

## Article

# Epidemiology of Hand, Foot, and Mouth Disease and Genetic Evolutionary Characteristics of Coxsackievirus A10 in Taiyuan City, Shanxi Province from 2016 to 2020

Jitao Wang <sup>1,2,\*</sup>, Hongyan Liu <sup>1,†</sup>, Zijun Cao <sup>1</sup>, Jihong Xu <sup>2</sup>, Jiame Guo <sup>2</sup>, Lifeng Zhao <sup>2</sup>, Rui Wang <sup>2</sup>, Yang Xu <sup>2</sup>, Ruihong Gao <sup>2</sup>, Li Gao <sup>2</sup>, Zhihong Zuo <sup>2</sup>, Jinbo Xiao <sup>3</sup>, Huanhuan Lu <sup>3</sup> and Yong Zhang <sup>3,4,\*</sup> 

<sup>1</sup> School of Public Health, Shanxi Medical University, 56 Xinjian South Road, Taiyuan 030001, China

<sup>2</sup> Taiyuan Center for Disease Control and Prevention, 89 Xinjian South Road, Taiyuan 030012, China

<sup>3</sup> WHO WPRO Regional Polio Reference Laboratory, National Health Commission Key Laboratory of Biosafety, National Health Commission Key Laboratory of Medical Virology, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

<sup>4</sup> Center for Biosafety Mega-Science, Chinese Academy of Sciences, Wuhan 430071, China

\* Correspondence: wangjitao99@126.com (J.W.); yongzhang75@sina.com (Y.Z.);

Fax: +86-0351-7822732 (J.W.); +86-10-58900184 (Y.Z.)

† These authors contributed equally to this work.

**Abstract:** In recent years, the prevalence of hand, foot, and mouth disease (HFMD) caused by enteroviruses other than enterovirus A71 (EV-A71) and coxsackievirus A16 (CVA16) has gradually increased. The throat swab specimens of 2701 HFMD cases were tested, the VP1 regions of CVA10 RNA were amplified using RT-PCR, and phylogenetic analysis of CVA10 was performed. Children aged 1–5 years accounted for the majority (81.65%) and boys were more than girls. The positivity rates of EV-A71, CVA16, and other EVs were 15.22% (219/1439), 28.77% (414/1439), and 56.01% (806/1439), respectively. CVA10 is one of the important viruses of other EVs. A total of 52 CVA10 strains were used for phylogenetic analysis based on the VP1 region, 31 were from this study, and 21 were downloaded from GenBank. All CVA10 sequences could be assigned to seven genotypes (A, B, C, D, E, F, and G), and genotype C was further divided into C1 and C2 subtypes, only one belonged to subtype C1 and the remaining 30 belonged to C2 in this study. This study emphasized the importance of strengthening the surveillance of HFMD to understand the mechanisms of pathogen variation and evolution, and to provide a scientific basis for HFMD prevention, control, and vaccine development.

**Keywords:** hand, foot, and mouth disease; coxsackievirus A10; RT-PCR; VP1 region; phylogenetic tree



**Citation:** Wang, J.; Liu, H.; Cao, Z.; Xu, J.; Guo, J.; Zhao, L.; Wang, R.; Xu, Y.; Gao, R.; Gao, L.; et al. Epidemiology of Hand, Foot, and Mouth Disease and Genetic Evolutionary Characteristics of Coxsackievirus A10 in Taiyuan City, Shanxi Province from 2016 to 2020. *Viruses* **2023**, *15*, 694. <https://doi.org/10.3390/v15030694>

Academic Editor: Mamta Chawla Sarkar

Received: 16 February 2023

Revised: 3 March 2023

Accepted: 6 March 2023

Published: 7 March 2023



**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Background

Hand, foot, and mouth disease (HFMD) is a global, acute febrile exanthematous infectious disease usually caused by human enterovirus (EV) and occurs mainly in young children less than 5 years of age [1]. HFMD is a mild disease but sometimes can cause severe complications that can even lead to death [2,3]. EV belongs to the genus *Enterovirus*, family *Picornaviridae*, order *Picornavirales*. EV is highly infectious to susceptible populations, and EVs that infect human beings can be divided into four species (A–D) [4]. EV-A consists of 25 viruses such as coxsackievirus A2 (CVA2)–CVA8, CVA10, CVA12, CVA14, CVA16, EV-A71, EV-A76, EV-A89–A92, EV-A114, and EV-A119–A125, that can cause HFMD, acute flaccid paralysis (AFP), herpetic angina, encephalitis meningitis, and other diseases. HFMD is one of the most common diseases caused by the members of EV-A [5,6]. Although EV-A71 and CVA16 were mainly responsible for causing HFMD [7–9], the recent increased frequency of CVA10-associated HFMD outbreaks worldwide, have changed this view [10,11]. In fact, the pathogen spectrum of HFMD continues to change, CVA10 even replaced EV-A71 and CVA16 as the dominant pathogen for HFMD in some areas. CVA10-associated HFMD outbreaks have been reported in past decades, in several countries including France,

Vietnam, Uruguay [12–14], as well as in Fujian Province, Wuhan City, and Shijiazhuang City in China [15–18]. However, the CVA10 genome is less stable than that of EV-A71, CVA16, and CVA6, with more frequent recombination [19]. HFMD associated with CVA10 is more severe, and more likely to cause serious nervous system diseases, such as aseptic meningitis and viral meningitis, especially in younger children, and is the new leading cause of severe HFMD in addition to EV-A71 [10,19]. Thus, more research on the genetic characteristics and evolution of the CVA10 epidemic is required.

