Circulation of Coxsackievirus A10 and A6 in Hand-Foot-Mouth Disease in China, 2009–2011

Qing-Bin Lu1,2,*, Xiao-Ai Zhang2,*, Ying Wo2, Hong-Mei Xu3, Xiu-Jun Li1, Xian-Jun Wang4, Shu-Jun Ding4, Xiao-Dan Chen2, Cui He2, Li-Juan Liu2, Hao Li2, Hong Yang2, Ting-Yu Li3, Wei Liu2*, Wu-Chun Cao2*

1 Department of Epidemiology and Health Statistics, Shandong University, Jinan, People’s Republic of China, 2 State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, People’s Republic of China, 3 Children’s Hospital, Chongqing Medical University, Chongqing, People’s Republic of China, 4 Department of Infectious Disease Control, Shandong Provincial Disease Prevention and Control Center, Jinan, People’s Republic of China

Abstract

Coxsackieviruses A10 (CV-A10) and A6 (CV-A6) have been associated with increasingly occurred sporadic hand-foot-mouth disease (HFMD) cases and outbreak events globally. However, our understanding of epidemiological and genetic characteristics of these new agents remains far from complete. This study was to explore the circulation of CV-A10 and CV-A6 in HFMD and their genetic characteristics in China. A hospital based surveillance was performed in three heavily inflicted regions with HFMD from March 2009 to August 2011. Feces samples were collected from children with clinical diagnosis of HFMD. The detection and genotyping of enteroviruses was performed by real-time PCR and sequencing of 5’ UTR/VP1 regions. Phylogenetic analysis and selection pressure were performed based on the VP1 sequences. Logistic regression model was used to identify the effect of predominant enterovirus serotypes in causing severe HFMD. The results showed 92.0% of 1748 feces samples were detected positive for enterovirus, with the most frequently presented serotypes as EV-71 (944, 54.0%) and CV-A16 (451, 25.8%). CV-A10 and CV-A6 were detected as a sole pathogen in 82 (4.7%) and 44 (2.5%) cases, respectively. Infection with CV-A10 and EV-71 were independently associated with high risk of severe HFMD (OR = 2.66, 95% CI: 1.40–5.06; OR = 4.81, 95% CI: 3.07–7.53), when adjusted for age and sex. Phylogenetic analysis revealed that distinct geographic and temporal origins correlated with the gene clusters based on VP1 sequences. An overall ν value of the VP1 was 0.046 for CV-A10 and 0.047 for CV-A6, and no positively selected site was detected in VP1 of both CV-A10 and CV-A6, indicating that purifying selection shaped the evolution of CV-A10 and CV-A6. Our study demonstrates variety of enterovirus genotypes as viral pathogens in causing HFMD in China. CV-A10 and CV-A6 were co-circulating together with EV-71 and CV-A16 in recent years. CV-A10 infection might also be independently associated with severe HFMD.

Citation: Lu Q-B, Zhang X-A, Wo Y, Xu H-M, Li X-J, et al. (2012) Circulation of Coxsackievirus A10 and A6 in Hand-Foot-Mouth Disease in China, 2009–2011. PLoS ONE 7(12): e52073. doi:10.1371/journal.pone.0052073

Editor: Chiyu Zhang, Institut Pasteur of Shanghai, Chinese Academy of Sciences, China

Received September 14, 2012; Accepted November 8, 2012; Published December 18, 2012

Copyright: © 2012 Lu et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported by the grants of China Mega-Project on Infectious Disease Prevention (2009ZX10004-204), National Science Fund for Distinguished Young Scholars (30725032) and Shandong Scientific and Technical Supporting Program (2009GG10002055). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: caowc@nic.bmi.ac.cn (WCC); liu_weis@sohu.com (WL)
† These authors contributed equally to this work.

Introduction

Hand-foot-mouth disease (HFMD) is a common disease characterized with fever, sore throat, general malaise and vesicular eruptions on hand, feet, oral mucosa and tongue. Since 1997, several large epidemics of HFMD have been reported in the Asia-Pacific region, especially in Southeast Asia. Although HFMD is classically a mild disease, outbreaks in Asia have been associated with a high incidence of fatal cardiopulmonary and neurologic complications. HFMD has now become a notifiable disease in many countries [1].

