CVA6	D3
KC866916	JB143090122	CVA6	D2
KP143077	AFP569/GD/CHN/2006	CVA6	D2
KJ577297	11MH15 Q2 2011	CVA6	D2
KC866917	JB143090083	CVA6	D2
KJ865427	109982/GZ/2010/A6	CVA6	D2
KC866914	JB143090156	CVA6	D2
MN845762	3/HLJ/CHN/2011	CVA6	D2
KC866903	JB143090062	CVA6	D2
JX495119	SHAPHC1366/SH/CHN/11	CVA6	D2
MK106189	54203/HeB/CHN/2012	CVA6	D2
MF285622	CVA6/S845/BJ/CHN/2010	CVA6	D2
MF596066	CVA6/S845VP1/BJ/CHN/2010	CVA6	D2
KY211711	54203/HeB/CHN/2012	CVA6	D2
KP143080	AFP147/GD/CHN/2007	CVA6	D2
AB779614	Kyoto1	CVA6	D1
LC126143	Hyogo1278	CVA6	D1
FR797988	ESP08/54,698	CVA6	D1
HE572917	CF165026 FRA10	CVA6	D1
JQ364887	96188/SD/CHN/1996/CA6	CVA6	C
KP143074	AFP560/GD/CHN/2004	CVA6	B
AY421764	Gdula	CVA6	A

[a] CVA6: Coxsackievirus A6.

2.5. Determination of the VP1 nucleotide sequence of CVA6 and CVA16

The VP1 genes of CVA6 and CVA16 were amplified using the following specific primers: CVA6 VP1–F:5′-ATATTA-TAGCTCTTGGAGCAGCA-3' (nucleotides 2353–2376) and VP1-R:5′-GCAGTAATACACTCCTGTTTGAC-3' (nucleotides 3502–3525). CVA16 VP1–F:5′-CTGGGTACTTTGACTATTACAC-3' (nucleotides 2261–2283) and VP1-R:5′-GTTGTTATCTTGTCTCTACTAGTG-3' (nucleotides 3304–3328). The RNA amplification of VP1 gene was performed using the OneStep RT-PCR kit (Takara, China) according to the manufacturer instructions. The reactions were incubated at 50 °C for 30 min, followed by 94 °C for 5 min. Thermocycling was performed for 35 cycles of 94 °C, 30 s; 55 °C, 30 s and 72 °C, 1 min 30 s in the C1000 Touch PCR (Biorad, USA). Amplicons were sequenced using an ABI3730XL Genetic Analyzer (Applied Biosystems, United States).

2.6. Phylogenetic analysis

The CVA6 and CVA16 VP1 sequences generated in this study were aligned with reference strains from worldwide in GenBank (Tables 1 and 2). A total of 225 CVA6 and 185 CVA16 VP1 sequences were included in the phylogenetic analysis, including the 166 CVA6 (GenBank accession numbers OR507398–OR507563) and 144 CVA16 (GenBank accession numbers OR507254–OR507397) sequences generated in this study. The sequences were aligned using Mega X (https://megasoftware.net/). Phylogenetic trees were constructed using the neighbor-joining method after estimating the genetic distance using the Kimura two-parameter method. A bootstrapping test was performed 1000 times. Phylogenetic trees were annotated and visualized using Itol (https://itol.embl.de/).

Table 2
Strains used for molecular analysis of CVA16[a].

