Sporadic Hand, Foot and Mouth Disease Cases Associated With Non-C4 Enterovirus 71 Strain in Xiamen, China From 2009-2018

Mengyuan Chen
Xiamen University
Shuizhen He
Xiamen Haicang Hospital
Qiang Yan
Xiamen University
Jianmei Zhang
Xiamen Center for Disease Control and Prevention
Caiyu Li
Xiamen University
Xiaosong Su
Xiamen University
Shiyin Zhang (zhangshiyin@xmu.edu.cn)
Xiamen University https://orcid.org/0000-0001-8291-7290
Tingdong Li
Xiamen University
Shengxiang Ge
Xiamen University
Min Chen
Xiamen Center for Disease Control and Prevention
Jun Zhang
Xiamen University
Ningshao Xia
Xiamen University

Research Article

Keywords: non-C4 EV71 strains, hand foot and mouth disease, human enteroviruses, phylogenetic analysis

Posted Date: February 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-231221/v1

Abstract

EV71 has caused large HFMD epidemics among young children and EV71 infection was the leading cause of severe cases and deaths. In mainland China, almost all EV71 strains were segregated into C4 sub-genotype and prevalence of non-C4 EV71 strains was still unclear in mainland China. This study aimed to comprehensively report this in Xiamen. 5'UTR and VP1 Sequences of the strains were amplified by RT-nested-PCR and then sequenced. 32 non-C4 EV71 strains were identified in Xiamen during the study. This study would provide important information for related fields and provide new insights of EV71 strains in China.

Introduction

Hand, foot and mouth disease (HFMD), caused by human enteroviruses (HEVs), is a common pediatric infectious disease. In recent years, mainland China has suffered large number of HFMD outbreaks (1, 2). From January 2008 to June 2019, 22.54 million HFMD cases including 3704 deaths were reported to the national surveillance system for HFMD in China established by Chinese Center for Disease Control and Prevention (Chinese CDC) (http://www.nhc.gov.cn/jkj/s2907/new_list.shtml?tdsourcetag=s_pcqq_aiomsg).

HFMD is caused by human enteroviruses (HEV) and the main pathogens in mainland China include Enterovirus 71 (EV71), Coxsackievirus A16 (CV16), Coxsackievirus A6 (CA6) and Coxsackievirus A10 (CA10). As severe cases, usually with clinical manifestations of nervous system syndromes and cardiopulmonary failure, were mostly caused by EV71, cases infected with EV71 were paid more attention by clinicians and epidemic patterns of EV71 in many countries and regions were thoroughly studied by researchers.

EV71 can be clustered into 3 genotypes, including A, B (B1-B5 sub-genotypes) and C (C1-C5 sub-genotypes). On present understanding, in other parts of Asia, including Taiwan Province in China, several sub-genotypes could circulate or co-circulate in a same area (3–9), while in mainland China almost all EV71 strains belonged to C4 sub-genotype (2, 10) and only individually sporadic cases caused by non-C4 strains were reported (11–13). The prevalence and risk factors of non-C4 strains were not clear. This might lead to bias in vaccine design, diagnostic reagents development and disease control and prevention strategies. This study aimed to study the prevalence of non-C4 strains through a 10-year epidemic study in Xiamen, a transport hub between Southeast Asia and mainland China that may stand in the forefront of epidemic trends.

Materials And Methods

In this study, deep monitoring system for HFMD pathogen spectrum was implemented in Xiamen since 2009. Based on this system, all EV71 sub-genotypes could be identified. HFMD cases diagnosed by clinicians were reported to Xiamen CDC and cases between 2008 and 2019 were analyzed. Two sampling

methods were adapted in this study: from January 2009 to June 2010, all samples were collected and detected, while random sampling method was took from July 2010 to March 2018 due to increase of cases.

