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BRIEF REPORT

Epidemiology and etiology of hand, foot, and mouth disease in Fujian province, 2008-2014

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Abstract Millions of cases of hand, foot, and mouth disease (HFMD) have been reported annually in mainland China since 2008. In this study, we investigated the epidemiology and etiology of an HFMD epidemic in Fujian province, which is located in subtropical southeastern China. Our study found similar epidemiological features of HFMD in southern areas of China, including seasonality and demographic distribution, as well as correlation between severity of illness and serotype. At least 22 serotypes of other enterovirus co-circulating with enterovirus 71 were found to belong to clade C4a, and those circulating with coxsackievirus A16 were associated with clades B1a and B1b.

Keywords: Hand, foot, and mouth disease · Enterovirus · Epidemiology · Etiology

Hand, foot, and mouth disease (HFMD) is a common viral disease that afflicts children. The symptoms of the disease are generally mild, self-limiting, and characterized by fever, herpangina and rash on extremities. A few patients may develop severe complications involving the nervous system, such as aseptic meningitis, brain stem encephalitis, acute flaccid paralysis, and neurogenic pulmonary edema, or even death [1]. Transmission of the virus via direct person-to-person contact or indirect transmission through contaminated objects leads to frequent outbreaks in crowded settings. In the late 1990s, Taiwan, Malaysia, and Singapore experienced large-scale outbreaks of HFMD [2–4]. In mainland China, HFMD was sporadically reported only during the 1980s and 90s [5–7]. No large-scale outbreaks were observed until sudden local outbreaks occurred in Shandong and Anhui provinces in 2007-2008 [8, 9]. The outbreaks and the subsequent widespread occurrence of HFMD posed a serious threat to children's health. HFMD was categorized as a notifiable infectious disease on May 2, 2008, in mainland China. Comprehensive and enhanced surveillance of the disease has been implemented nationwide since then. Systematic surveillance in recent years has revealed that HFMD is widespread in mainland China, and millions of cases, including hundreds of deaths, are reported annually.

HFMD is caused by human enteroviruses (EVs) belonging to the genus *Enterovirus* of the family *Picornaviridae*. The EV virion is composed of a capsid and a positive single-strand RNA genome whose sequence undergoes frequent variation. According to their antigenic properties and pathogenicity [10], EVs have been divided into numerous serotypes and grouped traditionally into poliovirus (PV, 3 serotypes), coxsackie virus A (CVA, 23 serotypes), coxsackievirus B (CVB, 6 serotypes), and echovirus (ECHO, 28 serotypes). Advances in molecular typing have led to the classification of enteroviruses into four species: A to D [11]. Genetically, the viruses with identical serotypes have been further divided into multiple genotypes or sub-genotypes. Among the multiple serotypes of EV, EV71 and CVA16 are the two major serotypes associated with HFMD. In addition to the two main

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serotypes, infection with multiple EVs (non-EV71 and non-CVA16) and such as CVA2, 4, 5, 6, 8, 10 and 16, CVB1-5, also results in HFMD pathogenesis with diverse clinical manifestations. The diversity of serotypes associated with HFMD and the occurrence of genetic mutations are regarded as important factors contributing to the HFMD epidemic. Co-circulation of multiple serotypes and genetic variation of EV contribute to the complex epidemiology of HFMD.

In addition to etiological factors, natural factors are thought to be involved in the HFMD epidemic. Previous studies have shown that climate and geographical location are important factors in the HFMD epidemic in mainland China [12]. The abundance of rainfall and higher average temperature in southern areas compared with the northern areas contributes to the higher incidence of HFMD [13–15]. Fujian is a coastal province located in southeastern China, with a typical sub-tropical monsoon climate. In recent years, the incidence of HFMD in the province has increased annually. However, it remains unclear whether the HFMD epidemic in Fujian is similar to that in southern China. Understanding the HFMD epidemic is important for effective prevention and control of the disease. We therefore investigated the HFMD epidemic in Fujian using descriptive epidemiology combined with laboratory monitoring.

This study was approved by the Ethics Review Committee of the Fujian Provincial Center for Disease Control and Prevention (CDC). Specimens were collected from patients who underwent regular medical examination at sentinel hospitals, and therefore, written informed consent was waived. All of the information collected from patients, including demographic data, clinical records, and laboratory findings was kept anonymous to protect their privacy.