The enterovirus coding region is divided into three subregions, P1, P2, and P3. P1 encodes four structural proteins (VP1, VP2, VP3, and VP4), whilst the non-structural proteins are encoded in the P2 (2A, 2B, and 2C) and P3 (3A, 3B, 3C, and 3D polymerase) regions. VP1, VP2, and VP3 are present on the outside of the capsid, whereas VP4 is internal. The VP1 protein of CVA10 can identify and bind to receptors on target cells, which contains many important neutralization epitopes [20,21]. Phylogenetic analysis based on the nucleotide sequence of the entire VP1 region of CVA10 (894 bp) is widely used to understand its genetic evolutionary characteristics and is a reliable and rigorous method for molecular epidemiological studies [18,22].

Taiyuan city, the capital of Shanxi Province, China, is located to the west of Taihang Mountain and the east of the Yellow River, on the Loess Plateau in Northwestern China. CVA10-associated HFMD had been reported in many provinces and regions of China, but only a few studies were published from Taiyuan City. In this study, we analyzed the epidemiological characteristics of HFMD and the genetic characteristics of CVA10 in Taiyuan City, Shanxi Province, from 2016–2020. The study emphasized the importance of strengthening the surveillance of HFMD to understand the mechanisms of pathogen variation and evolution, so as to provide a scientific basis for HFMD prevention, control, and vaccine development.

## 2. Methods

### 2.1. Epidemiological Information and Sample Collection

The epidemiological data were retrieved from the China Information System for Disease Control and Prevention and the population data came from the Taiyuan Statistical Yearbook. In ten hospitals in different districts and counties of Taiyuan City, specimens were collected from clinically diagnosed HFMD cases within three days after the onset of the disease and sent to Taiyuan Center for Disease Control and Prevention (Taiyuan CDC). At least five specimens were collected every month. Due to the impact of COVID-19, the number of HFMD cases and specimens in 2020 is limited. A total of 2701 throat swab specimens were sent to the virus microbiology laboratory of Taiyuan CDC for enterovirus nucleic acid testing within 24 h from 1 January 2016 to 31 December 2020. Upon receipt of specimens by the laboratory, each specimen was assigned a unique laboratory code and entered into the Gastrointestinal Information Database of Taiyuan CDC (Excel).

### 2.2. RNA Extraction and EV Identification

Viral RNA was extracted from the throat swab specimens using a MagMAX™-96 Viral RNA Isolation Kit (Thermo Fisher Scientific, Foster City, CA, USA) according to the manufacturer's instructions. RNA was eluted in a final volume of 50 µL of elution buffer and used immediately or stored at  $-80^{\circ}\text{C}$ . All steps of specimen preparation and RNA extraction were carried out in the biosafety cabinet. RNA was detected using three-channel real-time RT-PCR kits (Diagnostic Kit for pan-EV, EV-A71, CVA16, Shanghai ZJ Bio-Tech Co., Ltd., Shanghai, China). A 25 µL reaction system consisting of 1 µL enzyme mixture, 19 µL reaction solution, and 5 µL viral nucleic acid was prepared. PCR cycling parameters were set up as per the manufacturer's protocols:  $50^{\circ}\text{C}$  for 30 min,  $95^{\circ}\text{C}$  for 10 min, followed by 45 cycles of  $95^{\circ}\text{C}$  for 10 s, and  $55^{\circ}\text{C}$  for 40 s in a CFX96 Real-time Thermal Cycler (Bio-Rad, Hercules, CA, USA). A positive result was defined as a cycle threshold (Ct) value  $\leq 43$ , and the positive control was defined as a Ct value  $\leq 35$ . The negative specimens were not under the scope of our study. The specimens positive for

other EVs were detected using commercially available real-time RT-PCR kits (Nucleic Acid Detection Kit for CVA10, BioMax Biotechnology Co., Ltd., Beijing, China). A 20  $\mu$ L reaction system consisting of a 5  $\mu$ L primer probe mixture, 5  $\mu$ L RT-PCR reaction solution (containing enzymes), and 10  $\mu$ L viral nucleic acid was prepared. PCR cycling parameters were set up according to the instructions: 25 °C for 2 min, 53 °C for 10 min, 95 °C for 2 min, followed by 45 cycles of 95 °C for 5 s, and 60 °C for 30 s in a CFX96 Real-time Thermal Cycler (Bio-Rad, Hercules, CA, USA). A positive result was defined as a Ct value  $\leq$  37, and the positive control was defined as a Ct value  $\leq$  32. The negative specimens were not under the scope of our study.

### 2.3. VP1 Region Amplification and Nucleotide Sequencing

In total, 31 specimens were detected to amplify the entire VP1 region of CVA10 by using the One Step RT-PCR kits (CVA10 Target region (VP1) Molecular typing Kit, BioMax Biotechnology Co., Ltd., Beijing, China) with specific in-house primer pairs (forward primer with nucleotide position 2172–2192nt, CVA10-VP1-S: GCTCAGTAACACTCAYTTYCG, and reverse primer with nucleotide position 3376–3394nt, CVA10-VP1-A: CTCGAGAACTGT-CYTCCCA). The primers yielded a CVA10 amplification product of 1223 bp, spanning the entire VP1 region of CVA10 (894 bp). The reaction system for each tube consisted of 12.5  $\mu$ L PCR Buffer, 2  $\mu$ L PCR Enzyme, 0.5  $\mu$ L RT Enzyme, 3  $\mu$ L Primer Mix, and 7  $\mu$ L viral nucleic acid up to a final volume of 25  $\mu$ L. The amplification conditions were as follows: 45 °C for 10 min, 94 °C for 2 min, followed by 45 cycles of 98 °C for 10 s, 55 °C for 15 s, and 68 °C for 20 s in an ABI ProFlex™ 96-Well PCR System (Thermo Fisher Scientific, Foster City, CA, USA). Amplification products were determined using a 1% agarose gel electrophoresis image analysis system and sent to Tsingke (Beijing, China) Biotechnology Co., Ltd. for DNA bi-directional sequencing.

