Observational Study

Medicine®

OPEN

Molecular characteristics of hand, foot, and mouth disease for hospitalized pediatric patients in Yunnan, China

Yilin Zhao, MM[a,b], Haihao Zhang, MM[a,b], Hongbo Liu, MM[a,b], Jie Zhang, MM[a,b], Licun He, MS[a,b], Hao Sun, MD[a,b], Xiaoqin Huang, MM[a,b], Zhaoqing Yang, MD[a,b], Shaohui Ma, MM[a,b,*]

Abstract

Hand, foot, and mouth disease (HFMD) is a common infectious disease caused by multiple enteroviruses (EVs) in China. To better define the etiologic agents and clinical characteristics of HFMD, we conducted this study in Yunnan, China.

In this study, 1280 stool specimens were collected from pediatric patients hospitalized for treatment of HFMD in 2010. EV was detected with nested reverse transcription polymerase chain reaction and directly genotyped by gene sequencing of the viral protein 1 (VP1) region. Phylogenetic analysis was performed based on the VP1 partial gene and the clinical characteristics were analyzed using SPSS Software.

Of 1280 specimens, 1115 (87.1%) tested positive for EV. Seventeen different EV serotypes were detected. Coxsackievirus A16 (CA16) was the most frequently detected serotype (615/1115 cases, 55.1%), followed by enterovirus 71 (EV71; 392/1115, 35.2%), CA10 (45/1115, 4.0%), and CA4 (23/1115, 2.1%). Among the 709 severe cases, CA16, EV71, CA10, and CA4 accounted for 48.0%, 42.0%, 3.5%, and 2.3%, respectively. Of the 26 critical cases, 13 were caused by EV71, 9 by CA16, 2 by CA4, and 1 each were the result of CA10 and E9, respectively. All EV71, CA16, CA10, and CA4 isolates were highly homologous to the strains isolated from mainland China, and belonged to the C4a, B1a, G, and C genotypes, respectively.

Our study showed that EV71 and CA16 were the main causative agents for severe and critical HFMD, but other serotypes can also cause severe and critical cases.

Abbreviations: CA16 = coxsackievirus A16, EV = *Enterovirus*, EV71 = enterovirus 71, HFMD = hand, foot, and mouth disease, PCR = polymerase chain reaction, VP1 = viral protein 1.

Keywords: clinical features, *Enterovirus*, hand, foot, and mouth disease, pathogen, serotyping

1. Introduction

Hand, foot, and mouth disease (HFMD) is a common infectious disease in China, which mainly affects children younger than 5 years.[1–5] The symptoms are generally mild and self-limiting. A few patients may develop severe complications, including encephalitis, pneumonia, myocarditis, brain-stem encephalitis, and acute flaccid paralysis.[6,7]

HFMD is caused by more than 20 different enteroviruses (EVs), particularly enterovirus 71 (EV71) and coxsackievirus A16 (CA16). Other EVs including CA2, 4, 5, 6, 8, and 10, CB1–5, also result in HFMD pathogenesis with diverse clinical manifestations.[4,8,9] EV comprises more than 100 serotypes, all of which belong to the genus *Enterovirus* and the family *Picornavirus*. These viruses are characterized by a single positive-strand 7.4 kb genomic RNA with a long open reading frame that encodes 4 structural proteins [viral protein 1 (VP1) through VP4] and 7 nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D). VP1 is the immunodominant protein of the picornavirus capsid and contains serotype-specific information. Thus, the VP1 has been used for virus serotype identification and molecular epidemiology.[5,10,11]

Since 2008, more than 1 million cases have been reported annually in mainland China, with more than 100 deaths per year. Yunnan is a major tourism province located in Southwest China, with a typical subtropical plateau monsoon climate. More than 80,000 cases presenting with HFMD are reported each year. It, however, remains unclear whether the epidemic of HFMD in Yunnan is like that in the rest of Southwest China. Here we report the etiology and clinical characteristics of hospitalized children with HFMD in Yunnan, China. These results help further understand the molecular epidemiological features of HFMD and EV infection in Southwest China.

Editor: Bernhard Schaller.