Accession number	Strain name	Serotype	Genotype
MW197397	CVA16-132 GD-CHN 2019-02	CVA16	B1a
LC656490	14-WS-YN–CHN–2020	CVA16	B1a
OP373940	CVA16-2019-386-TY-SX-CHN	CVA16	B1a
MW179203	MY2019-19-CVA16	CVA16	B1a
OP373936	CVA16-2019-365-TY-SX-CHN	CVA16	B1a
LC533790	J115-WS-YN–CHN–2019	CVA16	B1a
MT119437	CVA16/11/CHN/GZ/2018	CVA16	B1a
LC656502	74-WS-YN–CHN–2020	CVA16	B1a
MZ043540	03EI-01-1-797	CVA16	B1a
MW197380	CVA16-45 GD-CHN 2019-01	CVA16	B1a
LC707410	81-QJ-YN–CHN–2021-CVA16	CVA16	B1a
OP373747	CVA16-2021-241-TY-SX-CHN	CVA16	B1b
MT119438	CVA16/12/CHN/GZ/2018	CVA16	B1b
OP373748	CVA16-2019-97-TY-SX-CHN	CVA16	B1b
MW197386	CVA16-76 GD-CHN 2019-02	CVA16	B1b
MT119434	CVA16/08/CHN/GZ/2018	CVA16	B1b
OP373774	CVA16-2019-266-TY-SX-CHN	CVA16	B1b
MW197396	CVA16-128 GD-CHN 2019-02	CVA16	B1b
OP373780	CVA16-2019-147-TY-SX-CHN	CVA16	B1b
MT553238	S7443/BJ/CHN/2019	CVA16	B1b
MW197407	CVA16-842 GD-CHN 2019-05	CVA16	B1b
MW197368	CVA16-R546 GD-CHN 2018-05	CVA16	B1b
MN886521	S7628/BJ/CHN/2019	CVA16	B1b
AB465368	576/Toyama/1988	CVA16	B2
AB634293	**Y92–2998**	CVA16	B2
AB634286	Y88-5375	CVA16	B2
AB634287	**Y92–2389**	CVA16	B2
AB465366	24/Toyama/1981	CVA16	B2
AB465367	379/Toyama/1984	CVA16	B2
AM292468	SB2239/SAR/00	CVA16	B2
AM292455	S10432/SAR/98	CVA16	B2
AB634322	721-Yamagata-1998	CVA16	B2
OP562188	S0969TW99	CVA16	B2
OP562190	N3649TW00	CVA16	B2
AB465369	107/Toyama/1990	CVA16	B2
LT617109	CVA16C CF193053 FRA 2012	CVA16	C
LT617115	CVA16C PAR190033 FRA 2014	CVA16	C
KF956720	FLA6916	CVA16	C
MG957117	SH-HP-16-51	CVA16	C
LT617105	CVA16C CF350028 FRA 2011	CVA16	C
U05876	G-10	CVA16	A

[a] CVA16: Coxsackievirus A16.

2.7. Statistical analysis

Microsoft Office (version 2010, Microsoft Corp) and SPSS (version 26.0, IBM Corp) were used for statistical analysis. Descriptive analyses were performed, and categorical variables were analyzed using the chi-squared test or Fisher's exact test. Statistical significance was defined as a two-sided P-value of <0.05.

3. Results

3.1. Changes in epidemiology of HFMD

A total of 25,354 suspected HFMD cases were reported to the surveillance system, and 16,530 laboratory-confirmed HFMD cases between January 2018 and December 2022. The annual distribution of the suspected and laboratory-confirmed HFMD cases during this period is shown in Fig. 1A. The numbers of laboratory-confirmed HFMD cases from 2018 to 2022 were 4084, 5084, 1622, 3565, and 2175, respectively. Since 2020, the number of laboratory-confirmed HFMD cases has markedly decreased compared with that in 2018 and 2019, and a noticeable 3-fold reduction from 2019 to 2020 was observed ($p < 0.001$). Under routine control, a comparison between cases in 2021 and the average number of cases in 2018–2019 showed that HFMD cases in 2021 decreased slightly in general (relative reduction:22.2 %).

The incidence of HFMD in Hubei showed seasonality, which generally peaked in the second quarter, except in 2020, and may have had a second small peak in the fourth quarter. In 2018 to 2019 and 2021 to 2022, the incidence of HFMD continued to decline from the first week and gradually increased from the ninth week. The ascending trend was obvious in the 12th week and peaked in the 19th to 27th weeks (April to June) and dwindled in the 28th week. There was a small peak in autumn and winter at the 44th to 50th weeks (November–December). However, in 2020, the HFMD epidemic peak was postponed, with a low epidemic situation in the first half, rising in the third quarter, and peaking in the fourth quarter (Fig. 1B).

There were no significant differences in the age and sex distribution of patients with HFMD between 2018 and 2022 (Table 3). Male cases were 1.39 times more common than females. Children aged 0–3 years accounted for 60.2 % of laboratory-confirmed cases, with an average of 10.3 % children under 1 year of age. Children aged 3–6 years accounted for 32.8 % of the cases, while only 7 % of the cases occurred in children aged >6 years.