Samples were examined using reverse transcription-nested-PCR (RT-nested-PCR) as we previously reported [14]. In this method, 5' UTR analysis could detect almost all sub-genotypes of EV71, while VP1 analysis for EV71 could only identify C4 and C5 sub-genotypes for specificity consideration. In this study, 55 EV71 samples that failed to be sub-genotyped were analyzed. Nucleic acids were extracted using GenMagBio Viral DNA/RNA Extraction Kit (GenMagBio, China), and 3-step RT-nested-PCR was performed by using non-EV71 VP1 primers (see Supplementary Data). The RT mixture for each tube consisted of 4 μl M-MLV Buffer, 4 μl dNTP mixture (10 mM each), 0.6 μl specific primer (10 μM), 0.2 μl Reverse Transcriptase M-MLV (TaKaRa), 0.2 μl Recombinant Rnasin Ribonuclease Inhibitor (Promega), 5 μl viral RNA and 6 μl nuclease-free water. RT reaction was performed at 50 ℃ for 30 min. The first round PCR mixture for each tube consisted of 2 μl 10× PCR Buffer, 2.4 μl dNTP mixture (2.5 mM each), 0.4 μl forward primer (10 μM), 0.4 μl reverse primer (10 μM), 0.2 μl rTaq (TaKaRa), 5 μl cDNA and 9.6 μl nuclease-free water. The PCR was conducted under these conditions: initial denaturation at 95°C for 10 min, followed by 35 cycles of 94°C for 40 s, 53°C for 40 s, 72°C for 1 min and final extension at 72°C for 10 min. A volume of 2 μl PCR product was used for the second round PCR with 5 μl 10× PCR Buffer, 6 μl dNTP mixture (2.5 mM each), 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), 0.5 μl rTaq (TaKaRa) and 34.5 μl nuclease-free water undergoing the same PCR conditions. The amplicons were purified and sequenced. All the sequences were submitted to GenBank sequence database (NCBI, USA) with given accession numbers MT861057-MT861109. Phylogenetic trees based on partial 5' UTR and VP1 sequences were constructed by using the Jukes-Cantor algorithm and the neighbor-joining method in MEGA 7.0 software (www.megasoftware.net). As the partial VP1 sequences located at different regions, 3 phylogenetic trees based on VP1 sequences were constructed.

Results

From January 2009 to September 2019, 64309 cases, including 124 severe cases, were reported to Xiamen CDC. As country level, the HFMD cases in Xiamen are generally on a rise. From January 2009 to March 2018, throat swabs from 7203 cases were tested. Among the cases, 5315 were detected as HEV positive (73.8%). CA6 (33.63%), EV71 (28.45%), CA16 (23.62%) and CA10 (4.98%) were the primary pathogens (Fig. 1).

As EV71 was the first primary pathogen from 2009 to 2015 and EV71 infections accounted for the majority of severe cases, each EV71 strain was sub-genotyped. The vast majority belonged to C4 sub-genotype. However, a small portion of the strains were failed to be sub-genotyped. Further studies indicated these strains belonged to non-C4 strains which were particularly uncommon in mainland China. 32 non-C4 strains were identified in Xiamen (Table 1). These cases, all belonged to mild type, showed similar clinical manifestations with the patients infected with other HEVs.

Table 1
Genotyping results of non-C4 EV-A71 strains in Xiamen from 2008–2018

Strains	Isolation year	5'UTR	VP1	Note
2009–3159	2009	India Orphan Strains		
2009–3711	2009	India Orphan Strains		
2009–3956	2009	India Orphan Strains		
2009–4425	2009	India Orphan Strains		
2009–4633	2009	A		
2009–5442	2009	India Orphan Strains		
2009–5463	2009	India Orphan Strains	B5	Recombinant strain
2009–5535	2009	B5	B5	
2010 – 140	2010	India Orphan Strains		
2010–6086	2010	India Orphan Strains		
2010–6089	2010	C4	B5	Recombinant strain
2010–6187	2010	India Orphan Strains		
2010–7223	2010	India Orphan Strains		
2010–7590	2010	India Orphan Strains		
2010–7635	2010	India Orphan Strains		
2011-31	2011	India Orphan Strains	C5	Recombinant strain
2011-43	2011	B4	B3	Recombinant strain
2011 – 432	2011		B5	
2011 – 558	2011		B3	
2012 – 109	2012	B3		
2012-14	2012	B3		
2012 – 203	2012	B5		
2012 – 290	2012	B5	B5	
2012 – 339	2012	B5		
2012 – 351	2012	B5	B5	
2012 – 354	2012	B5	B5	
2012 – 356	2012	B5	B5	

Strains	Isolation year	5'UTR	VP1	Note
2012 – 376	2012	B5	B5	
2012-97	2012	B5	B3	
2013 – 284	2013	B5		
2014-4	2014		C2	
2015 – 216	2015	B5	B5	

According to the VP1 sequences, 3 B3 strains, 10 B5 strains, 1 C2 strains, 1 C5 strains were identified. Among the 15 strains, the 5' UTR and VP1 sequences of 4 strains were clustered into different sub-genotypes. This indicated inner-genotyped recombination events happened between 5' UTR and VP1. According to the VP1 sequences, most Xiamen B5 strains were closely related to strains detected in Taiwan, while another 2 Xiamen B5 strains had a close relationship with Southeast Asia strains. All Xiamen B5 strains isolated from 2010–2012 were closely grouped into a sub-lineage. The Xiamen C2 strain was closely related to Philippines strains while the Xiamen C5 strain to Vietnam strains. The Xiamen B3 strains were segregated into 2 sub-lineages.