This work was supported by the Basic Research Projects of Yunnan Province, China (Grant Number: 2017FA006) and Chinese Academy of Medical Sciences (CAMS) Initiative for Innovative Medicine (2016-I2M-3-026).

Ethical approval: All experimental protocols involving human samples were approved by the Human Ethics Commission at the Institute of Medical Biology, Chinese Academy of Medical Sciences.

The authors have no conflicts of interest to disclose.

[a] Institute of Medical Biology, Chinese Academy of Medical Sciences, Peking Union Medical College, [b] Yunnan Key Laboratory of Vaccine Research Development on Severe Infectious Disease, Kunming, Yunnan, PR China.

[*] Correspondence: Shaohui Ma, Institute of Medical Biology, Chinese Academy of Medical Sciences, Peking Union Medical College, 935 Jiao Ling Road, Kunming, Yunnan Province 650118, PR China (e-mail: shaohuima70@hotmail.com).

Medicine (2018) 97:31(e11610)

Received: 2 August 2017 / Accepted: 28 June 2018

http://dx.doi.org/10.1097/MD.0000000000011610

2. Subjects and methods

2.1. Subjects and sample collection

From January 1 to December 31, 2010, 1280 stool specimens were collected from children admitted with HFMD to a pediatric hospital in Yunnan, China. Stool samples were collected during the acute phase of the illness and sent to our laboratory within 24 hours. During this time, the clinical data of all patients were gathered including age, sex, and clinical symptoms. All stool samples were stored at −80°C. This work was supported by the Basic Research Projects of Yunnan Province, China and Chinese Academy of Medical Sciences Initiative for Innovative Medicine.

2.2. RNA extraction and amplification

To identify the EV serotypes associated with HFMD, a nested polymerase chain reaction (PCR) was performed as previously reported.[11] Briefly, 1 g of stool was suspended in 5 mL of phosphate-buffered saline, and the suspensions were centrifuged at 3000 × *g* for 30 minutes at 4°C. The supernatant was collected and stored at −80°C. Viral RNA was extracted using a QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA) as per the manufacturer's instructions. The partial VP1 gene sequences of EV were amplified by nested reverse transcription PCR, using PrimeScript One Step RT-PCR Kit Ver.2 (TakaRa, Dalian, China) and 2×TSINGKE Master Mix (TSINGKE, Beijing, China) as described previously.[12]

2.3. Sequencing and molecular typing

All positive PCR products were sequenced directly using an ABI 3730XL automatic sequencer (Applied Biosystems, Foster City, CA) and all sequences were analyzed using the Enterovirus Genotyping Tool for serotyping.[13]

2.4. Statistical analyses

All statistical analyses were performed using the Statistic Package for Social Science (SPSS) 20.0 software (IBM, Ammonk, N.Y.). The data were statistically analyzed by chi-square test and *t* test, and the statistical significance was defined as $P < .05$.

2.5. Phylogenetic analyses

Phylogenetic analyses of the 4 predominant serotypes EV71, CA16, CA10, and CA4 were performed using Molecular Evolutionary Genetic Analysis (MEGA) version 7.0 software (http://www.megasoftware.net/) applying a neighbor-joining method with 1000 duplicates. Reference sequences were obtained from GenBank.

The sequences of the EV described in this study were deposited in the GenBank database under the accession number: MF150562-MF150639.

3. Results

Among the 1280 patients, 541 (42.27%) were general patients, 709 (55.39%) had severe clinical manifestations, 26 (2.03%) were critically ill, and 4 (0.31%) died. Patients' age ranged between 4 months and 13 years; the mean age was 2.60 ± 1.41 years. The sex ratio was 1.66:1, with 799 boys and 481 girls. The median hospital stay was 8.96 days. Although HFMD cases occurred during the year, a seasonal increase in HFMD cases was observed in May, accounting for 20.7% of all cases, and there was a small increase in November, with the greatest number of severe cases occurring in June (Fig. 1).