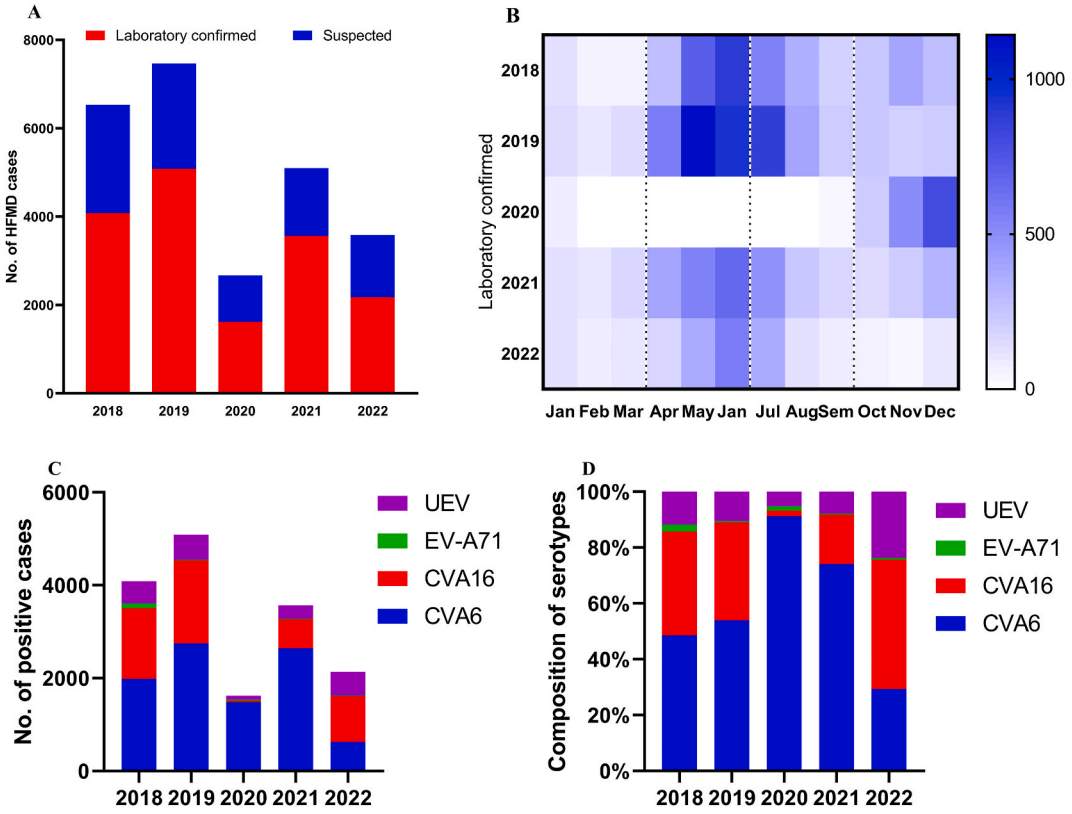

Fig. 1. Distribution of enterovirus-associated HFMD cases in Hubei Province, from 2018 to 2022. (A) Yearly distribution of suspected and laboratory-confirmed HFMD cases; (B) Monthly heatmap of laboratory-confirmed HFMD cases; (C) Number of different serotypes in all laboratory-confirmed cases; (D) Composition of different serotypes in all laboratory-confirmed cases.

Table 3
Patient characteristics of laboratory confirmed HFMD[a] cases from 2018 to 2022.

	2018	2019	2020	2021	2022	P value
Gender						
Male, n (%)	2364 (57.9)	2911 (57.3)	961 (59.2)	2073 (58.1)	1228 (56.4)	0.445
Female, n (%)	1720 (42.1)	2173 (42.7)	661 (40.8)	1492 (41.9)	947 (43.6)	
Age						
<1 year, n (%)	411 (10.1)	503 (9.9)	186 (11.5)	374 (10.5)	234 (10.8)	0.386
1–3 year, n (%)	1994 (48.8)	2568 (50.5)	804 (49.6)	1801 (50.5)	1081 (49.7)	0.509
3–6 year, n (%)	1398 (34.2)	1661 (32.7)	511 (31.5)	1141 (32.0)	713 (32.8)	0.193
>6 year, n (%)	281 (6.9)	352 (6.9)	121 (7.4)	249 (7.0)	147 (6.7)	0.936

[a] HFMD: Hand, foot, and mouth disease.