### 2.4. Phylogenetic Analyses

Sequences were spliced by Sequencher software (version 5.4.6), and EV-types were verified by the enterovirus automated genotyping tool [23]. BioEdit7.2.5 was used for amino acid sequence alignment of the CV-A10 VP1 region. Phylogenetic trees were constructed based on the VP1 sequences of the CVA10 identified in this study and the sequences downloaded from the GenBank database using the MEGA software (Version 11.0.11) [24]. Phylogenetic analysis using the neighbor-joining (NJ) method was performed based on the Kimura 2-parameter model, and the reliability was evaluated by 1000 bootstrap replicates. Bootstrap values greater than 80% were considered statistically significant for grouping. Genotype differences were computed by the group mean distance computing method in MEGA software.

### 2.5. Nucleotide Accession Number

All the CVA10-Taiyuan sequences obtained during this study were submitted in GenBank under accession numbers OP244619–OP244649, which are shown in Supplementary Table S1.

### 2.6. Statistical Analyses

Data analyses were performed using IBM SPSS Statistics software (Version 26.0). The Pearson's Chi-Square Test was used to analyze the data, and the value of  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Epidemiology Features

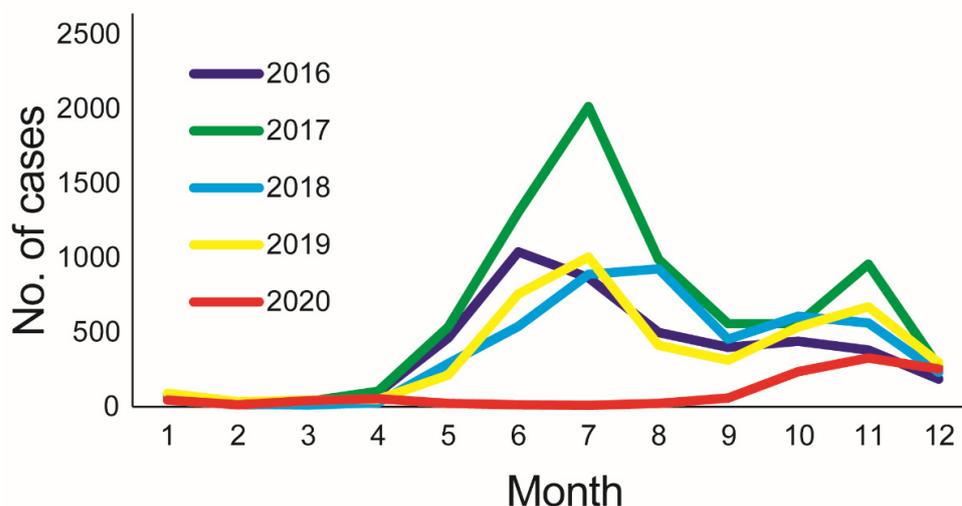
A total of 22,044 clinical HFMD cases were reported to the China Information System for Disease Control and Prevention in Taiyuan during 2016–2020. The average annual HFMD incidence rate was 86.45 per 100,000 population (range 20.68–148.49), and the incidence rate increased from 2016 to 2017 and decreased from 2017 to 2020, with a peak in

2017. Among these HFMD cases, 13,152 were males and 8892 were females, with an average annual male-to-female ratio of 1.48 (range 1.36–1.56). The average annual incidence in males (101.10 per 100,000 population) was higher than in females (71.18 per 100,000 population) and this difference was statistically significant ( $\chi^2 = 660.330, p < 0.01$ ). Of all cases, 81.65% (17,998) were children aged 1–5, and the average annual incidence is higher in boys than in girls. The majority (99.35%) of them were scattered children (49.33%), kindergarten children (41.74%), and school-going students (8.27%) (Table 1). Although HFMD cases occurred throughout the year, two seasonal peaks were observed from June to August and from October to November during 2016–2019, respectively. The annual HFMD cases were lowest ( $n = 1100$ ) in 2020 due to the impact of the COVID-19 pandemic, with the peak in November (Figure 1). There is no relationship between the age and serotype of viruses according to the existing data of statistical analysis ( $p < 0.05$ ).