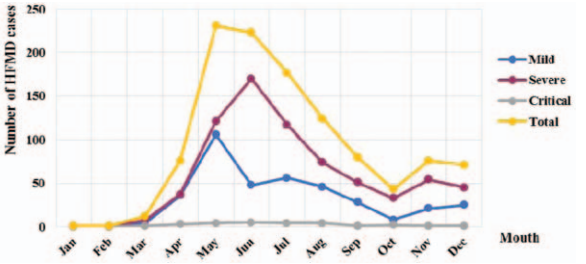

Figure 1. The temporal distribution of the mild, severe, and critical cases identified in HFMD patients in Yunnan, China, 2010. HFMD = hand, foot, and mouth disease.

The examination of stool specimens from these 1280 patients revealed the presence of EV in 1115 cases (87.1%). Among these 1115 patients, there were 376 general patients, 709 severely ill patients, 26 critically ill patients, and 4 deaths. Further VP1 sequencing and molecular typing showed that all sequences obtained were assigned to 17 different EV serotypes (Table 1). CA16 was the most frequently detected serotype (615/1115, 55.1%), followed by EV71 (392/1115, 35.2%), CA10 (45/1115, 4.0%), and CA4 (23/1115, 2.1%). Among the 709 severe cases, CA16, EV71, CA10, and CA4 accounted for 48.0%, 42.0%, 3.5%, and 2.3%, respectively ($P < .0001$). In addition, another 13 EVs also resulted in severe clinical manifestations. Of the 26 critical cases, 13 were caused by EV71, 9 by CA16, 2 by CA4, and 1 each were the result of CA10 and E9. Two deaths were the result of EV71 infection, whereas CA16 and CB2 caused 1 death each.

EV71 infection more often results in meningoencephalitis and hyperarousal ($P < .0001$), whereas CA4 is more likely to cause fever, vomiting, and convulsion. For CA10, the male/female ratio of patients was highest, where it reached 2.21:1. Moreover, the 26 critical cases frequently showed coma and/or convulsions (Table 2).

Phylogenetic analyses showed that EV71 viruses belonged to the C4a subgenotype, CA16 viruses belonged to the B1a genotype, CA10 viruses belonged to the G genotype, and CA4 viruses were assigned to the C genotype (Fig. 2), and the EV71, CA16, CA10, and CA4 strains were highly homologous with the strains isolated from mainland China.

4. Discussion

In this study, the epidemiological characteristics of the HFMD epidemic in Yunnan province of China showed some similarities with previous reports.[2,6,14] The occurrence of HFMD had apparent seasonal distribution in Yunnan, China. The number of HFMD cases reached a maximum in May; we also observed a small increase in November, which is a common phenomenon in the southern provinces.[3,15–17] This may be due to the different climate conditions between the southern and northern provinces of China.[4,15,16] In addition, the mean age of all hospitalized pediatric patients was 2.60 ± 1.41 years old and 76.87% of all reported HFMD severe and critical cases were younger than 3 years old, which is also consistent with the reports from other countries and regions.[18–21] Most patients with HFMD need to be transferred to a comprehensive hospital, in which patients

Table 1

The pathogens identified in patients with hand-foot-mouth disease in Yunnan, China, 2010.

No. (%) cases Serotype	Mild case n = 541	Severe case n = 709	Critical case n = 26	Death case n = 4	Total n = 1280
EV71	79 (14.60)	298 (42.03)	13 (50.00)	2 (50.00)	392 (30.63)
CA16	265 (48.99)	340 (47.95)	9 (34.63)	1 (25.00)	615 (48.05)
CA10	19 (3.51)	25 (3.53)	1 (3.84)	0 (0.00)	45 (3.52)
CA4	5 (0.92)	16 (2.26)	2 (7.69)	0 (0.00)	23 (1.80)
CA2	0 (0.00)	1 (0.14)	0 (0.00)	0 (0.00)	1 (0.08)
CA5	3 (0.56)	6 (0.85)	0 (0.00)	0 (0.00)	9 (0.70)
CA6	1 (0.18)	5 (0.71)	0 (0.00)	0 (0.00)	6 (0.47)
CA8	2 (0.37)	5 (0.71)	0 (0.00)	0 (0.00)	7 (0.54)
CA12	0 (0.00)	2 (0.28)	0 (0.00)	0 (0.00)	2 (0.15)
CA13	0 (0.00)	1 (0.14)	0 (0.00)	0 (0.00)	1 (0.08)
CA24	0 (0.00)	1 (0.14)	0 (0.00)	0 (0.00)	1 (0.08)
CB2	0 (0.00)	1 (0.14)	0 (0.00)	1 (25.00)	2 (0.15)
CB4	0 (0.00)	1 (0.14)	0 (0.00)	0 (0.00)	1 (0.08)
E9	0 (0.00)	3 (0.42)	1 (3.84)	0 (0.00)	4 (0.31)
E11	0 (0.00)	1 (0.14)	0 (0.00)	0 (0.00)	1 (0.08)
E16	2 (0.37)	2 (0.28)	0 (0.00)	0 (0.00)	4 (0.31)
E30	0 (0.00)	1 (0.14)	0 (0.00)	0 (0.00)	1 (0.08)
Total infection	376 (69.50)	709 (100.00)	26 (100.00)	4 (100.00)	1115 (87.11)
Nonenterovirus	165 (30.50)	0 (0.00)	0 (0.00)	0 (0.00)	165 (12.89)