3.2. Changes in the enterovirus distribution of different serotypes in Hubei Province from 2018 to 2022

As shown in Fig. 1C and D, CVA6 was the main causative agent, usually followed by CVA16 and other serotypes in 2018–2021. The proportion of CVA6 cases accounted for 86 % in 2020 and 75 % in 2021, which was significantly higher than the 37.8 % in 2018 and 35 % in 2019 ($P < 0.001$). However, CVA16 was the dominant serotype by 2022, accounting for 45 % of the HFMD cases, whereas CVA6 accounted for only 29 %. CVA16 accounted for 2 % only in 2020, which was notably lower than in other years.

Different serotypes of enteroviruses contribute differently during different seasons. CVA6 was prevalent throughout the year, consistent with the prevalence of HFMD. Whereas CVA16 was prevalent in the first half of the year and peaked in the second quarter of the year. In 2020, the peak for CVA6 was delayed to the fourth quarter, which was in accordance with HFMD epidemics (Fig. 2A). CVA16 showed two similar peaks in the second and fourth quarters of 2021, while CVA6 was the dominant serotype. The CVA16 epidemic trend in 2022 was similar to that in 2018, and became the dominant serotype (Fig. 2B). The proportion and absolute quantity of CVA6 are expected to decrease significantly by 2022.

3.3. Phylogenetic analysis of CVA6 and CVA16

In total, 166 and 144 representative strains of CVA6 and CVA16, respectively, were collected for phylogenetic analysis from 2018 to 2022. Phylogenetic analysis indicated that CVA6 belonged to sub-genotype D3 and CVA16 belonged to sub-genotypes B1b and B1a. CVA6 was distributed in four major branches, showing 91.7–99.9 % identity for nucleotides and 98.0–100 % identity for amino acids, while CVA16 was distributed in two major branches of B1b and one major branch of B1a, showing 84.9–99.9 % identity for nucleotides and 96.8–100 % identity for amino acids. Most strains were similar to those from other provinces of mainland China (Figs. 3A and 4A).

The CVA6 strains were distributed in clusters A to C from 2018 to 2019, whereas circulating strains were no longer detected in cluster D. From 2018 to 2021, the identified strains merged and evolved into clusters B and C. In cluster D, strains were obtained between 2020 and 2022, and a subgroup of CVA6 strains emerged in 2022 (Fig. 3B).

A temporal evolutionary pattern of B1b of CVA16 emerged from clusters A to B, whereas strains from 2018 to 2019 were mostly distributed in cluster B. Strain B1b of CVA16 from 2021 to 2022 was detected in cluster A. For B1a of CVA16, only one cluster was detected, but a subgroup of B1a of CVA16 strains emerged in 2022. Only B1a was detected in 2020 (Fig. 4B).