Beyond that, VP1 sequences of 17 strains were failed to be amplified. The 5' UTR phylogenetic analysis showed that 1, 2 and 3 strains clustered with genotype A, sub-genotype B3 and B5, while 11 showed higher homology with India orphan strain (Table 1 and Fig. 2a-2d) (2 C4 strains were excluded according to VP1 phylogenetic analysis).

Discussion

Asia was the EV71-associated-HFMD stricken area. In Southeast Asia, including Taiwan Province of China, several sub-genotypes of EV71 strains co-circulated in a same region. However, the mainland China showed entirely different epidemic patterns. As currently known, except for single cases, EV71 strains were all belonged to C4 sub-genotype. Rare non-C4 EV71 isolated from different years and areas were reported (11–13). We had reported the first imported B5 strains in Xiamen (isolated in 2009) that displayed a close genetic relationship with the 2006–2008 Taiwan strains and Singapore strains (15). Five years later, the second imported B5 strain which shared the highest nucleotide identity with Vietnam strains in 2011–2013 was reported in Chongqing (11). Two C2 strains were respectively isolated in Shandong (in 1998) and Beijing (in 2015) (12, 13).

For high sensitivity, detection reagents used in mainland China were usually developed for specifically detecting EV71 C4 strains. The prevalence and risk factors of non-C4 strains were not clear. What's more, EV71 vaccine was designed based on C4 strain and cross protections against non-C4 EV71 strains was unknown. As very few researchers systematically concerned prevalence of non-C4 EV71 strains in

mainland China, deep surveillance of non-C4 EV71 strains would provide important information. This study reported the prevalence of non-C4 strains through a 10-year epidemic study in Xiamen.

In this study, we firstly systematically reported non-C4 EV71 cases in a same region of mainland China. 32 non-C4 EV71 strains, covering B3, B5, C2, C3 and India orphan strain, had been identified. The non-C4 strains accounted for 2.12% (32/1512, far greater than other districts) of EV71 strains. The non-C4 strains had closed relationships with South-east Asia strains. There might be two reasons. On one hand, a deep, comprehensive and long-term surveillance system for pathogen spectrum of HFMD had been performed in Xiamen for 10 years. As all sub-genotypes of HEV could be identified under our system, non-C4 EV71 strains wouldn't be ignored. On another hand, as Xiamen city is an important tourist city and a key transportation hub between mainland China and Southeast Asia, Xiamen may stands at the forefront of EV71 epidemic changes. Epidemic patterns of HEV in Xiamen might be affected by imported HEV strains from Southeast Asia. A study in Taiwan showed large epidemics occurred just after minorities of strains were identified a few years ago. This suggested that large epidemics caused by non-C4 EV71 strains can occur at any time and enough attention still should be given.

This study provided new understanding of EV71 epidemic patterns in mainland China, and also provides important information for formulating EV71 prevention and control strategies, and developing vaccine and diagnostic reagents.

Declarations

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

Animal and human rights statement

This article does not contain any studies with human or animal subjects performed by any of the authors.

Fundings

This work was supported by the National Natural Science Foundation of China (31971369), the Key Program of the National Natural Science Foundation of Fujian Province (2019J02004) and the CAMS Innovation Fund for Medical Sciences (2019RU022).

References

1. Xing W, Liao Q, Viboud C, et al (2014) Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. Lancet Infect Dis 14:308-18.