CA16 = coxsackievirus A16, EV71 = enterovirus 71.

with HFMD might be timeously diagnosed and treated, and thus decreasing the risk of severe HFMD.[22,23] Thus, these pediatric patients should be targeted as the main population in any prevention program.

In addition to EV71 and CA16, other EVs, including CA4, CA6, CA12, and CB3, are also associated with HFMD and cocirculated in outbreaks and sporadic cases.[14,16,19,20,24] In the present study, we identified 17 serotypes of EVs, including EV71, CA16, CA10, CA4, CA2, CA5, CA6, CA8, CA12, CA13, CA24, CB2, CB4, E9, E11, E16, and E30, from stool samples of patients hospitalized for HFMD in Yunnan, China. Of these, CA16 was the most frequently detected serotype, followed by EV71, CA10, and CA4. However, in Yunnan, during 2016, 11 serotypes were identified from hospitalized patients with HFMD with serious symptoms. Of these serotypes EV71 was the most frequently identified, followed by CA16, CA6, and CA10 (data not shown). In Suzhou, EV71 was the most frequently detected serotype, followed by CA16 in the period from January 2008 to December 2013.[25] From May 2010 to April 2011 in Shanghai, EV71 was predominant, followed by CA10, CA6, and CA16 in admitted patients with HFMD.[26] In Beijing, the most commonly detected serotype from hospitalized children was CA6, followed by EV71 in 2013.[27] Since 2013, CA6 has displaced EV71 and CA16 to become the prevalent pathogenic serotype in HFMD cases in some provinces of China and elsewhere.[28–30] The prevalent serotypes were, however, different in hospitalized and not hospitalized children.[31] In addition, the causative agents of HFMD often overlap with those of aseptic meningitis including CB3, CB5, E9, and E30, which are often associated with outbreaks of aseptic meningitis in China.[32–37] This is not unexpected as EVs have long been identified as the main agents of aseptic meningitis.[12,38–41] Thus, it is possible that some of these serotypes may become predominant in HFMD in the future and we should pay special attention to the EVs associated with aseptic meningitis.

EV71 has been identified as the most prevalent serotype causing severe and critical cases of HFMD, and CA16 is thought to be associated with mild HFMD and self-limited symptoms. There have been reports of CA16, CA10, and CA4 being associated with severe HFMD independently of EV71.[5,29,30,42] In the present study, we found that CA16 was the prevalent serotype causing severe and critical cases of HFMD, rather than EV71, this kind of preference may be limited to specific populations. In addition, other serotypes including CA4, CA10, and E9, were also associated with severe and critical cases. In particular, CB2 and CA16 had a strong association with fatal cases. It, however, remains difficult to distinguish between these serotypes in the clinical context. Long-term attention should also be paid to preventing infections with serotypes other than EV71 and CA16 in the future. In addition, for the age distribution of the children with HFMD, there were no significant difference among the positive rate of EV71, CA16, CA10, and CA4. As for disease severity across the EV strains and symptoms in different age groups, there was also no statistically significance. We, however, found that fever (>38°C), oral ulcer, and hand and foot rash were the most frequent symptoms for hospitalized pediatric patients. And limb sharking, startle, and vomiting were the most frequent severe symptoms for children aged 1 to 2 years. Meningoencephalitis was frequently observed in patients infected by EV71. Thus, the duration of fever 3 days (>38°C), EV71 infection, limb sharking, startle, and vomiting are risk factors for severe HFMD, which is also consistent with the previous report.[43]