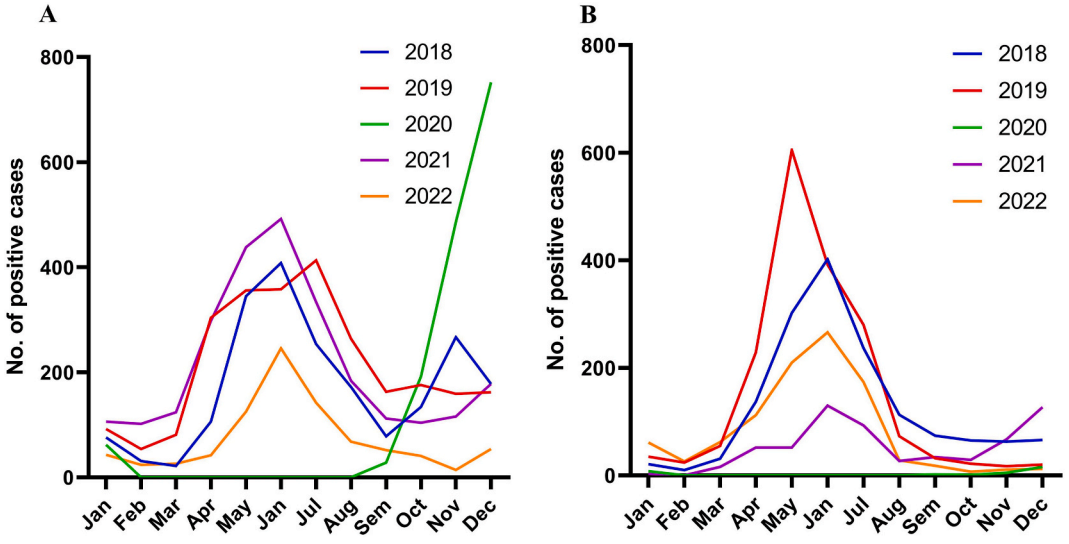

Fig. 2. Seasonal features of enterovirus by serotype in Hubei Province from 2018 to 2022. (A) CVA6; (B) CVA16.

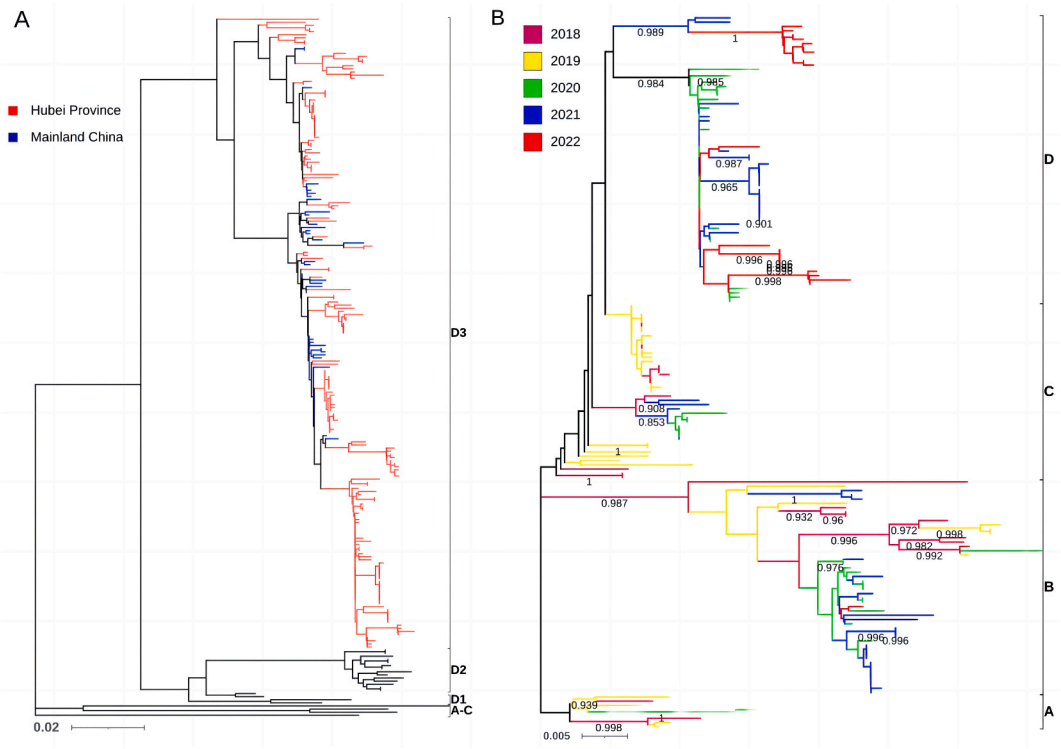

Fig. 3. Phylogenetic analysis of CVA6 VP1 sequences. (A) Branches are colored by region which include indicated Hubei strains; (B) Phylogenetic tree of CVA6 strains circulating in Hubei Province from 2018 to 2022.