The records for reported HFMD cases were retrieved according to the date of onset of illness from May 2, 2008, to December 31, 2014, and downloaded from the National Disease Surveillance Information Report Administration System of China. The definitions of mild, severe and fatal cases were adopted according to the *Guidelines for response to hand-foot-and-mouth disease epidemic* (http://www.chinacdc.cn/jkzt/crb/bl/szkb/jszl_2275/200906/t20090612_24707.html) issued by the China CDC. Epidemiological characteristics including temporal and spatial distribution, demographic features, and severity of illness were descriptively analyzed. Data sorting and statistics were performed using Microsoft Excel. The correlation between the serotype and severity of illness was analyzed by linear regression using GraphPad Prism (Ver. 5.0), and the geographic distribution of the disease was analyzed using ArcGIS (Ver.10.2).

To obtain the viral isolates for etiological characterization of HFMD, throat and/or cloacal swab specimens were collected from HFMD patients through the surveillance network of sentinel hospitals and laboratories at the municipal CDCs of Fujian. The swabs were transferred to 3-5 mL of DMEM (Gibco) containing 5% FBS (Pan Biotech) and delivered to laboratories within 12 h for molecular diagnosis using one-step RT-PCR or real-time one-step RT-PCR according to the *Guidelines for response to hand-foot-and-mouth disease epidemic*. The positive specimens were inoculated in parallel on human rhabdomyosarcoma cells (RD, ATCCRCCL-136) and human epidermoid cancer cells (HEp-2, ATCCRCCL-23) for viral isolation. Cellular infection and viral propagation were confirmed by observing cytopathic effects. One-step RT-PCR was used to amplify the RNA extracted from the supernatant with the following universal primers for EV detection: PE2 (5'-TCCGGCCCCTGAATGCGGCTAATCC-3') and PE1 (5'-ACACGGACACCCAAAGTAGTCGGTCC-3').

To analyze the genetic variation of the main serotypes of EVs, 179 strains of EV71 and 60 strains of CVA16 isolated from HFMD patients with various clinical outcomes were randomly selected during 2010-2014. Complete *VP1* gene amplification and sequencing were performed using the EV71-specific primers EV71/VP1F (5'-CAGGAAACAGCTATGACGCAGCCCAAAAGAACTTCAC-3') and EV71/VP1R (5'-AGTCACGACGTTGTAAAGTCGCGAGAGCTGTCTTC-3') and the CVA16-specific primers CVA16/VP1F(5'-CAGGAAACAGCTATGACAGCCCAGGACAACTTCAC-3') and CVA16/VP1R (5'-AGTCACGACGTTGTAGCCGATTCACTACCCTAT-3'). After agarose gel separation and column purification, all of the amplicons were subjected directly to bidirectional sequencing on a Genetic Analyzer 3500 platform (Life Technologies) with M13-RV and M13-M primers fused to the 5' termini of forward and reverse primers (underlined), respectively. All of the viral sequences obtained in this study were deposited in the GenBank database (accession nos. KU595714-595892 and KU595958-596017). Together with the sequences of genotype/subgenotype reference strains and viral isolates found in other provinces of China, VP1 sequences obtained in this study were aligned using MEGA6.0 software (Ver. 6.0). Phylogenetic trees of EV71 and CVA16 were reconstructed by the neighbor-joining method (Kimura 2-parameter model) and validated by bootstrap tests (1,000 repeats).

To determine the serotype spectrum of other EVs (non-EV71 and non-CVA16) in Fujian, partial VP1 fragments obtained from 407 strains of other EVs from HFMD patients during 2011-2014 were amplified by one-step RT-PCR with various primers as reported previously [16, 17]. The amplicons were gel-purified and subsequently inserted into pMD20T vector (TaKaRa) by A-T cloning. The recombinant plasmids were purified and subjected to

bidirectional sequencing using M13-RV and M13-M4 primers flanking the insertion site. All of the sequences of other EVs were submitted to the online Enterovirus Genotyping Tool (http://www.rivm.nl/mpf/enterovirus/typingtool) for viral serotyping.

A total of 448,788 cases, including 2,211 severe cases and 88 deaths, were reported in Fujian between May 2, 2008 and Dec 31, 2014. Overall, the incidence rates of HFMD increased from 4.77 to 30.14 per 10,000 annually between 2008 and 2014. However, the case-severity, case-fatality and severity-fatality rates decreased after 2010 (Table S1, Supplementary Material). The temporal distribution indicated that the HFMD epidemic in Fujian had clear seasonality. The incidence peaked in May, with a secondary peak in September each year (Fig. 1). However, the distinct temporal distribution between EV71 and CVA16 reported previously [18] was not observed in Fujian. The spatial distribution indicated that HFMD was spread across nine cities and one pilot zone in the province, with incidence rates varying between subregions during the period of 2010-2014. Overall, the incidence rates in coastal areas were slightly higher than in inland areas (Figure S1, Supplementary Material). We speculate that this might be related to the higher population density and abundant rainfall in coastal areas [13]. The demographic distribution (Table S2) indicated that males outnumbered females, with a ratio of 1.72 (1.60-1.87, male/female). Among the reported cases, children younger than 4 years accounted for 56.15% (322,478 cases) and the 1-year age group was the most affected (149,220 cases, 33.25%). Individual children accounted for 77.96%, followed by kindergarten children, who accounted for 19.22%. Etiological monitoring revealed that the serotype distribution of EVs co-circulating in Fujian changed greatly during 2008-2014. In brief, EV71 was predominant during 2008-2009 and tended to decrease after 2010 despite its predominance until the third quarter of 2012. EV71 was replaced by other EVs after the fourth quarter of 2012, except for the period from the fourth quarter of 2013 to the first half of 2014, during which CVA16 was most prevalent (Fig. S2).