When the VP1 sequences from the predominant EV71, CA16, CA4, and CA6 strains isolated from other provinces of China where compared, there were no characteristic mutations and all Yunnan isolates belonged to the corresponding predominant genogroups, respectively. This indicated that those predominant genogroups are relatively stable and could form the basis for preventative programs in China.

Thus, surveillance of the etiology might help with predicting potential outbreak serotypes and aid in effectively developing related vaccines, except the EV71 vaccine. In addition to the host and natural factors, including young age and a confirmed

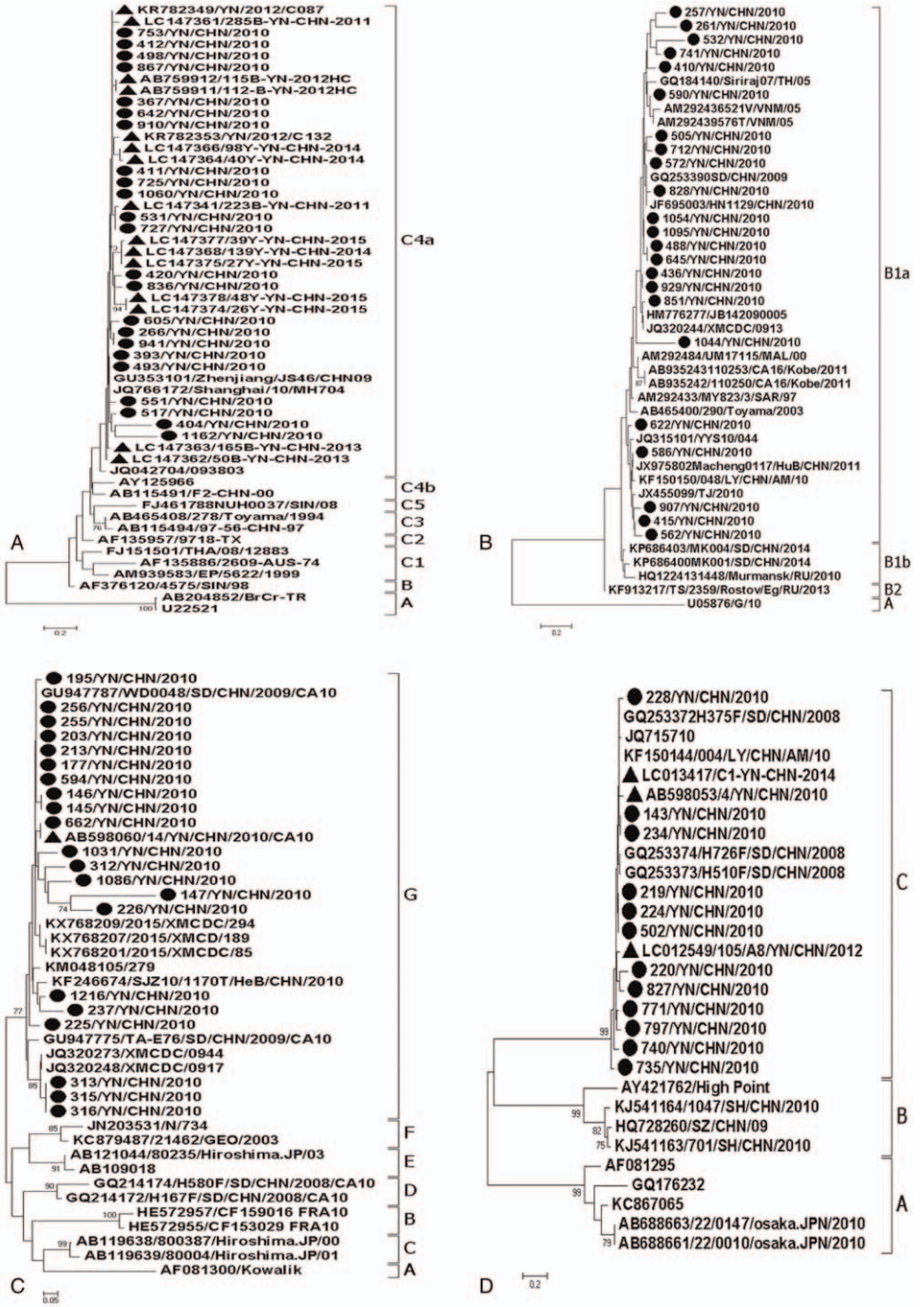

Figure 2. Phylogenetic trees of human enterovirus 71 (EV71), coxsackievirus A16 (CA16), CA10, and CA4 based on the partial viral protein 1 (VP1) sequences by the neighbor-joining algorithm implemented in Molecular Evolutionary Genetic Analysis (MEGA; version 7.0) using the Kimura 2-parameter substitution model and 1000 bootstrap pseudoreplicates, respectively. A, EV71. B, CA16. C, CA10. D, CA4. Only strong bootstrap values (>75%) are shown. • indicates strains isolated in this investigation, ▲ indicates strains isolated in other studies in Yunnan.

diagnosis at first visit to hospital, virus factors may also be responsible for the different clinical phenotypes observed in hospitals in China.[22,43]

Acknowledgments

The authors thank Zengqing Du from the department of infectious diseases of Kunming Children's Hospital, China for sample collection.

Author contributions

Conceptualization: Hongbo Liu.