Historically, outbreaks of HFMD were mainly caused by two types of enterovirus A species, enterovirus 71 (EV-71) and coxsackievirus A16 (CV-A16), with differing ratios. In recent years, coxsackieviruses A10 (CV-A10) and A6 (CV-A6), in addition to EV-71 and CV-A16, have been associated with increasingly occurred sporadic HFMD cases and outbreak events globally [2,3,4,5,6,7,8,9,10,11]. In the largest outbreak of HFMD in Singapore in 2008, the most prevalent virus serotypes were demonstrated to be CV-A6 and CV-A10, accounting for 35.3% of the detected cases [3]. Large outbreaks of HFMD were reported to be caused by the co-circulating of CV-A10 and CV-A6 in Finland [11]. A sentinel surveillance study performed in France found CV-A10 and CV-A6 to be the most predominant HEV serotypes, which were also responsible for the outbreak events in 2010 [12]. One study performed in India in 2012 documented CV-A16 and CV-A6 as major, while CV-A10 and EV-71 as rare viral pathogens of HFMD [10]. One onychomadesis outbreak that occurred in 2008 in Spain was demonstrated to be associated with an outbreak of HFMD primarily caused by CV-A10 [5]. During 2008, an outbreak of HFMD with onychomadesis as a common feature occurred in Finland was identified to be caused by CV-A6 [2]. CV-A6, as the main serotype, also caused outbreaks of HFMD in Taiwan, 2010 [8] and in Japan, 2010 [9]. All of these previous studies provided strong evidence of CV-A6 and CV-A10 infections as new and important causes of HFMD.
of HFMD, thus highlighting the necessity of comprehensive surveillance of all HEVs circulation in HFMD epidemics.

In China, there have been large outbreaks of HFMD every year in the past 3 years, each involving more than 500,000 cases, with an increasing number of neurologic symptoms and deaths reported. HFMD has become an important public health concern in China mainland. According to previous surveillance, EV-71 and CV-A16 have co-circulated as two most frequent HEV types in causing repeated HFMD outbreak in different areas [13,14,15,16]. A few studies have also attempted to clarify the roles of other enteroviruses types, and identified only minor roles of CV-A10 and CV-A6 in China [15,17]. Since most of the studies were performed before 2009 based on a small sample size, we have herein broadened these analyses to include more regions and over a longer time span in order to provide a more comprehensive overview of the viral pathogens of HFMD, with an emphasis to explore the prevalence of CV-A10 and CV-A6 in causing HFMD, as well as their epidemiological, clinical and genetic characteristics.

Materials and Methods

Sample Collection

The sentinel surveillance was performed from March 2009 to August 2011. The children suffering HFMD in three pediatric hospitals, which were set as the sentinel sites under national surveillance program for HFMD in Chongqing municipality (southwest China), Henan (central China) and Shandong province (east China) were recruited into the study. The patients were identified according to the diagnostic criteria defined as follows: maculopapular of vesicular rash on the palms and/or soles and vesicles or ulcers in the mouth. Children with serious complications, including encephalitis, meningitis, acute flaccid paralysis, cardiorespiratory failure or death, were considered as severe HFMD. By the standard criteria in different hospitals, meningitis was defined as pleocytosis in cerebrospinal fluid analysis, encephalitis was characterized by the presence of altered level of consciousness, personality changes, or hallucinations. Children diagnosed with HFMD, but without above mentioned serious complications, were classified as mild HFMD.

Medical records of the patients were reviewed by physicians to collect the demographic data, the clinical symptoms and signs, laboratory findings, clinical diagnoses, and outcomes. Written informed consents were acquired from parents or guardians of all participants. The study was approved by the Ethics Review committee of Chongqing Medical University, Jining Infectious hospital and Armed Police Henan Hospital.

Detection and Genotyping of HEV

Feces samples were collected and screened for HEV. Briefly, RNA were extracted from each specimen by using QIAamp® MiniElute Virus Spin Kits (QIAGEN, Hilden, Germany) and the cDNA sample was synthesized using SuperScript® III First-Strand Synthesis System for Reverse Transcription Polymerase Chain Reaction (RT-PCR) (Invitrogen, America).

The detection of HEV and further classification of EV-71 and CV-A16 for HEV-positive samples were performed by real-time PCR using previously described primers, respectively [18]. To further identify the HEV serotypes other than EV-71 and CA-A16, RT-PCR specific for a partial sequence of the 5’UTR was performed for other HEV-positive samples by using previously reported primers [19]. The amplicons were subject for sequencing and BLAST.