The case-severity and case-fatality rates of HFMD in Fujian varied each year. The rates peaked in 2010 and declined subsequently (Table S1). The correlation between disease severity and EV serotype revealed that EV71 was the leading etiological agent associated with severity and fatality of HFMD cases in Fujian. During 2010-2014, EV71 infection accounted for 40.23% (5,648/14,040) of mild cases. However, the proportion of EV71 infections increased significantly to 72.8% (1,194/1,640) and 98.59% (70/71) in severe and fatal cases, respectively. Linear regression analysis revealed that the incidence of EV71 infections was 75.74% (2,548/3,364), 43.88% (624/1,422), 50.19% (1,979/3,943), 23.65% (808/3,417) and 26.44% (953/3,605) each year from 2010 to 2014, which was strongly correlated with annual case-severity rates of HFMD ($r^2 = 0.9254$, $p = 0.0089$). By contrast, CVA16 infection mainly caused mild HFMD (22.93%, 3,220/14,040) and accounted only for 7.01% (115/1,640) of severe cases, with no deaths. Other EVs also caused mild HFMD (36.84%, 5,172/14,040) and accounted for 20.18% of severe cases (331/1,640) and 1.41% of all of the deaths (1/71). Notably, the case-severity and case-fatality rates in 2008-2009, compared with those in 2010-2014, were relatively low in spite of the higher incidence of EV71 (Table S1). The underlying causes included neglect of most patients due to the relatively mild manifestation of other EVs and poor surveillance networks in the initial stages of enhanced disease surveillance. The quality of reporting was

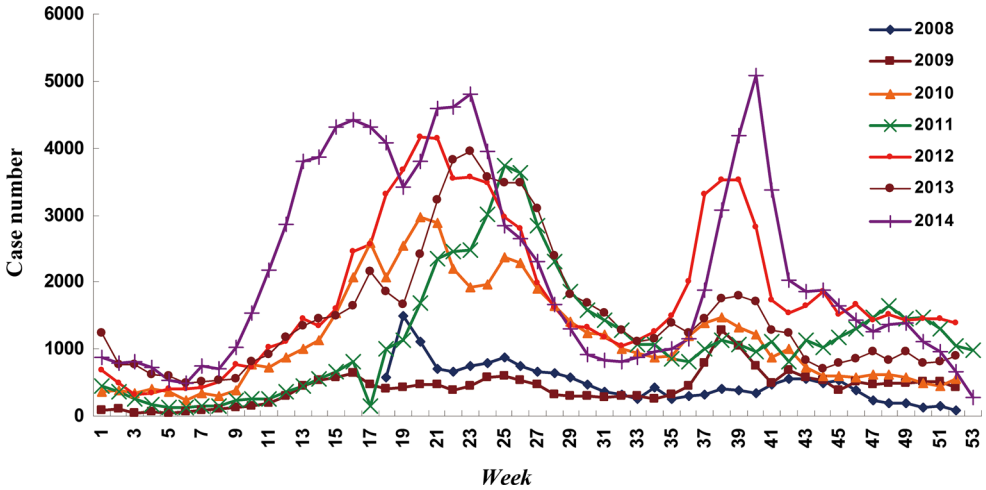


Fig. 1 Weekly distribution of HFMD in Fujian, 2008-2014. Case registries were collected and sorted by the date of disease onset since May 2, 2008