2. Liu S-L, Pan H, Liu P, et al (2015) Comparative epidemiology and virology of fatal and nonfatal cases of hand, foot and mouth disease in mainland China from 2008 to 2014. Rev Med Virol 25:115-28.
3. Chia MY, Chiang PS, Chung WY, et al (2014) Epidemiology of enterovirus 71 infections in Taiwan. Pediatr Neonatol 55:243-9.
4. Wu WH, Kuo TC, Lin YT, et al (2013) Molecular epidemiology of enterovirus 71 infection in the central region of Taiwan from 2002 to 2012. PLoS One 8:e83711.
5. Huang SW, Hsu YW, Smith DJ, et al (2009) Reemergence of enterovirus 71 in 2008 in taiwan: dynamics of genetic and antigenic evolution from 1998 to 2008. J Clin Microbiol 47:3653-62.
6. Wu Y, Yeo A, Phoon MC, et al (2010) The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. Int J Infect Dis 14:e1076-81.
7. Donato C, Hoi le T, Hoa NT, et al (2016) Genetic characterization of Enterovirus 71 strains circulating in Vietnam in 2012. Virology 495:1-9.
8. Chua KB, Kasri AR (2011) Hand foot and mouth disease due to enterovirus 71 in Malaysia. Virologica Sinica 26:221-8.
9. McMinn P, Lindsay K, Perera D, et al (2001) Phylogenetic analysis of enterovirus 71 strains isolated during linked epidemics in Malaysia, Singapore, and Western Australia. J Virol 75:7732-8.
10. Liu W, Wu S, Xiong Y, et al (2018) Co-circulation and genomic recombination of coxsackievirus A16 and enterovirus 71 during a large outbreak of hand, foot, and mouth disease in Central China. PLoS One. Obiology 9:2337.
11. Yang Q, Zhang Y, Yan D, et al (2016) Isolation of an imported subgenotype B5 strain of human enterovirus A71 in Chongqing City, China, 2014. Virol J 13:115.
12. Tao Z, Wang H, Xu A (2012) Identification of a C2 subgenogroup strain of enterovirus 71 in a retrospective study in Shandong Province, China, from 1990 to 2010. J Clin Microbiol 50:1823-4.
13. Li J, Li Y, Zhang S, et al (2018) Analysis of an Imported Subgenotype C2 Strain of Human Enterovirus 71 in Beijing, China, 2015. Front Microbiol 9:2337
14. Ge S, Yan Q, He S, et al (2013) Specific primer amplification of the VP1 region directed by 5' UTR sequence analysis: enterovirus testing and identification in clinical samples from hand-foot-and-mouth disease patients. J Virol Methods 193:463-9.
15. He S, Yan Q, Xu X, et al (2013) Isolation and identification of an EV71-B5 strain. Strait journal of prevention medicine 04:1-4.

Figures

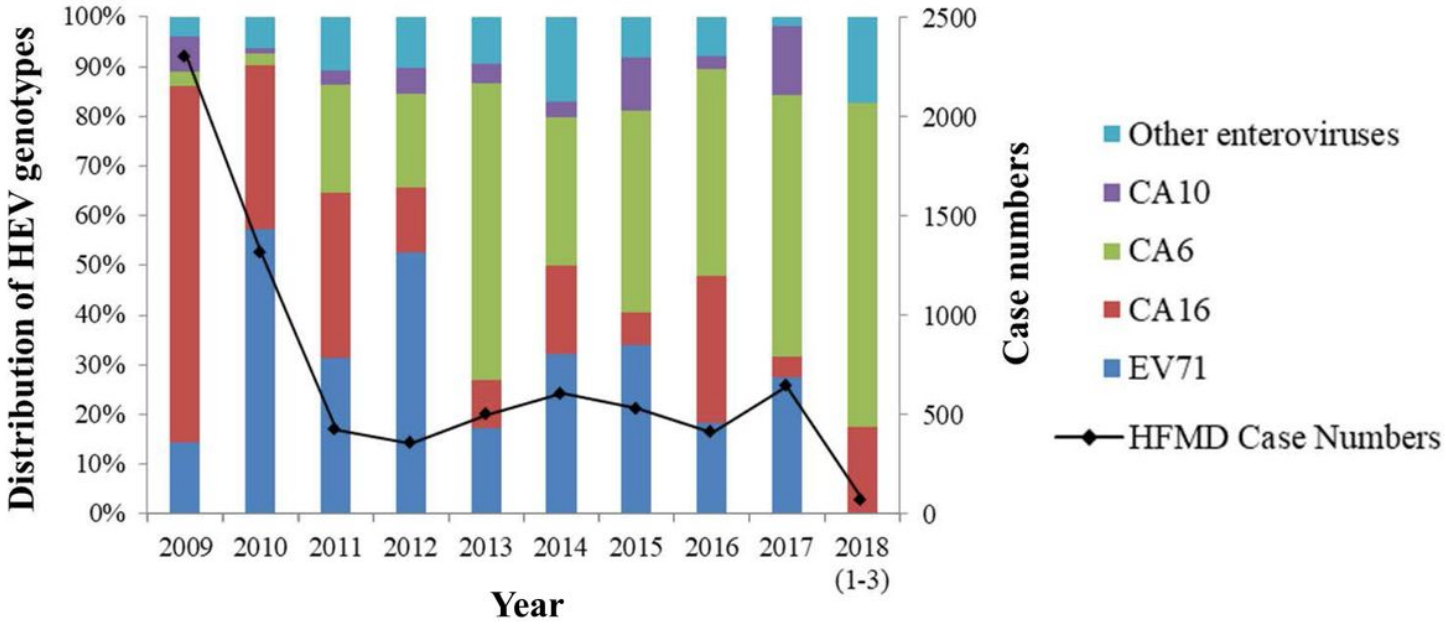

Figure 1

Year distribution of detected HFMD cases (the continuous line) and pathogen spectrum of HEV (the histogram) in Xiamen, China from January 2009 to March 2018.