Sequence Analysis of CV-A10 and CV-A6

The VPI sequences for CV-A10 and CV-A6 positive samples were amplified by semi-nested PCR using the previously described primers 222, 224, 486, 488, AN88 and AN89 [16,20]. The genomic sequences were assembled using Lasergene’s DNA SeqMan software (version 7.1.0, DNA Star Inc, Madison, WI, USA). The sequences obtained from the study were submitted to NCBI and the GenBank Accession Numbers were JX947632–JX947838. All comparison alignments were performed and phylogenetic tree was constructed by neighbor-joining method with 1000 bootstrap replications using CLC genomics Workbench (version 5.1, developed by CLC bio). Similarities between strains were calculated by using BioEdit (version 7.1.3, www.mbio.ncsu.edu/bioedit/bioedit.html).

Statistical Analysis

Descriptive statistics were performed, with continuous variables summarized as median and range, and categorical variables summarized as frequencies and proportions. Chi-square test/Fisher exact or non-parametric test was used to see the difference between two groups. Logistic regression model was used to identify the association between severe HFMD and the viral pathogens after adjusting for age and sex. All the statistical analyses were conducted by SAS 9.13 (SAS Institute, Cary, North Carolina).

Results

From March 2009 to August 2011, a total of 1748 children were recruited in the study with age ranging from 1 month to 15 years (median: 2 years) and 1125 (64.4%) were male. Altogether 92.0% of the 1748 feces samples were detected to be positive for HEV, with the most frequently presented serotypes as EV-71 (944, 54.0%) and CV-A16 (451, 25.8%). In addition, CV-A10 and CV-A6 were detected as a sole pathogen in 82 (4.7%) and 44 (2.5%) cases, respectively (Figure 1). The detailed distribution of enterovirus types in each province were shown in Figure 2.

The sex and age distribution of HFMD cases studied is shown in Table 1. The median age of CV-A10 positive cases was 23 months (range 7 months to 85 months), which was close to the median age of all HEV positive cases (24 months, range
Figure 1. The enterovirus type identified in hand-foot-mouth diseases patients during Mar 2009 to Aug 2011, China. EV, enterovirus; CV, coxsackievirus; ECV, echovirus. doi:10.1371/journal.pone.0052073.g001

Figure 2. The detailed distribution of enterovirus types in each province. EV, enterovirus; CV, coxsackievirus; ECV, echovirus. doi:10.1371/journal.pone.0052073.g002
The median age of CV-A6 positive cases was 18 months, which was lower than the patients with other infection groups. No significant differences were identified among different enterovirus serotypes in terms of gender distribution. Respiratory and digestive syndromes were evenly distributed among each group, while syndrome of cyclic system and nervous system were significantly overrepresented among the patients infected with EV-71/CV-A10 than in CV-A16/CV-A6.

Similar to EV-71 and CV-A16, the prevalent season for CV-A10 and CV-A6 was warm season from April to August, with the proportion of CV-A10 and CV-A6 among the total enterovirus types attaining peak both in May for CV-A10 (51.2%) and CV-A6 (41.2%), respectively (Figure 3 and Table 2). When compared geographically, the proportions of CV-A10 and CV-A6 were significantly higher in Shandong Province than in other two provinces. When compared temporally, the proportions of CA-10, EV-71 and CV-A16 were different among the three years (Table 2).

There were 725 (41.5%) patients diagnosed as severe HFMD. The risk factors for severe HFMD were evaluated based on the multivariate logistic regression analysis (Table 3). When adjusted for the effect from age and gender, CV-A10 and EV-71 were independently associated with higher risk of severe HFMD (OR = 2.66, 95%CI: 1.40–5.06; OR = 4.81, 95%CI: 3.07–7.53).

Identity analyses based on 5’UTR sequences revealed both CV-A10 and CV-A6 were highly conserved within the same serotype.

Two phylogenetic trees were constructed by the VP1 nucleotide sequences of CV-A10 and CV-A6 from the present study and those downloaded from GenBank, respectively.