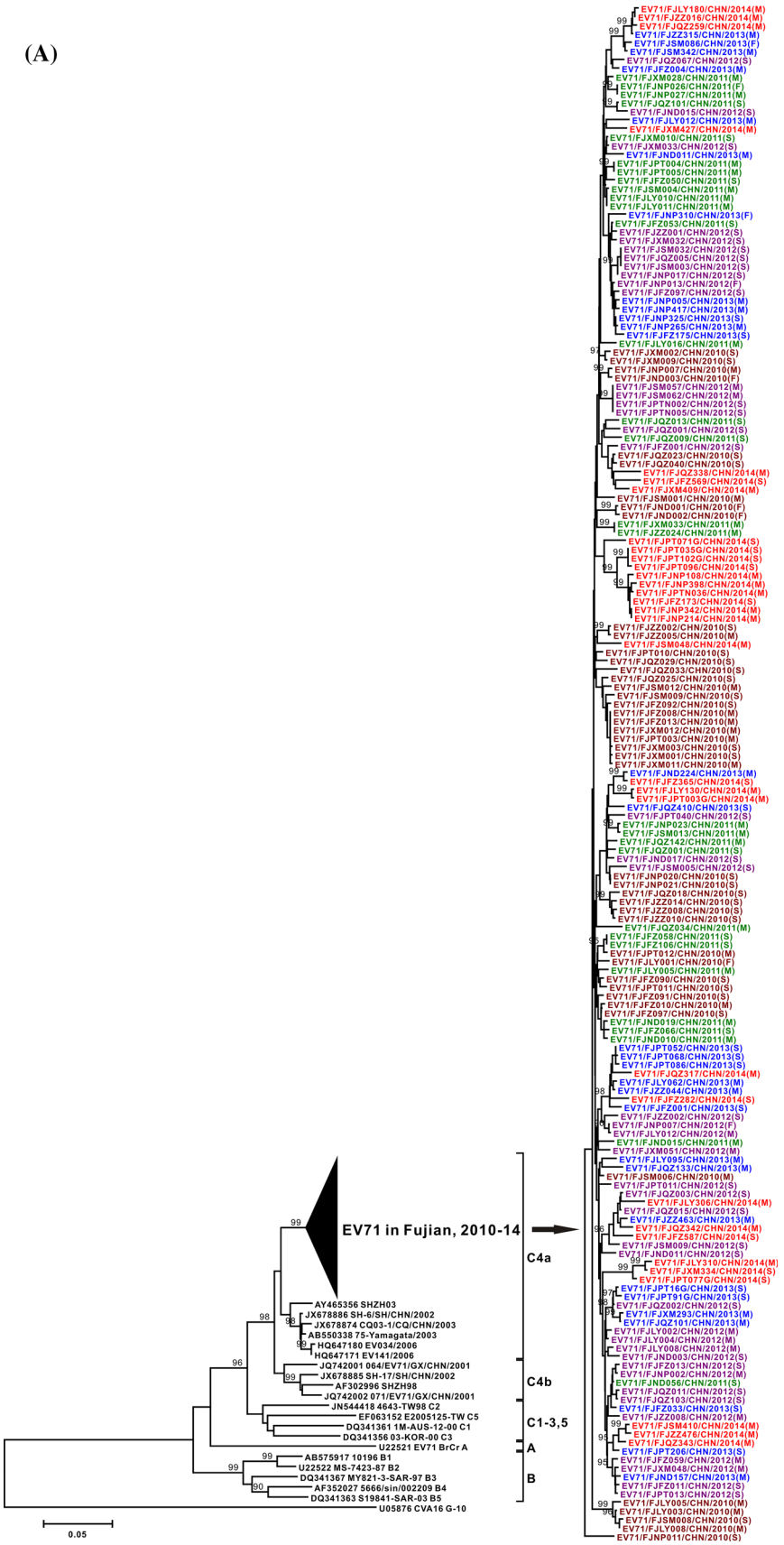
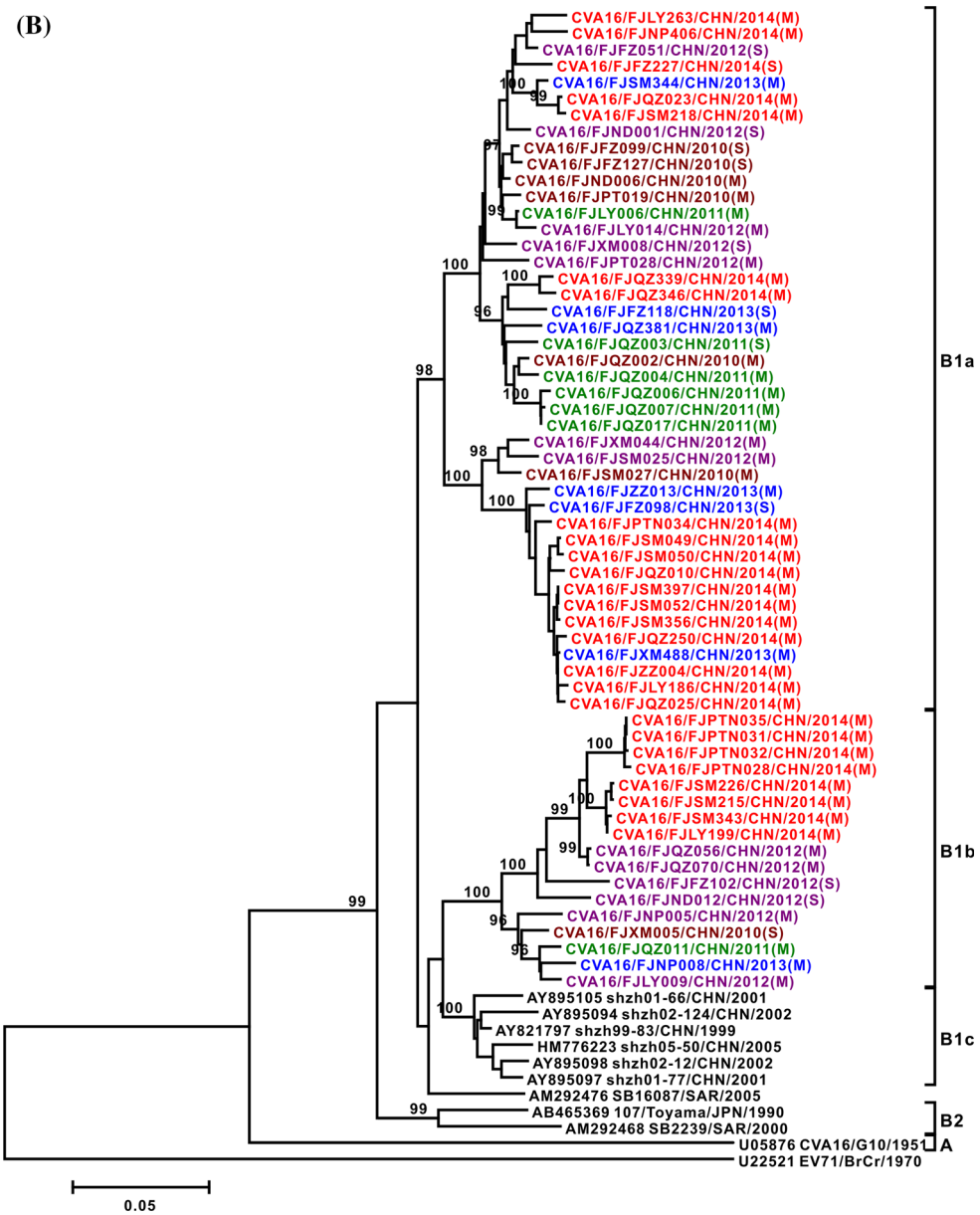