Data curation: Hongbo Liu, Jie Zhang.
Funding acquisition: Zhaoqing Yang, Shaohui Ma.
Investigation: Yilin Zhao, Haihao Zhang, Jie Zhang, Licun He.
Project administration: Xiaoqin Huang.
Software: Haihao Zhang, Hao Sun.
Writing – original draft: Yilin Zhao.
Writing – review and editing: Shaohui Ma.

Table 2

Comparison of the demographic and the clinical data from patients infected with different enteroviruses.

	EV71	CA16	CA10	CA4	*P*
No. positive cases	392	615	45	23	
No. severe cases	298 (76.0)	340 (55.3)	25 (55.6)	16 (69.6)	<.0001
No. critical case	13 (3.3)	9 (1.5)	1 (2.2)	2 (8.7)	.047
Age (years)	2.70±1.51	2.50±1.26	2.39±1.22	2.51±1.26	.057
Male/female ratio	1.58	1.67	2.21	1.86	.771
Fever (>38°C)	365 (93.1)	523 (85.0)	40 (88.9)	22 (95.7)	.001
Oral ulcer	382 (97.4)	590 (95.3)	42 (93.3)	19 (82.6)	.010
Hand and foot rash	368 (93.9)	582 (94.6)	41 (91.1)	19 (82.6)	.091
Meningoencephalitis	284 (72.4)	277 (45.0)	14 (31.1)	11 (47.8)	<.0001
Startle	199 (50.8)	222 (36.1)	16 (35.6)	9 (39.1)	<.0001
Decreased mental status	59 (15.1)	59 (9.6)	9 (20.0)	4 (17.4)	.013
Coma	9 (2.3)	5 (0.8)	1 (2.2)	1 (4.3)	.064
Limb sharking	103 (26.3)	84 (13.7)	5 (11.1)	3 (13.0)	<.0001
Vomiting	79 (20.2)	84 (13.7)	6 (13.3)	6 (26.1)	.022
Headache	47 (16.1)	48 (7.8)	0 (0)	1 (4.3)	.010
Convulsion	13 (3.3)	9 (1.5)	1 (2.2)	3 (13.0)	.009
Death	2 (0.5)	1 (0.2)	0	0	

CA16 = coxsackievirus A16, EV71 = enterovirus 71.

References

[1] Wang Z, Lv H, Zhu W, et al. Epidemiologic features of enterovirus 71-associated hand-foot-and-mouth disease from 2009 to 2013 in Zhejiang, China. Int J Environ Res Public Health 2017;14:pii: E33.