**Table 1.** Distribution of reported and detected HFMD cases in Taiyuan from 2016 to 2020.

Variable	2016	2017	2018	2019	2020	Total
HFMD Reported Cases	4472	7400	4648	4424	1100	22,044
Incidence rate/10 <sup>5</sup>	92.17	148.49	90.87	84.56	20.68	86.45
Gender						
Male (Incidence rate/10 <sup>5</sup> )	2728 (110.15)	4395 (172.94)	2723 (104.72)	2673 (100.44)	633 (23.19)	13,152 (101.10)
Female (Incidence rate/10 <sup>5</sup> )	1744 (73.43)	3005 (123.04)	1925 (76.55)	1751 (68.12)	467 (18.04)	8892 (71.18)
Gender ratio	1.56	1.46	1.41	1.53	1.36	1.48
Residence						
Scattered children	2290	3691	2459	1896	539	10,875
Kindergarten children	1839	3204	1722	2012	425	9202
School students	316	458	443	489	118	1824
Other	27	47	24	27	18	143
Age Group (%)						
<1	197 (4.41%)	572 (7.73%)	291 (6.26%)	188 (4.25%)	65 (5.91%)	1313 (5.71%)
1–<2	915 (20.46%)	1655 (22.36%)	1135 (24.42%)	786 (17.77%)	204 (18.55%)	4695 (20.71%)
2–<3	810 (18.11%)	1060 (14.32%)	742 (15.96%)	693 (15.66%)	162 (14.73%)	3467 (15.76%)
3–<4	893 (19.97%)	1620 (21.89%)	760 (16.35%)	910 (20.57%)	244 (22.18%)	4427 (20.19%)
4–<5	819 (18.31%)	1110 (15.00%)	711 (15.30%)	623 (14.08%)	169 (15.36%)	3432 (15.61%)
5–<6	346 (7.74%)	638 (8.62%)	401 (8.63%)	515 (11.64%)	77 (7.00%)	1977 (8.73%)
≥6	492 (11.00%)	745 (10.07%)	608 (13.08%)	709 (16.03%)	179 (16.27%)	2733 (13.29%)
Specimens	586	604	616	603	292	2701
EV-positive (%)	420 (71.67%)	328 (54.30%)	311 (50.49%)	227 (37.65%)	153 (52.40%)	1439 (53.28%)
EV-A71(%) <sup>a</sup>	123 (29.29%)	69 (21.04%)	22 (7.07%)	3 (1.32%)	2 (1.31%)	219 (15.22%)
CVA16 (%) <sup>b</sup>	106 (25.24%)	50 (15.24%)	130 (41.80%)	112 (49.34%)	16 (10.46%)	414 (28.77%)
Other EVs (%) <sup>c</sup>	191 (45.48%)	209 (63.72%)	159 (51.13%)	112 (49.34%)	135 (88.24%)	806 (56.01%)
CVA10 (%) <sup>d</sup>	13 (6.81%)	0 (0.00%)	3 (1.89%)	14 (12.50%)	1 (0.74%)	31 (3.85%)

Notes: “a, b, c” was the proportion of EV-A71, CVA16, and other EVs in positive numbers, respectively. “d” was the proportion of CVA10 in other EVs.



**Figure 1.** Monthly distribution of HFMD cases from 2016–2020.

### 3.2. Laboratory Detection

A total of 2701 throat swab specimens were collected from HFMD patients during 2016–2020, among them, 1439 (53.28%) were positive for human enterovirus (EV-positive), with 219 (15.22%) were associated with EV-A71, 414 (28.77%) with CVA16, and 806 (56.01%) with other EVs other than EV-A71 and CVA16. The proportion of other EVs was significantly higher than that of EV-A71 and CVA16 in Taiyuan during 2016–2020. CVA10 (31 cases) accounted for 2.15% of all confirmed cases (Table 1).

### 3.3. Phylogenetic Analysis of the VP1 Region of CVA10

The entire VP1 sequences of 31 CVA10 were amplified and sequenced in our study. The VP1 nucleotide sequences among CVA10 strains shared 92.6% to 100% nucleotide identity, corresponding to a 96.9% to 100% amino acid identity. A similar analysis with the prototype strain Kowalik showed a 75.9% to 77.0% nucleotide identity, corresponding to a 91.2% to 93.6% amino acid identity. The highest nucleotide similarity with the genotype C downloaded from the GenBank was 91.7% to 100% and amino acid similarity of 96.9% to 100%. A total of 52 CVA10 strains were used for phylogenetic analysis for the VP1 region, including the 31 CVA10 strains identified in this study and 21 reference strains of CVA10 downloaded from GenBank.

The mean distance of nucleotides and amino acid sequences within and between seven genotypes of CVA10 was calculated using MEGA 11.0.11 with a p-distance model (Table 2). A difference of at least 15% in the VP1 region was used to discriminate genotypes [25]. The results showed that the mean nucleotide distance between genotypes was more than 15%, and the mean nucleotide distance within genotypes was 3.1–8.6%. The average nucleotide divergences between genotype A and genotype G is the largest (30.5%), the difference between genotype C and genotype G is the smallest (17.5%), and the difference between other genotypes is between 18.3% and 29.9%.

**Table 2.** The average evolutionary divergence of nucleotide and amino acid sequences within and between genotypes based on the CVA10 VP1 coding gene.