Fig. 2 VP1-based phylogenetic analysis of EV71 (**A**) and CVA16 (**B**) in Fujian, 2010-2014. The viruses isolated in 2010, 2011, 2012, 2013, and 2014 are colored brown, green, purple, blue and red, respectively. Viruses isolated from mild (M), severe (S), and fatal (F) cases, are shown. Bootstrap values greater than 95% are displayed above the nodes. Reference sequences of various genotypes or subgenotypes downloaded from GenBank and the corresponding accession numbers are indicated with the virus designation

Fig. 2 continued



improved by adjusting the surveillance strategy of China CDC as well as development of diagnostic methods for other EVs.

VP1-based phylogenetic analysis of EV71 and CVA16 was conducted to investigate the phylogenesis of the two main serotypes of EV71 and CVA16 in this study. The reconstructed phylogenetic tree indicated that all of the EV71 viruses circulating in Fujian during 2010-2014 belonged to clade C4a and were not clustered by clinical severity or time of appearance (Fig. 2A). The phylogenetic tree of CVA16 indicated that the viruses circulating in Fujian during the same period belonged to two clades, B1a and B1b. Similar to EV71, the viruses of CVA16 showed no clustering by clinical severity or time of appearance (Fig. 2B). Genetically, EV71 was classified into genotypes A, B and C, and the latter two were further divided into subgenotypes B1-5 and C1-5. Molecular epidemiology suggested that the two clades, C4a and C4b, of subgenotype C4 of EV71 were associated with a persistent epidemic in mainland China [19], and the clade shift from C4b to C4a occurred around 2004 [20]. CVA16 comprised genotypes A and B, and the latter included two subgenotypes, B1 and B2. Two clades in the B1 subgenotype (B1b and B1a) were associated with the HFMD epidemic and circulated in mainland China [21]. Therefore, the EV71 and CVA16 circulating in Fujian were genetically similar to those circulating synchronously nationwide. Despite the circulation of many genetically heterogenous viral strains,