| Table 1. The demographic and clinical characteristics of patients with different enteroviruses infection. |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristics                                    | CV-A10 (n = 82) | CV-A6 (n = 44) | EV-71 (n = 944) | CV-A16 (n = 451) | P               |
| Age, months (median, range)                       | 25(7–85)       | 18(10–59)      | 24(2–168)       | 24(1–178)       | 0.128           |
| Sex, male (%)                                     | 54(65.9)       | 27(61.4)       | 598(63.4)       | 311(69.0)       | 0.211           |
| Clinical manifestations                            |                |                |                |                 |                 |
| Respiratory system                                | 17(21.5)       | 7(16.7)        | 198(25.6)       | 94(21.9)        | 0.281           |
| Digestive system                                  | 8(10.1)        | 1(2.4)         | 42(5.5)         | 21(4.9)         | 0.224           |
| Cyclic system                                     | 11(13.9)       | 4(9.5)         | 125(16.3)       | 32(7.5)         | <0.001          |
| Nervous system                                    | 24(29.3)       | 8(18.2)        | 497(52.4)       | 101(22.4)       | <0.001          |
| Outcome                                           |                |                |                |                 |                 |
| Mild                                               | 52(63.4)       | 36(81.8)       | 438(46.4)       | 335(74.3)       |                 |
| Severe                                             | 30(36.6)       | 8(18.2)        | 506(53.6)       | 116(25.7)       |                 |

Note: respiratory system syndromes were defined as the presence of at least one of the followings: cough, bronchitis or other upper respiratory tract disease, or pneumonia; digestive system syndromes were defined as the presence of at least one of the followings: diarrhea or vomit; cyclic system syndromes were defined as the presence of at least one of the followings: myocarditis or cardiac damage; nervous syndromes were defined as the presence of at least one of the followings: meningitis, encephalitis, brain myelitis, coma, acute flaccid paralysis or seizures.

doi:10.1371/journal.pone.0052073.t001

Figure 3. The temporal distribution of HEV types in hand-foot-mouth diseases patients during March 2009 to August 2011, China.
doi:10.1371/journal.pone.0052073.g003
(Figure 4 and Figure 5). The CV-A10 sequences were assigned to ten clusters (A–J) with clear geographical and temporal specific distributions (Figure 4): All Chinese CV-A10 strains were segregated into four distinct branches: Cluster A comprising the strains in this study and other strains from Yunnan, Fujian, and Shandong, China during 2009–2011; Cluster C including strain detected exclusively from Chongqing in 2010; Cluster F with strains from Yunnan, Shandong and Taiwan, China before 2009; Cluster H with one strain from Yunnan, 2008, together with the strains from India. The temporal characteristics were also obvious, with the strains of same geographic origin whereas different sampling time were clustered separately, for example, the Shandong strains isolated in 2010/2011 and 2008 were classified into different clusters (A and F, respectively) Cluster B, includes the 2008 strains from Spain and one 2010 strain from France; Cluster D includes the 2003 strain from Japan; Cluster E includes two 2006 strains from Central Africa Republic; Cluster G mainly includes the 2000/2001 strains from Japan; Cluster I includes the European strains; Cluster J includes one strain from USA.

Global selective pressure was examined on the determinant encoding VP1 for CV-A10 (564 bp, nt 1–564 of VP1) and for CV-A6 (552 bp, nt 1–552 of VP1). Before making the inference of positive selection, recombination should be taken into account. Table 2.

Table 2. The composition of the enteroviruses types in the children with HFMD in each sampling month and region.

| Date       | CV-A10 | CV-A6 | EV-71 | CV-A16 | Other types | Negative | Total |
|------------|--------|-------|-------|--------|-------------|----------|-------|
| n          | %      | n     | %     | n      | %           | n        | n     |
| Mar-09     | 0      | 0.0   | 0     | 0      | 0.0         | 7        | 53.8  |
| Apr-09     | 2      | 1.7   | 1     | 0.9    | 69          | 59.5     | 15    |
| May-09     | 0      | 0.0   | 3     | 5.6    | 21          | 38.9     | 14    |
| Jun-09     | 0      | 0.0   | 1     | 9.1    | 3           | 27.3     | 2     |
| Jul-09     | 0      | 0.0   | 0     | 0.0    | 55          | 43.0     | 60    |
| Aug-09     | 0      | 0.0   | 0     | 0.0    | 4           | 44.4     | 1     |
| Sep-09     | 0      | 0.0   | 0     | 0.0    | 1           | 100.0    | 0     |
| Oct-09     | 0      | 0.0   | 0     | 0.0    | 1           | 100.0    | 0     |
| Nov-09     | 0      | 0.0   | 0     | 0.0    | 22          | 46.8     | 15    |
| Dec-09     | 0      | 0.0   | 1     | 7.1    | 1           | 9        | 64.3  |
| Mar-10     | 0      | 0.0   | 0     | 0.0    | 3           | 75.0     | 1     |
| Apr-10     | 1      | 1.4   | 2     | 2.9    | 56          | 81.2     | 4     |
| May-10     | 4      | 3.1   | 6     | 4.7    | 90          | 69.8     | 12    |
| Jun-10     | 0      | 0.0   | 1     | 2.3    | 36          | 83.7     | 3     |
| Jul-10     | 0      | 0.0   | 0     | 0.0    | 6           | 100.0    | 0     |
| Aug-10     | 0      | 0.0   | 0     | 0.0    | 0           | 0.0      | 0     |
| Year       | 2      | 0.6   | 5     | 1.5    | 160         | 48.2     | 96    |
| Region     |        |       |       |        |             |          |       |
| Chongqing  | 26     | 3.4   | 15    | 1.9    | 446         | 57.9     | 208   |
| Shandong   | 53     | 7.2   | 27    | 3.6    | 318         | 42.9     | 221   |
| Henan      | 3      | 1.3   | 2     | 0.8    | 180         | 75.9     | 22    |