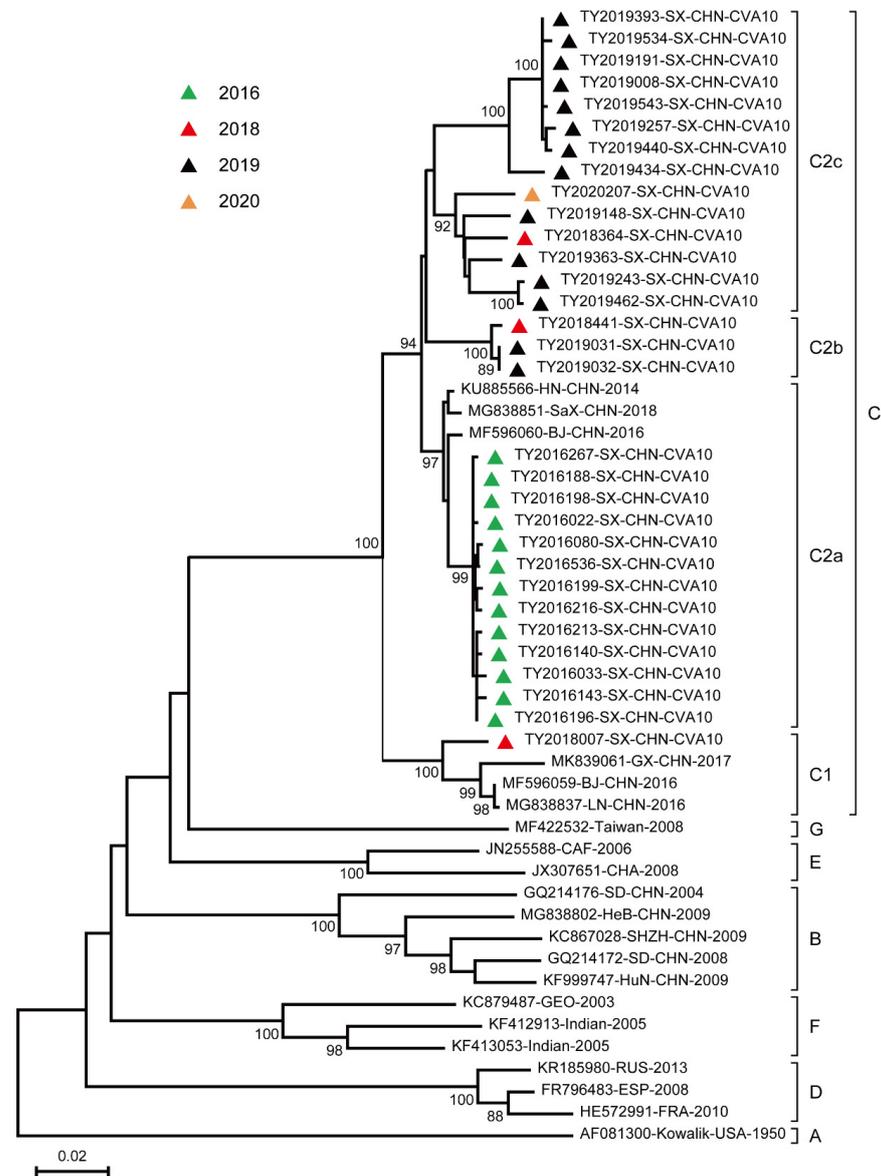
Genotypes	A	B	C	D	E	F	G
A	/	9.4%	8.0%	9.5%	8.1%	7.5%	9.8%
B	29.6%	7.3% (2.0%)	5.5%	8.4%	5.1%	5.4%	6.5%
C	28.7%	21.6%	4.0% (1.2%)	8.1%	4.1%	4.8%	5.7%
D	29.9%	24.1%	24.3%	3.1% (1.2%)	7.8%	6.5%	7.3%
E	27.8%	21.6%	18.3%	23.3%	7.3% (3.5%)	3.6%	6.6%
F	27.9%	21.7%	20.5%	21.8%	21.0%	8.6% (2.1%)	6.0%
G	30.5%	19.1%	17.5%	24.6%	19.3%	20.5%	/

Note: The left-lower data in italics are nucleotide diversity. The right-upper data in normal font are amino acid sequence diversity. Genotype F is based on the available partial VP1 gene of CVA10 from GenBank, the others used the complete VP1 region.

As entire VP1 sequences of CVA10 available in the public database were limited, to investigate the origin and spread of CVA10 strains in the world, three representative strains of genotype F on partial VP1 were selected to perform the phylogenetic analysis. Seven genotypes (A, B, C, D, E, F, and G) were assigned with a mean group distance of 17.5–30.5%, and the distribution of these genotypes showed geographic and temporal circumscribed characterization. Genotype A was only composed of the prototype strain Kowalik isolated in the US in 1950. Foreign epidemic strains such as African viruses, European viruses, and Indian viruses are mainly clustered in genotypes D, E, and F. According to continuous HFMD surveillance, genotype B circulating mainly in China completely disappeared during 2004–2009 [18].

Thirty-one Taiyuan CVA10 strains along with the domestic reference strains obtained on GenBank belonged to C1 (one strain) and C2 subtypes (30 strains) within genotype C. The only CVA10 belonging to subtype C1 was acquired in 2018. Subtype C2 can be further

divided into three evolutionary branches (C2a, C2b, and C2c) (Figure 2). An apparent evolutionary trend could be observed from 2016–2020. All the CVA10 strains detected in 2016 belonged to C2a. One CVA10 strain detected in 2018 and two CVA10 strains detected in 2019 belonged to C2b, whereas the remaining CVA10 strains detected between 2018 and 2020 belonged to C2c.



**Figure 2.** Phylogenetic analysis based on the complete and the available partial VP1 of CVA10. Taiyuan strains of different years were revealed by different colors according to the figure. The CVA10 nucleotide sequences in this study are represented by Serial number-Province-Country-Genotype.

### 3.4. Antigenic Differences among Different Genotypes of CVA10

The 162–176 residue of CVA10 VP1 shows the effective neutralization of the CVA10 type. It is in the EF loop of VP1 protein and is a specific linear neutralizing epitope on CVA10 VP1 [26]. The amino acid sequence analysis of CVA10 genotypes including Taiyuan CVA10 showed that the linear neutralizing epitope was highly conservative in CVA10 (Figure 3).



and specific therapeutic drugs for CVA10-associated HFMD, only a few studies were published from Taiyuan City. Hence, in this study, we conducted a comprehensive analysis of the epidemiological characteristics and molecular evolution characteristics of CVA10 in Taiyuan City from 2016 to 2020.

The results indicated that more infections were found in males than in females. Except for sex differences at the level of host immune status, another explanation could be related to the extra outdoor activities, including more frequent exposure to unclean toys and facilities by boys, which increases the risk of infection compared to girls [32]. Of all HFMD cases in Taiyuan City, children aged 1–5 accounted for the majority (81.65%) of the cases due to low herd immunity, and the majority (99.35%) among them were scattered children, kindergarten children, and school students. Such high incidence rates suggest that this particular age group should be used as a key population for HFMD control and prevention. Health education programs should be conducted to prevent HFMD transmission not only for children at kindergartens and schools but also for community families with young children. There were obvious seasonal features in the HFMD epidemic. Although HFMD cases in Taiyuan City were reported throughout the year, two seasonal peaks were observed from June to August and from October to November during 2016–2019, respectively. The main peak might be due to an increase in viral transmission because of extremes temperature [33]. The second peak may be due to a shift in climate from warm dry conditions to cold wet conditions, mainly in the southern provinces. However, this phenomenon is relatively rare in Shanxi, a northern province of China. Nevertheless, epidemiological surveillance and prevention efforts should be strengthened before the peak of the disease each year.