Table 1 Summary of other enterovirus serotypes detected in HFMD patients reported in Fujian, 2011-2014

Serotype	2011		2012		2013		2014		Total	
	No. of cases	(%)	No. of cases	(%)	No. of cases	(%)	No. of cases	(%)	No. of cases	(%)
CVA2	3	3.90	3	1.46	4	4.30	0	0.00	10	2.46
CVA4	0	0.00	31	15.12	0	0.00	2	6.25	33	8.11
CVA5	1	1.30	2	0.98	6	6.45	1	3.13	10	2.46
CVA6	23	29.87	103	50.24	56	60.22	17	53.13	199	48.89
CVA8	1	1.30	3	1.46	0	0.00	0	0.00	4	0.98
CVA9	3	3.90	2	0.98	0	0.00	1	3.13	6	1.47
CVA10	32	41.56	38	18.54	26	27.96	7	21.88	103	25.31
CVA12	4	5.19	1	0.49	0	0.00	0	0.00	5	1.23
CVA21	0	0.00	0	0.00	1	1.08	0	0.00	1	0.25
CVB1	3	3.90	0	0.00	0	0.00	0	0.00	3	0.74
CVB2	1	1.30	0	0.00	0	0.00	0	0.00	1	0.25
CVB3	0	0.00	7	3.41	0	0.00	0	0.00	7	1.72
CVB4	0	0.00	5	2.44	0	0.00	0	0.00	5	1.23
CVB5	3	3.90	1	0.49	0	0.00	2	6.25	6	1.47
ECHO3	0	0.00	1	0.49	0	0.00	0	0.00	1	0.25
ECHO6	1	1.30	2	0.98	0	0.00	1	3.13	4	0.98
ECHO7	0	0.00	1	0.49	0	0.00	0	0.00	1	0.25
ECHO9	0	0.00	1	0.49	0	0.00	0	0.00	1	0.25
ECHO16	0	0.00	2	0.98	0	0.00	0	0.00	2	0.49
ECHO25	0	0.00	2	0.98	0	0.00	0	0.00	2	0.49
ECHO30	2	2.60	0	0.00	0	0.00	0	0.00	2	0.49
EV68	0	0.00	0	0.00	0	0.00	1	3.13	1	0.25
Total	77	100.00	205	100.00	93	100.00	32	100.00	407	100.00

no viral strain of EV71 or CVA16 demonstrated superior dissemination because no temporal changes in severity or incidence were observed.

The spread of other EV (non-EV71 and non-CVA16) infections among HFMD patients has been reported in other regions of mainland China [22, 23]. Etiological monitoring revealed a similar phenomenon in Fujian in recent years (Fig. S2). We identified 22 serotypes of EV in Fujian based on partial VP1 sequences derived from 407 strains of other EVs isolated from HFMD patients during 2011-2014, including nine serotypes of CVA, five of CVB, seven of ECHO and one of EV68 (Table 1). Among the other EVs, CVA6 and CVA10 accounted for 48.89% (199/407) and 25.31% (103/407), respectively. The serotypes of other predominant EVs were CVA10 (41.56%, 32/77) in 2011 and CVA6 since 2012 (48.89-60.22%).

Based on genetic differences, more than 100 serotypes of EV have been identified so far [24]. HFMD is caused by multiple EV serotypes that cause diverse clinical manifestations [25, 26], which can differ even between similar EV serotypes. For example, an outbreak of viral encephalitis caused by ECHO30, which is difficult to distinguish from HFMD, was reported previously in Fujian. We postulated that co-circulation of multiple serotypes of EVs and the lack of cross-immunity has resulted in a considerable increase of HFMD in Fujian since 2011. Co-circulation of multiple EV serotypes is a diagnostic challenge, especially for atypical HFMD cases [26], as well as disease management and control.

Rapid advances in vaccine development against EV71 have been reported in mainland China, Taiwan and Singapore [27]. The inactivated EV71 vaccine derived from clade C4a of the C4 subgenotype, which was predominant in mainland China, showed efficient protection against viral infection and partial cross-protection against viruses of other genotypes [28–30]. Fortunately, two EV71 vaccines developed by different institutions were licensed toward the end of 2015 in mainland China. Considering that EV71 is the leading cause of severe HFMD, affordable vaccination has the potential to greatly reduce the severity and fatality of HFMD.

In summary, we investigated the epidemiology of HFMD in Fujian during the period of 2008-2014. The temporal and spatial distribution, demographics, and correlation between severity of illness and viral serotype in Fujian were consistent with those of an HFMD epidemic in

the southern areas of China [31]. Nonetheless, the increasing incidence of HFMD, co-circulation of multiple serotypes, potential genetic variation among EVs similar to seasonal influenza viruses [32], and underlying epidemiological factors are still challenges for disease prevention and control. Continuous epidemiological and etiological surveillance of HFMD is, therefore, imperative.