[2] Weng Y, Chen W, Huang M, et al. Epidemiology and etiology of hand, foot, and mouth disease in Fujian province, 2008–2014. Arch Virol 2016;162:1–8.

[3] Xia Y, Shan J, Ji H, et al. Study of the epidemiology and etiological characteristics of hand, foot, and mouth disease in Suzhou City, East China, 2011–2014. Arch Virol 2016;161:1933–43.

[4] Zhuang ZC, Kou ZQ, Bai YJ, et al. Epidemiological research on hand, foot, and mouth disease in Mainland China. Viruses 2015;7:6400–11.

[5] Huang Y, Zhou Y, Lu H, et al. Characterization of severe hand, foot, and mouth disease in Shenzhen, China, 2009–2013. J Med Virol 2015;87: 1471–9.

[6] Ni H, Yi B, Yin J, et al. Epidemiological and etiological characteristics of hand, foot, and mouth disease in Ningbo, China, 2008–2011. J Clin Virol 2012;54:342–8.

[7] Nasri D, Bouslama L, Pillet S, et al. Basic rationale, current methods and future directions for molecular typing of human enterovirus. Expert Rev Mol Diagn 2007;7:419–34.

[8] Klein M, Chong P. Is a multivalent hand, foot, and mouth disease vaccine feasible? Hum Vaccin Immunother 2015;11:2688–704.

[9] Guan H, Wang J, Wang C, et al. Etiology of multiple non-EV71 and non-CVA16 enteroviruses associated with hand, foot and mouth disease in Jinan, China, 2009–June 2013. PLoS One 2015;10:e0142733.

[10] Bruu AL. Enteroviruses: Polioviruses, Coxsackieviruses, Echoviruses and Newer Enteroviruses. A Practical Guide to Clinical Virology, Second Edition. John Wiley & Sons, Ltd, 2007: 44–45.

[11] Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol 2006; 44:2698–704.

[12] Ma S, Zhang Y, Du C, et al. Dynamic constitution of the pathogens inducing encephalitis in hand, foot and mouth disease in Kunming, 2009–2011. Jpn J Infect Dis 2015;68:504–10.

[13] Kroneman A, Vennema H, Deforche K, et al. An automated genotyping tool for enteroviruses and noroviruses. J Clin Virol 2011;51:121.

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

[15] Deng T, Huang Y, Yu S, et al. Spatial-temporal clusters and risk factors of hand, foot, and mouth disease at the district level in Guangdong Province, China. PLoS One 2013;8:e56943.

[16] Zhang W, Huang B, She C, et al. An epidemic analysis of hand, foot, and mouth disease in Zunyi, China between 2012 and 2014. Saudi Med J 2015;36:593–8.

[17] Zou XN, Zhang XZ, Wang B, et al. Etiologic and epidemiologic analysis of hand, foot, and mouth disease in Guangzhou city: a review of 4,753 cases. Braz J Infect Dis 2012;16:457–65.

[18] Chan KP, Goh KT, Chong CY, et al. Foot and mouth disease caused by human enterovirus 71, Singapore. Emerg Infect Dis 2003;9:78–85.

[19] Nguyen NT, Pham HV, Hoang CQ, et al. Epidemiological and clinical characteristics of children who died from hand, foot and mouth disease in Vietnam, 2011. BMC Infect Dis 2014;14:341.

[20] Kwisung P, Baeckhee L, Kyoungah B, et al. Enteroviruses isolated from herpangina and hand-foot-and-mouth disease in Korean children. Virol J 2012;9:205.

[21] Tian H, Zhang Y, Shi Y, et al. Epidemiological and aetiological characteristics of hand, foot, and mouth disease in Shijiazhuang City, Hebei province, China, 2009–2012. PLoS One 2017;12:e0176604.

[22] Song CL, Cheng YB, Chen D, et al. Risk factors for death in children with severe hand, foot and mouth disease [in Chinese]. Zhongguo Dang Dai Er Ke Za Zhi 2014;16:1033–6.

[23] Yang S, Li P, Li Y, et al. Clinical manifestations of severe enterovirus 71 infection and early assessment in a Southern China population. BMC Infect Dis 2017;17:153.