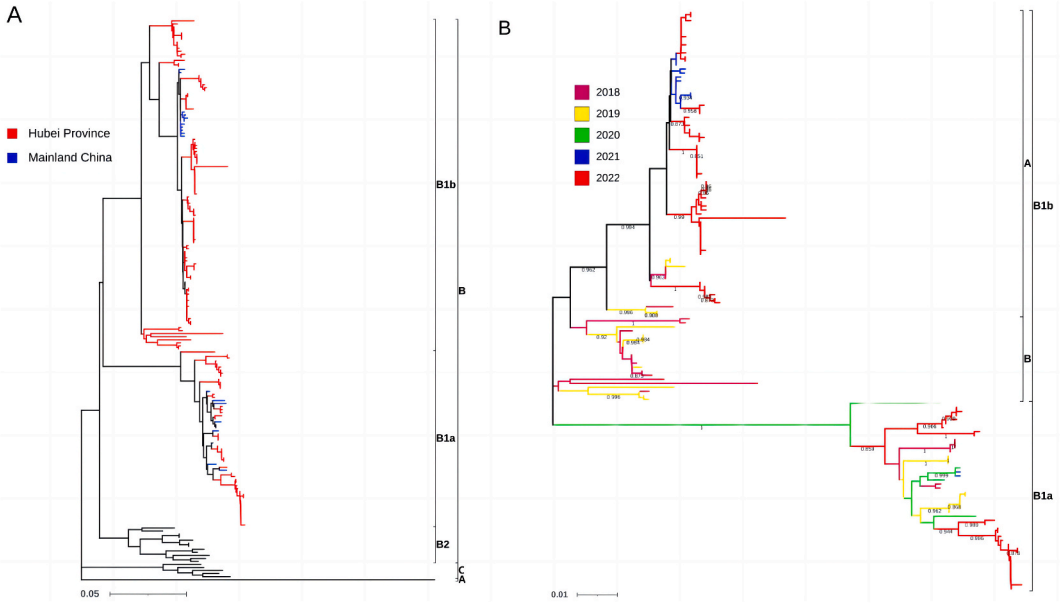

Fig. 4. Phylogenetic analysis of CVA16 VP1 sequences. (A) Branches are colored by region which include indicated Hubei strains. (B) Phylogenetic tree of CVA16 strains circulating in Hubei Province from 2018 to 2022.

4. Discussion

The COVID-19 pandemic has affected almost all countries and areas. NPIs in response to COVID-19 pandemic led to important changes in people's interactions, and changes in the epidemiology of communicable diseases have been reported in many countries.

This study observed a sharp decline in laboratory-confirmed cases of HFMD from 2020 to 2022 compared to 2018 and 2019, with a 3-fold reduction in 2020. The HFMD epidemic, including epidemic peaks and enteroviruses serotypes, also changed after the outbreak of COVID-19. These data implied that NPIs related to COVID-19 also play a role in mitigating HFMD transmission.

Consistent with other studies, we found that the first HFMD epidemic peak was delayed to the fourth quarter of 2020 [10,11]. NPIs, including the closure of schools and suspension of public leisure entertainment, were strictly enforced in the first half of 2020. NPIs are considered important measures for efficiently controlling the spread of HFMD [12,13]. Zhao et, el. found that kindergarten closures were associated with a more than 75 % reduction in HFMD incidence in Hubei Province in 2020 [10]. HFMD in Guangzhou in 2020 also reached its lowest level compared to the five-year average [11]. The effective reproduction number of HFMD dropped to zero after the COVID-19 outbreak in six cities in China [14]. However, in other countries, the number of HFMD cases has decreased. In America rhino/enterovirus detection continued, but with a substantially lower frequency of 4.27 % between April 2020 and March 2021, compared with an annual range of 8.65–18.28 % from January 2015 to March 2020 [15]. In Italy, search peaks of HFMD for both Wikipedia and Google occurred during November to December during the autumn-winter season and June during the spring-summer season, except for the period from June 2020 to June 2021, probably owing to the restrictions of the COVID-19 pandemic [16]. Despite the closure of all schools, nurseries, and infant institutions lasting only in the first three quarters of 2020 and fourth quarter of 2022, other restrictions such as social distancing, hand hygiene measures, mandatory use of masks, and restrictions on commercial activities lasting from 2020 to 2022, which could be the reason for the peak of HFMD in 2021 and 2022, are still lower than those in 2018 and 2019.