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Compliance with ethical standards

Conflict of interest The authors declare the absence of any conflicts of interest.

References

1. Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T (2010) Clinical features, diagnosis, and management of enterovirus 71. Lancet Neurol 9:1097–1105
2. Chan KP, Goh KT, Chong CY, Teo ES, Lau G, Ling AE (2003) Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerg Infect Dis 9:78–85
3. Herrero LJ, Lee CS, Hurrelbrink RJ, Chua BH, Chua KB, McMinn PC (2003) Molecular epidemiology of enterovirus 71 in peninsular Malaysia, 1997–2000. Arch Virol 148:1369–1385
4. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, Wang JR, Shih SR (1999) An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. N Engl J Med 341:929–935
5. He YQ, Feng SJ, Liu K, Yang H (2003) Surveillance of Enterovirus Type 71 in shenzhen in 2001. Dis Surveill 18:287–289
6. Lin SE, Zhang Q, Xie HP, Xie JP, He JX, Dong QL, Fang ZY (2004) Phylogenetic analysis of enterovirus 71 isolated from patients with hand, foot and mouth disease in Guangdong and Fujian provinces 2000–2001. Zhong hua shi yan he lin chuang bing du xue za zhi 18:227–229
7. Zheng ZM, Zhang JH, Zhu WP, He PJ (1989) Isolation of enterovirus type 71 from the vesicle fluid of an adult patient with hand-foot-mouth disease in China. Virol Sin 4:375–382
8. Yang F, Ren L, Xiong Z, Li J, Xiao Y, Zhao R, He Y, Bu G, Zhou S, Wang J, Qi J (2009) Enterovirus 71 outbreak in the People's Republic of China in 2008. J Clin Microbiol 47:2351–2352
9. Zhang Y, Tan XJ, Wang HY, Yan DM, Zhu SL, Wang DY, Ji F, Wang XJ, Gao YJ, Chen L, An HQ, Li DX, Wang SW, Xu AQ, Wang ZJ, Xu WB (2009) An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. J Clin Virol Off Publ Pan Am Soc Clin Virol 44:262–267
10. Khetsuriani N, Lamonte-Fowlkes A, Oberst S, Pallansch MA, Centers for Disease C, Prevention (2006) Enterovirus surveillance–United States, 1970–2005. Morb Mortal Wkly Rep Surveill Summ 55:1–20
11. Pallansch MA, Oberste MS, Whittion JL (2013) Enteroviruses: Polioviruses, Coxsackieviruses, Echoviruses, and Newer Enteroviruses, 6th edn. Lippincott Williams & Wilkins, USA
12. Wang Y, Feng Z, Yang Y, Self S, Gao Y, Longini IM, Wakefield J, Zhang J, Wang L, Chen X, Yao L, Stanaway JD, Wang Z, Yang W (2011) Hand, foot, and mouth disease in China: patterns of spread and transmissibility. Epidemiology 22:781–792
13. Deng T, Huang Y, Yu S, Gu J, Huang C, Xiao G, Hao Y (2013) Spatial-temporal clusters and risk factors of hand, foot, and mouth disease at the district level in Guangdong Province, China. PloS One 8:e56943
14. Ni H, Yi B, Yin J, Fang T, He T, Du Y, Wang J, Zhang H, Xie L, Ding Y, Gu W, Zhang S, Han Y, Dong H, Su T, Xu G, Cao G (2012) Epidemiological and etiological characteristics of hand, foot, and mouth disease in Ningbo, China, 2008–2011. J Clin Virol Off Publ Pan Am Soc Clin Virol 54:342–348
15. Zhuang ZC, Kou ZQ, Bai YJ, Cong X, Wang LH, Li C, Zhao L, Yu XJ, Wang ZY, Wen HL (2015) Epidemiological research on hand, foot, and mouth disease in mainland China. Viruses 7:6400–6411
16. Oberste MS, Maher K, Williams AJ, Dybdahl-Sissoko N, Brown BA, Gookin MS, Penaranda S, Mishrik N, Uddin M, Pallansch MA (2006) Species-specific RT-PCR amplification of human enteroviruses: a tool for rapid species identification of uncharacterized enteroviruses. J Gen Virol 87:119–128
17. Oberste MS, Nix WA, Maher K, Pallansch MA (2003) Improved molecular identification of enteroviruses by RT-PCR and amplicon sequencing. J Clin Virol Off Publ Pan Am Soc Clin Virol 26:375–377
18. Liu W, Wu S, Xiong Y, Li T, Wen Z, Yan M, Qin K, Liu Y, Wu J (2014) 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 9:e96051
19. Zhang Y, Tan X, Cui A, Mao N, Xu S, Zhu Z, Zhou J, Shi J, Zhao Y, Wang X, Huang X, Zhu S, Zhang Y, Tang W, Ling H, Xu W (2013) Complete genome analysis of the C4 subgenotype strains of enterovirus 71: predominant recombination C4 viruses persistently circulating in China for 14 years. PloS One 8:e56341
20. Zhang Y, Zhu Z, Yang W, Ren J, Tan X, Wang Y, Mao N, Xu S, Zhu S, Cui A, Zhang Y, Yan D, Li Q, Dong X, Zhang J, Zhao Y, Wan J, Feng Z, Sun J, Wang S, Li D, Xu W (2010) An emerging recombinant human enterovirus 71 responsible for the 2008 outbreak of hand foot and mouth disease in Fuyang city of China. Virol J 7:94
21. Chen X, Tan X, Li J, Jin Y, Gong L, Hong M, Shi Y, Zhu S, Zhang B, Zhang S, Zhang Y, Mao N, Xu W (2013) Molecular epidemiology of coxsackievirus A16: intratype and prevalent intertype recombination identified. PloS One 8:e82861
22. Guan H, Wang J, Wang C, Yang M, Liu L, Yang G, Ma X (2015) Etiology of multiple non-EV71 and non-CVA16 enteroviruses associated with hand, foot and mouth disease in Jinan, China, 2009-June 2013. PloS One 10:e0142733
23. Yang XH, Yan YS, Weng YW, He AH, Zhang HR, Chen W, Xu JY, Lin QC, Zhou Y (2012) Molecular analysis of echovirus 30 responsible for the outbreak of aseptic meningitis in Fujian Province, 2011. Chin J Zoonosis 28:265–269
24. Oberste MS, Maher K, Kilpatrick DR, Flemister MR, Brown BA, Pallansch MA (1999) Typing of human enteroviruses by partial sequencing of VP1. J Clin Microbiol 37:1288–1293
25. Cabrerizo M, Tarrago D, Munoz-Almagro C, Del Amo E, Dominguez-Gil M, Eiros JM, Lopez-Miragaya I, Perez C, Reina J, Otero A, Gonzalez I, Echevarria JE, Trallero G (2014) Molecular epidemiology of enterovirus 71, coxsackievirus A16 and A6 associated with hand, foot and mouth disease in Spain. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 20:O150–O156
26. Yan X, Zhang ZZ, Yang ZH, Zhu CM, Hu YG, Liu QB (2015) Clinical and etiological characteristics of atypical hand-foot-and-mouth disease in children from Chongqing, China: a retrospective study. Biomed Res Int 2015:802046