As one of the important viruses of other EVs, the entire *VP1* sequences of 31 CVA10 were amplified and sequenced from 2016–2020. CVA10 strains in this study belonged to C1 and C2 subtypes in genotype C and were also the predominant genotype during 2010–2016 in China [18]. Only one CVA10 isolated in 2018 belonged to subtype C1 and the others to subtype C2. Subtype C1 in our study was closely related to other domestic strains, such as Beijing, Guangxi, and Liaoning [34]. As the spread of subtype C2, it has been divided into 3 branches (C2a, C2b, and C2c). Significant branch shifts can be observed from 2016 to 2020, C2b and C2c co-circulate in 2018 and which branch will become mainstream requires our continuous surveillance.

According to the mean distance of nucleotides and amino acid sequences within and between seven genotypes of CVA10, it was found that the mean nucleotide distance within the genotypes was 3.1–7.3% except for genotype F (8.6%), which might be due to the available partial *VP1* of genotype F, it is necessary to conduct continuous surveillance to get the complete *VP1* gene. The nucleotide identity between subtype C1 and C2 was 92.6% to 100%, while the amino acid identity was 96.9% to 100%, and the nucleotide identity within subtype C2 was 94.4% to 100%, while the amino acid identity was 97.6% to 100%, showing that synonymous mutations existed generally.

Previous studies have shown that the 162–176 residues in the *VP1* protein of CVA10 are a specific linear neutralizing epitope. Neutralizing epitopes play a key role in vaccine effectiveness and can be used as biomarkers to monitor vaccine effectiveness [35]. The amino acid sequence analysis of CVA10 genotypes including Taiyuan CVA10 showed that the linear neutralizing epitope (162–176 residues in *VP1*) was highly conservative, which might be beneficial to the development of the CVA10 vaccine. However, thus far, the research on the CVA10 epitopes is still very insufficient. One of the future research focuses is still the research on the CVA10 epitopes in order to strengthen the monitoring of the variation of the epitopes, which is also very important for the research of the CVA10 vaccine.

This study had some limitations. First, the proportion of laboratory-confirmed HFMD cases was small due to the limited sample size. Second, because there is no complete *VP1* sequence of genotype F in GenBank, partial *VP1* region sequences of genotype F of CVA10 were used for analysis in this study. However, to fully understand the genetic variation and

evolution of CVA10, research based on the entire VP1 region or even a full-length genome is necessary.

## 5. Conclusions

The study emphasized the importance of strengthening the surveillance of the HFMD pathogen spectrum in order to understand the mechanisms of pathogen variation and evolution, so as to provide a scientific basis for HFMD prevention, control, and vaccine development. The reported HFMD cases associated with CVA10 in this study raise concerns about the role of CVA10 in explaining new HFMD cases and outbreaks and call for enhanced surveillance of the HFMD pathogen spectrum and surveillance of enterovirus etiology.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/v15030694/s1>, Table S1. GenBank accession numbers were assigned for all the CVA10-Taiyuan sequences based on the entire VP1 region sequenced in this study.

**Author Contributions:** Data curation, Z.C. and H.L. (Huanhuan Lu); Funding acquisition, J.X. (Jihong Xu); Investigation, L.G.; Methodology, J.X. (Jinbo Xiao); Project administration, J.G.; Resources, R.G.; Software, L.Z.; Supervision, Y.X.; Validation, Z.Z.; Visualization, R.W.; Writing—original draft, H.L. (Hongyan Liu); Writing—review & editing, J.W. and Y.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported National Key Research and Development Program of China (Project No. 2021YFC2302003), and Natural Science Foundation of Beijing (Project No. L192014).

**Institutional Review Board Statement:** The study did not involve human participants or human experimentations, the only human materials used were the throat swab specimens collected from HFMD patients for public health purposes. Specimens were analyzed after informed and verbal consent obtained from the parents or guardians of the children. The study was conducted according to the guidelines of the Declaration of Helsinki, and experimental procedures were approved by the the Institutional Review Board and Human Research Ethics Committee of the Taiyuan Center for Disease Control and Prevention.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. All the CVA10-Taiyuan sequences obtained during this study were submitted in GenBank under accession numbers OP244619–OP244649.

**Acknowledgments:** The authors are grateful to county CDC staffs in Taiyuan City who were responsible for the specimen collection process.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

## Abbreviations

HFMD: hand, foot, and mouth disease; EV-A71: enterovirus A71; CVA16: coxsackievirus A16; CVA10: coxsackievirus A10; RT-PCR: reverse transcription polymerase chain reaction; EV: enterovirus; CVA2: coxsackievirus A2; AFP: acute flaccid paralysis; CDC: Center for Disease Control and Prevention; Ct value: Cycle threshold value; NJ: neighbor joining.

## References

1. Shah, V.A.; Chong, C.Y.; Chan, K.P.; Ng, W.; Ling, A.E. Clinical characteristics of an outbreak of hand, foot and mouth disease in Singapore. *Ann. Acad. Med. Singap.* **2003**, *32*, 381–387. [[PubMed](#)]
2. Xing, W.; Liao, Q.; Viboud, C.; Zhang, J.; Sun, J.; Wu, J.T.; Chang, Z.; Liu, F.; Fang, V.J.; Zheng, Y.; et al. Hand, foot, and mouth disease in China, 2008–2012: An epidemiological study. *Lancet Infect. Dis.* **2014**, *14*, 308–318. [[CrossRef](#)] [[PubMed](#)]
3. Zhang, Y.; Xu, W.B. Molecular epidemiology of enteroviruses associated with hand, foot, and mouth disease in the mainland of China. *Biomed. Environ. Sci. BES* **2013**, *26*, 875–876. [[CrossRef](#)] [[PubMed](#)]