Additional statistics:

P < 0.001

doi:10.1371/journal.pone.0052073.t002

PLOS ONE | www.plosone.org 5 December 2012 | Volume 7 | Issue 12 | e52073
The result of SBP test showed that there was no evidence for recombination in VP1 gene of CV-A10/CV-A6. An overall $v$ value of the VP1 was 0.046 (95% CI: 0.038–0.056) for CV-A10 and 0.047 (95% CI: 0.039–0.057) for CV-A6, indicating that the amino acids in the antigenic determinant were under purifying selection. Site-by-site analyses were performed to determine whether any specific site or residue of the VP1 region evolved under positive selection. No positively selected site was detected from CV-A10 dataset through the methods of SLAC, FEL and REL. Similarly, no positively selected site was detected for CV-A6, and although only one positively selected site (codon site: 12) was detected by SLAC method ($dN$-$dS$ = 24.45, $P \leq 0.001$), this site was not detected by FEL or REL methods. There were no positively selected site to detect by the codon-substitution model using PAML package.

### Discussion

HFMD is a common disease among children, especially under 5 years. HFMD is benign and self-limiting, but severe HFMD with complications are also developed. Numerous major epidemics of HFMD have occurred in eastern and southeastern Asian countries and regions in the past decade, with EV-71 as the most commonly responsible enterovirus type [21,22,23,24]. However, other types, especially CV-A10 and CV-A6, began to co-circulate with increasingly frequencies in recent years, turning into equally common causes of HFMD as CV-16 and EV-71 in certain regions [12,21,25]. In mainland China, the surveillance on HFMD was mostly focused on EV-71 and CV-A16, therefore, information on the pathogenic role of other enteroviruses, their geographic distribution and epidemiological profiles are also still lacking. Our study, based on the latest surveillance in three heavily inflicted regions with HFMD, demonstrated that the previously infrequently detected CV-A10 and CV-A6, are becoming important HFMD pathogens, although the major HFMD pathogens remained to be EV-71 and CV-A16. Therefore, virological surveillance to detect more serotypes concurrently is necessary and physicians should be aware of these emerging pathogens.

### Table 3. Logistic regression analysis on the effect of predominant enterovirus serotypes in severe HFMD.

| Variable       | Unadjusted | Adjusted |
|----------------|------------|----------|
|                | OR 95%CI   | $P$      | OR 95%CI | $P$ |
| Age, months    |            |          |          |     |
| $\leq 12$      | 4.26 2.77–6.54 <0.001 | 4.25 2.72–6.64 <0.001 |
| 12–24          | 2.05 1.41–2.99 <0.001 | 2.08 1.41–3.07 <0.001 |
| 24–36          | 1.78 1.20–2.65 0.004 | 1.85 1.22–2.79 0.004 |
| 36–48          | 2.22 1.45–3.39 <0.001 | 2.16 1.39–3.36 <0.001 |
| $>48$          | 1.0        | 1.0      |          |     |
| Sex, male/female | 0.87 0.71–1.06 0.168 | 0.94 0.75–1.17 0.556 |
| Enterovirus    |            |          |          |     |
| EV-71          | 4.58 2.97–7.07 <0.001 | 4.81 3.07–7.53 <0.001 |
| CV-A16         | 1.37 0.86–2.19 0.182 | 1.51 0.94–2.45 0.092 |
| CV-A10         | 2.29 1.24–4.22 0.008 | 2.66 1.40–5.06 0.003 |
| CV-A6          | 0.88 0.37–2.11 0.776 | 1.20 0.48–3.02 0.700 |