[24] Xu M, Su L, Cao L, et al. Genotypes of the enterovirus causing hand foot and mouth disease in Shanghai, China, 2012–2013. PLoS One 2015;10: e0138514.

[25] Chen Z, Sun H, Yan Y, et al. Epidemiological profiles of hand, foot, and mouth disease, including meteorological factors, in Suzhou, China. Arch Virol 2015;160:315–21.

[26] Xu M, Su L, Cao L, et al. Enterovirus genotypes causing hand foot and mouth disease in Shanghai, China: a molecular epidemiological analysis. BMC Infect Dis 2013;13:489.

[27] Gu H, Liu Z, Zhang L, et al. The etiological and clinical characteristics of hospitalized children with hand, foot and mouth disease in Beijing in 2013 [in Chinese]. Zhonghua Er Ke Za Zhi 2015;53:459–63.

[28] He YQ, Chen L, Xu WB, et al. Emergence, circulation, and spatiotemporal phylogenetic analysis of coxsackievirus A6- and coxsackievirus A10-associated hand, foot, and mouth disease infections from 2008 to 2012 in Shenzhen, China. J Clin Microbiol 2013;51: 3560–6.

[29] Zeng H, Lu J, Zheng H, et al. The epidemiological study of coxsackievirus A6 revealing hand, foot and mouth disease epidemic patterns in Guangdong, China. Sci Rep 2015;5:10550.

[30] Yang Q, Ding J, Cao J, et al. 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;87:954–60.

[31] Wang J, Teng Z, Cui X, et al. Epidemiological and serological surveillance of hand-foot-and-mouth disease in Shanghai, China, 2012–2016. Emerg Microbes Infect 2018;7:8.

[32] Zheng S, Ye H, Yan J, et al. Laboratory diagnosis and genetic analysis of a family clustering outbreak of aseptic meningitis due to echovirus 30. Pathog Glob Health 2016;110:233–7.
[33] Zhang W, Lin X, Jiang P, et al. Complete genome sequence of a coxsackievirus B3 recombinant isolated from an aseptic meningitis outbreak in eastern China. Arch Virol 2016;161:2335–42.
[34] Lee CJ, Huang YC, Yang S, et al. Clinical features of coxsackievirus A4, B3 and B4 infections in children. PLoS One 2014;9:e87391.
[35] Liu N, Jia L, Yin J, et al. An outbreak of aseptic meningitis caused by a distinct lineage of coxsackievirus B5 in China. Int J Infect Dis 2014;23:101–4.
[36] Golitsyna LN, Fomina SG, Novikova NA, et al. Molecular genetic echovirus 9 variants identified in patients with aseptic meningitis in Russia in 2007–2009 [in Russian]. Vopr Virusol 2011;56:37–42.
[37] Milia MG, Cerutti F, Gregori G, et al. Recent outbreak of aseptic meningitis in Italy due to Echovirus 30 and phylogenetic relationship with other European circulating strains. J Clin Virol 2013;58:579–83.
[38] Rasti M, Samarbafzadeh AR, Makvandi M, et al. Relative frequency of enteroviruses in children with aseptic meningitis referred to Aboozar Hospital in Ahvaz. Jundishapur J Microbiol 2012;5:456–9.
[39] Palacios G, Oberste MS. Enteroviruses as agents of emerging infectious diseases. J Neurovirol 2005;11:424–33.
[40] Cabrerizo M. Importance of enteroviruses in neuropaediatrics: from polioviruses to other enteroviruses [in Spanish]. Rev Neurol 2017;64: S35–8.
[41] Cordey S, Schibler M, L'Huillier AG, et al. Comparative analysis of viral shedding in pediatric and adult subjects with central nervous system-associated enterovirus infections from 2013 to 2015 in Switzerland. J Clin Virol 2017;89:22–9.
[42] Li JS, Dong XG, Qin M, et al. Outbreak of febrile illness caused by coxsackievirus A4 in a nursery school in Beijing, China. Virol J 2015;12:92.
[43] Fang Y, Wang S, Zhang L, et al. Risk factors of severe hand, foot and mouth disease: a meta-analysis. Scand J Infect Dis 2014;46:515–22.