4. Knowles, N.J.; Hovi, T.; Hyypiä, T.; King, A.M.Q.; Lindberg, A.M.; Pallansch, M.A.; Palmenberg, A.C.; Simmonds, P.; Skern, T.; Stanway, G.; et al. Picornaviridae. In *Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses*; King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J., Eds.; Elsevier: San Diego, CA, USA, 2012; pp. 855–880.
5. Chang, L.Y.; Lin, T.Y.; Hsu, K.H.; Huang, Y.C.; Lin, K.L.; Hsueh, C.; Shih, S.R.; Ning, H.C.; Hwang, M.S.; Wang, H.S.; et al. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. *Lancet* **1999**, *354*, 1682–1686. [[CrossRef](#)]
6. Palacios, G.; Oberste, M.S. Enteroviruses as agents of emerging infectious diseases. *J. Neurovirol.* **2005**, *11*, 424–433. [[CrossRef](#)]
7. Ji, T.; Han, T.; Tan, X.; Zhu, S.; Yan, D.; Yang, Q.; Song, Y.; Cui, A.; Zhang, Y.; Mao, N.; et al. Surveillance, epidemiology, and pathogen spectrum of hand, foot, and mouth disease in mainland of China from 2008 to 2017. *Biosaf. Health* **2019**, *1*, 32–40. [[CrossRef](#)]
8. Zhang, Y.; Tan, X.J.; Wang, H.Y.; Yan, D.M.; Zhu, S.L.; Wang, D.Y.; Ji, F.; Wang, X.J.; Gao, Y.J.; Chen, L.; et al. An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. *J. Clin. Virol.* **2009**, *44*, 262–267. [[CrossRef](#)]
9. Zhang, Y.; Wang, D.; Yan, D.; Zhu, S.; Liu, J.; Wang, H.; Zhao, S.; Yu, D.; Nan, L.; An, J.; et al. Molecular evidence of persistent epidemic and evolution of subgenotype B1 coxsackievirus A16-associated hand, foot, and mouth disease in China. *J. Clin. Microbiol.* **2010**, *48*, 619–622. [[CrossRef](#)]
10. Chen, M.; He, S.; Yan, Q.; Xu, X.; Wu, W.; Ge, S.; Zhang, S.; Chen, M.; Xia, N. Severe hand, foot and mouth disease associated with Coxsackievirus A10 infections in Xiamen, China in 2015. *J. Clin. Virol.* **2017**, *93*, 20–24. [[CrossRef](#)] [[PubMed](#)]
11. Munivenkatappa, A.; Yadav, P.D.; Nyayanit, D.A.; Majumdar, T.D.; Sangal, L.; Jain, S.; Sinha, D.P.; Shrivastava, A.; Mourya, D.T. Molecular diversity of Coxsackievirus A10 circulating in the southern and northern region of India [2009–17]. *Infect. Genet. Evol.* **2018**, *66*, 101–110. [[CrossRef](#)]
12. Hoa-Tran, T.N.; Nguyen, A.T.; Dao, A.T.H.; Kataoka, C.; Ta, H.T.T.; Nguyen, H.T.V.; Takemura, T.; Nguyen, T.T.T.; Vu, H.M.; Nguyen, T.T.H.; et al. Genetic characterization of VP1 of coxsackieviruses A2, A4, and A10 associated with hand, foot, and mouth disease in Vietnam in 2012–2017: Endemic circulation and emergence of new HFMD-causing lineages. *Arch. Virol.* **2020**, *165*, 823–834. [[CrossRef](#)] [[PubMed](#)]
13. Mirand, A.; Henquell, C.; Archimbaud, C.; Ughetto, S.; Antona, D.; Bailly, J.L.; Peigue-Lafeuille, H. Outbreak of hand, foot and mouth disease/herpangina associated with coxsackievirus A6 and A10 infections in 2010, France: A large citywide, prospective observational study. *Clin. Microbiol. Infect.* **2012**, *18*, E110–E118. [[CrossRef](#)] [[PubMed](#)]
14. Lizasoain, A.; Mir, D.; Martinez, N.; Colina, R. Coxsackievirus A10 causing hand-foot-and-mouth disease in Uruguay. *Int. J. Infect. Dis.* **2020**, *94*, 1–3. [[CrossRef](#)]
15. Chen, W.; Weng, Y.; He, W.; Zhu, Y.; Zhang, Y.; Huang, M.; Xie, J.; Zheng, K.; Yan, Y. Molecular epidemiology of hand-foot-mouth disease associated pathogen Coxsackievirus A10 identified in Fujian province, 2011–2014. *Zhonghua Liu Xing Bing Xue Za Zhi* **2016**, *37*, 563–567. [[CrossRef](#)] [[PubMed](#)]
16. Tian, H.; Zhang, Y.; Sun, Q.; Zhu, S.; Li, X.; Pan, Z.; Xu, W.; Xu, B. Prevalence of multiple enteroviruses associated with hand, foot, and mouth disease in Shijiazhuang city, Hebei province, China: Outbreaks of coxsackieviruses a10 and b3. *PLoS ONE* **2014**, *9*, e84233. [[CrossRef](#)] [[PubMed](#)]
17. Yang, Q.; Ding, J.; Cao, J.; Huang, Q.; Hong, C.; Yang, B. Epidemiological and etiological characteristics of hand, foot, and mouth disease in Wuhan, China from 2012 to 2013: Outbreaks of coxsackieviruses A10. *J. Med Virol.* **2015**, *87*, 954–960. [[CrossRef](#)] [[PubMed](#)]
18. Ji, T.; Guo, Y.; Huang, W.; Shi, Y.; Xu, Y.; Tong, W.; Yao, W.; Tan, Z.; Zeng, H.; Ma, J.; et al. The emerging sub-genotype C2 of CoxsackievirusA10 Associated with Hand, Foot and Mouth Disease extensively circulating in mainland of China. *Sci. Rep.* **2018**, *8*, 13357. [[CrossRef](#)]
19. Bian, L.; Gao, F.; Mao, Q.; Sun, S.; Wu, X.; Liu, S.; Yang, X.; Liang, Z. Hand, foot, and mouth disease associated with coxsackievirus A10: More serious than it seems. *Expert Rev. Anti-Infect. Ther.* **2019**, *17*, 233–242. [[CrossRef](#)]
20. Chen, J.; Ye, X.; Zhang, X.Y.; Zhu, Z.; Zhang, X.; Xu, Z.; Ding, Z.; Zou, G.; Liu, Q.; Kong, L.; et al. Coxsackievirus A10 atomic structure facilitating the discovery of a broad-spectrum inhibitor against human enteroviruses. *Cell Discov.* **2019**, *5*, 4. [[CrossRef](#)]
21. Zhu, L.; Sun, Y.; Fan, J.; Zhu, B.; Cao, L.; Gao, Q.; Zhang, Y.; Liu, H.; Rao, Z.; Wang, X. Structures of Coxsackievirus A10 unveil the molecular mechanisms of receptor binding and viral uncoating. *Nat. Commun.* **2018**, *9*, 4985. [[CrossRef](#)]
22. Oberste, M.S.; Maher, K.; Kilpatrick, D.R.; Flemister, M.R.; Brown, B.A.; Pallansch, M.A. Typing of human enteroviruses by partial sequencing of VP1. *J. Clin. Microbiol.* **1999**, *37*, 1288–1293. [[CrossRef](#)]
23. Kroneman, A.; Vennema, H.; Deforche, K.; Avoort, H.V.D.; Penaranda, S.; Oberste, M.S.; Vinje, J.; Koopmans, M. An automated genotyping tool for enteroviruses and noroviruses. *J. Clin. Virol.* **2011**, *51*, 121–125. [[CrossRef](#)] [[PubMed](#)]
24. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [[CrossRef](#)]
25. Brown, B.A.; Oberste, M.S.; Alexander, J.P., Jr.; Kennett, M.L.; Pallansch, M.A. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. *J. Virol.* **1999**, *73*, 9969–9975. [[CrossRef](#)]
26. Zhu, H.; Liu, X.; Wu, Y.; He, Y.; Zheng, H.; Liu, H.; Liu, Q. Identification of a neutralizing linear epitope within the VP1 protein of coxsackievirus A10. *Virol. J.* **2022**, *19*, 203. [[CrossRef](#)] [[PubMed](#)]