HFMD is caused by various enteroviruses, mainly involving CVA16 and EV-A71. EV-A71 was gradually superseded by other serotypes, such as CVA6 after application of the EV-A71 inactivated vaccine [17]. Our investigation showed that CVA6 was the dominant serotype of HFMD in Hubei Province, followed by CVA16. Previous studies have reported that CVA6 has gradually replaced EV-A71 and CVA16 as the predominant serotypes [18,19]. The prevalence of HFMD varies seasonally, and different enterovirus serotypes are prevalent in different seasons. CVA6 circulates throughout the year, with an outbreak occurring in the first peak in the second quarter and a smaller second peak in the fourth quarter. In contrast, CVA16 mainly spread during the first half of the year. In addition, we found that CVA16 was more susceptible to NPIs than CVA6. All cases in Shanghai in 2020 were diagnosed as CVA6 [20] and CVA16 circulating was at a very low epidemic level in many cities in China in 2020 and 2021 [19,21]. CVA6 transmission throughout the year had little effect on NPIs except for the closure of all schools, nurseries, and infant institutions in the first half of 2020. CVA6 continue to be prevalent as a major serotype in 2020 and 2021, same as 2018 and 2019. However, an interesting finding was that the number of CVA6 cases presented a substantially lower decline than that of CVA16 in 2022. A possible explanation for this may be that schools, nurseries, and infant institutions were closed again. The specific reasons for this need to be explored further. The above evidence suggests that the implementation of epidemic prevention measures not only leads to a decline in HFMD but may also change the composition of different enterovirus serotypes that cause HFMD.

Most strains in our study were consistent with those from other provinces of mainland China. The CVA6 strain reported in our study belongs to sub-genotype D3. As reported in previous studies, sub-genotype D3 may have greater transmissibility and can spread frequently among countries [22,23]. Most CVA6 isolates after 2018 in China belonged to sub-genotype D3 and showed a temporal evolutionary pattern [24]. For CVA16, B1a and B1b were the main evolutionary branches in China, which is consistent with our results. Interestingly, B1b was not detected in 2020 but was detected in other years. This finding was also reported in a previous study on the epidemiology of CVA16 in Taiyuan, Shanxi [21]. A possible explanation for this may be that the COVID-19 outbreak reduced the spread of the virus. The evolutionary branch of B1b in Hubei after 2018 remained stable and was the dominant subtype. This finding is consistent with those of other regions in China in recent years [25]. There was no significant mutation in the gene sequence, and only slight differences were found in VP1 sequences in different years. The closure of all schools, nurseries, and infant institutions that prevented the pandemic of HFMD in the first half of 2020 led to a change in the prevalence of CVA6 and CVA16.

This study had some limitations. First, HFMD is a self-limiting disease with a small number of cases requiring hospitalization, which has resulted in patients with mild symptoms being reluctant to visit hospitals owing to the COVID-19 pandemic. Second, laboratories in hospitals or the Centers for Disease Control and Prevention might have been overburdened by large-scale testing for COVID-19, consequently leading to reduced testing for other viruses. Despite these limitations, the results obtained in our study offer some insights into conducting subsequent long-term studies.

5. Conclusion

In conclusion, HFMD in Hubei Province decreased sharply during the COVID-19 pandemic, and a series of NPIs, especially the closure of all schools and nursery and infant institutions adopted during the COVID-19 pandemic, may contribute to the reduction of enterovirus transmission. The long-term closure of schools, nurseries, and infant institutions may significantly change the prevalence of enterovirus serotypes that cause HFMD.

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Ethical approval statement

This study was reviewed and approved by the Ethics Committee of Hubei Center for Disease Control and Prevention, with approval number 2021-034-02. All patients or their parents for sample collection. provided informed consent to participate in the study.

Data availability statement

The data of gene sequences that support the findings of this study are openly available in Genbank (accession numbers OR507254-OR507563). Other data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Kangping Zhou: Writing – original draft, Visualization, Resources, Data curation. **Zhihong Ding:** Resources, Investigation, Data curation. **Bin Hu:** Validation, Investigation. **Jianbo Zhan:** Validation, Investigation. **Kun Cai:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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