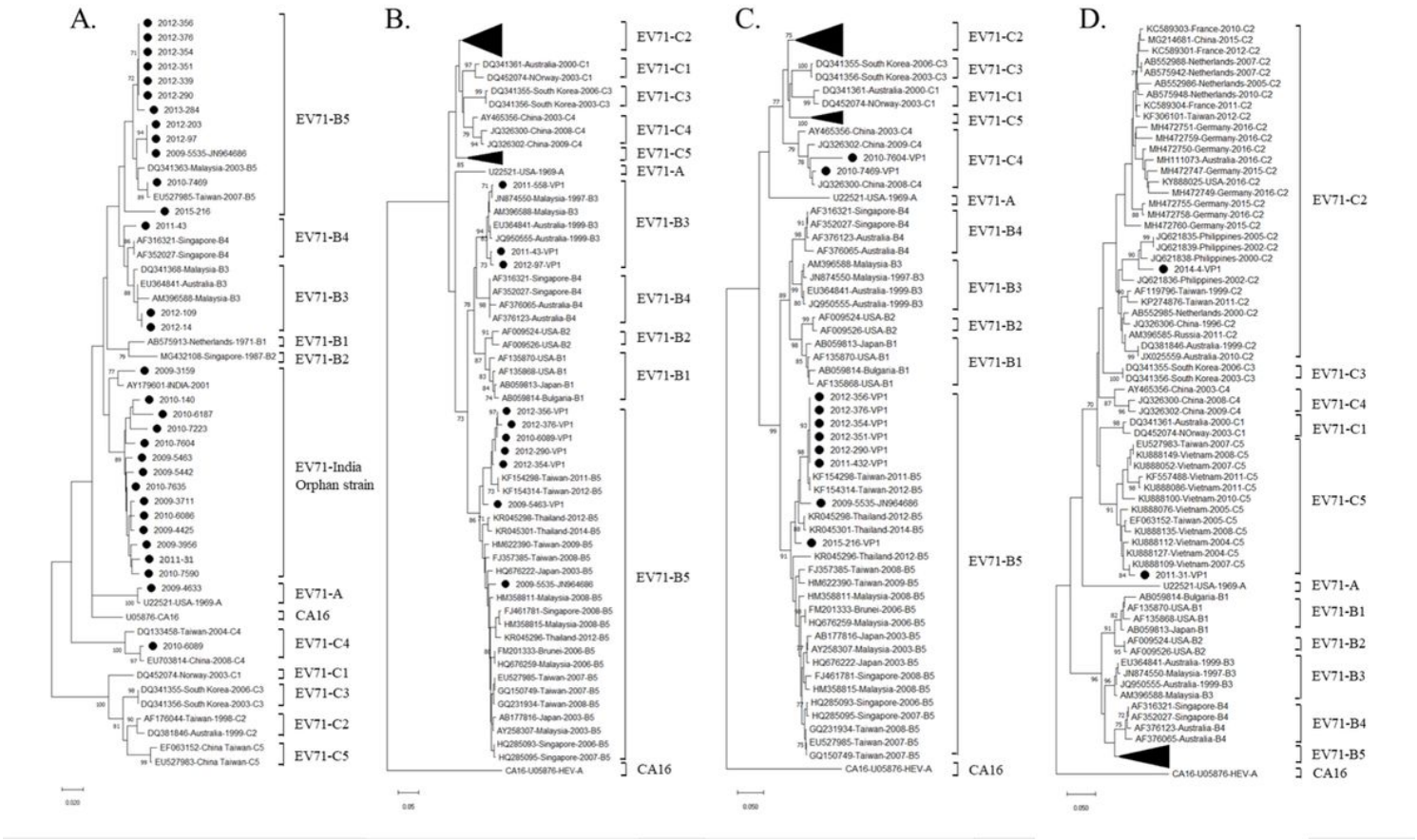

Figure 2

Neighbor-joining phylogenetic trees constructed based on the basis of partial 5’ UTR (A) (nt 206-500, responding to EV71 prototype strain BrCr U22521), partial VP1 sequences (B) (nt 2643-2832), partial VP1 sequences (C) (nt 2667-3130) and partial VP1 sequences (D) (nt 2822-3033) of EV71 strains which identified as different sub-genotypes by using MEGA 7.0. Sequences of this study are labeled with ●.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.docx