27. Klein, M.; Chong, P. Is a multivalent hand, foot, and mouth disease vaccine feasible? *Hum. Vaccines Immunother.* **2015**, *11*, 2688–2704. [[CrossRef](#)] [[PubMed](#)]
28. Liu, C.C.; Chow, Y.H.; Chong, P.; Klein, M. Prospect and challenges for the development of multivalent vaccines against hand, foot and mouth diseases. *Vaccine* **2014**, *32*, 6177–6182. [[CrossRef](#)]
29. Zhou, X.H.; Jia, C.L.; Huang, R.; Zhu, L. Etiological analysis of hospitalized patients with hand-foot-mouth disease in Shanxi province in 2017. *Shanxi Med. Univ.* **2020**, *51*, 879–882. [[CrossRef](#)]
30. Ren, H.; Liu, Y.; Wang, X.C.; Li, M.C.; Quan, D.C.; Rao, H.X.; Luo, T.E.; Zhao, J.F.; Li, G.H.; Qiu, L. Epidemiological characteristics and Spatial-temporal clustering of hand, foot and mouth disease in Shanxi province, 2009–2020. *Zhonghua Liu Xing Bing Xue Za Zhi* **2022**, *43*, 1753–1760. [[CrossRef](#)]
31. Mao, Q.; Wang, Y.; Bian, L.; Xu, M.; Liang, Z. EV-A71 vaccine licensure: A first step for multivalent enterovirus vaccine to control HFMD and other severe diseases. *Emerg. Microbes Infect.* **2016**, *5*, e75. [[CrossRef](#)]
32. Yin, D.Q.; Wang, C.B.; Wang, C.B.; Xiao, Z.; Ji, S.X. Epidemiology Characteristics of Human Coxsackievirus A16 and Enterovirus 71 Circulating in Linyi, China, from 2009 to 2017. *Jpn. J. Infect Dis.* **2018**, *71*, 470–473. [[CrossRef](#)] [[PubMed](#)]
33. Zhuang, D.; Hu, W.; Ren, H.; Ai, W.; Xu, X. The influences of temperature on spatiotemporal trends of hand-foot-and-mouth disease in mainland China. *Int. J. Environ. Health Res.* **2014**, *24*, 1–10. [[CrossRef](#)] [[PubMed](#)]
34. Zhang, Q.; Yu, W.; Zhou, X.T.; Lei, L.; Mao, L.L.; Yao, W.Q. Genetic Characteristics of VP1 Region of Coxsackievirus A10 Strains in Liaoning Province during 2013–2018. *J. Microbiol.* **2021**, *41*, 76–83.
35. Liu, C.C.; Chang, H.W.; Yang, G.; Chiang, J.R.; Chow, Y.H.; Sai, I.H.; Chang, J.Y.; Lin, S.C.; Sia, C.; Hsiao, C.H.; et al. Development of a quantitative enzyme linked immunosorbent assay for monitoring the Enterovirus 71 vaccine manufacturing process. *J. Virol. Methods* **2011**, *176*, 60–68. [[CrossRef](#)] [[PubMed](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.