27. Liang Z, Mao Q, Gao F, Wang J (2013) Progress on the research and development of human enterovirus 71 (EV71) vaccines. Front Med 7:111–121
28. Mao Q, Cheng T, Zhu F, Li J, Wang Y, Li Y, Gao F, Yang L, Yao X, Shao J, Xia N, Liang Z, Wang J (2013) The cross-neutralizing activity of enterovirus 71 subgenotype c4 vaccines in healthy chinese infants and children. PloS One 8:e79599
29. Meng FY, Li JX, Li XL, Chu K, Zhang YT, Ji H, Li L, Liang ZL, Zhu FC (2012) Tolerability and immunogenicity of an inactivated enterovirus 71 vaccine in Chinese healthy adults and children: an open label, phase 1 clinical trial. Hum Vaccines Immunother 8:668–674
30. Zhu FC, Meng FY, Li JX, Li XL, Mao QY, Tao H, Zhang YT, Yao X, Chu K, Chen QH, Hu YM, Wu X, Liu P, Zhu LY, Gao F, Jin H, Chen YJ, Dong YY, Liang YC, Shi NM, Ge HM, Liu L, Chen SG, Ai X, Zhang ZY, Ji YG, Luo FJ, Chen XQ, Zhang Y, Zhu LW, Liang ZL, Shen XL (2013) Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 381:2024–2032
31. Xing W, Liao Q, Viboud C, Zhang J, Sun J, Wu JT, Chang Z, Liu F, Fang VJ, Zheng Y, Cowling BJ, Varma JK, Farrar JJ, Leung GM, Yu H (2014) Hand, foot, and mouth disease in China, 2008-12: an epidemiological study. Lancet Infect Dis 14:308–318
32. Carrat F, Flahault A (2007) Influenza vaccine: the challenge of antigenic drift. Vaccine 25:6852–6862