Previous studies demonstrated that EV-71 is more likely to cause serious complications than other enterovirus types and usually leads to meningoencephalitis, pulmonary hemorrhage, circulation failure and death. One case-control study also revealed that EV-71 was significantly associated with an increased risk of severe HFMD (OR = 39.17, 95%CI: 9.80–156.52) [26]. In our study, CV-A10 was demonstrated to be associated with severe complications defined by the same criteria, although with a less effect than that of EV-71. Further
Our study identified distinct clusters of CV-A10 and CV-A6 strains that related to their geographic origins. Moreover, CV-A10 also displayed diverse genetic characteristics regarding their temporal sources. According to previous studies from Yang et al. and Wang et al., CV-A10 analyzed during 2008–2009 in Shandong and during 2009 in Beijing [16,17] formed different clusters with strains from Japan, respectively, therefore displaying clear cut temporal distribution. Hu et al. found natural recombination is a frequent event in human enterovirus A evolution [27]. Based on the current analysis, no evidence of recombination was revealed for VP1 gene of CV-A10. Further evolutionary studies, representing much more geographic locations and genetic information, would help to improve our understanding of its evolutionary relationships. Due to the limited VP1 nucleotide sequences of CV-A6 submitted to GenBank, the temporal characteristic of CV-A6 cannot be inferred, which warrants further investigation in the future.

CV-A10 and CV-A6, both belonging to enterovirus A group, had the similar global ω and no positively selected sites were detected. Our study shows that purifying selection plays an important part in shaping the evolution of CV-A10 and CV-A6. This constrained mutation of the two genotypes can be explained by the limited size and the genetic architecture of the viral genome which is overlapping between structural and functional domains like other viruses [20,29].

In summary, our study demonstrates variety of enterovirus genotypes in the pathogens of HFMD in China based on more than two-year surveillance. CV-A10 and CV-A6 were co-circulating with EV-71 and CV-A16 in recent three years. More wide regional surveillance is warranted to predict their potential in causing outbreak event, as reported from other countries. CV-A10 infection might also be associated with severe HFMD. Its diverse genetic characteristics, as well as distinct geographical distribution were also disclosed. Further genomic analysis and molecular epidemiological data of CV-A10 might help to track the spread of the virus across the country.

Author Contributions
Conceived and designed the experiments: WL WCC. Performed the experiments: QBL XAZ YW XDC CH LJL HL HY. Analyzed the data: QBL XAZ WL WCC. Contributed reagents/materials/analysis tools: HMX XJL XJW SJD TYL. Wrote the paper: QBL XAZ WL WCC.

References
1. Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, et al. (2010) Virology, epidemiology, pathogenesis, and control of enterovirus 71. Lancet Infect Dis 10: 778–790.
2. Østergaard R, Vuorinen T, Linna M, Susi P, Hyypia T, et al. (2009) Coxsackievirus A6 and hand, foot, and mouth disease, Finland. Emerg Infect Dis 15: 1485–1488.
3. Wu Y, Yeo A, Phoon MC, Tan EL, Poh CL, 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: e1076–1081.
4. Bracho MA, González-Candelas F, Valero A, Córdoba J, Salazar A (2011) Enterovirus co-infections and onychomadesis after hand, foot, and mouth disease, Spain, 2008. Emerg Infect Dis 17: 2223–2231.
5. Bracho MA, González-Candelas F, Valero A, Córdoba J, Salazar A (2011) Onychomadesis outbreak in Valencia, Spain associated with hand, foot, and mouth disease caused by enteroviruses. Pediatr Dermatol 28: 1–5.
6. Lo SH, Huang YC, Huang CG, Tsao KC, Li WC, et al. (2011) Clinical and epidemiologic features of Coxsackievirus A6 infection in children in northern Taiwan between 2004 and 2009. J Microbiol Immunol Infect 44: 232–237.
7. Tryfonos C, Richter J, Koptides D, Yiangou M, Christodoulou CG (2011) Molecular typing and epidemiology of enteroviruses in Cyprus, 2003-2007. J Med Microbiol 60: 1433–1440.
8. Wei SH, Huang YP, Liu MC, Tsou TP, Lin HC, et al. (2011) An outbreak of coxsackievirus A6 hand, foot, and mouth disease associated with onychomadesis in Taiwan, 2010. BMC Infect Dis 11: 346.
9. Fujimoto T, Iizuka S, Enomoto M, Abe K, Yamashita K, et al. (2012) Hand, foot, and mouth disease caused by coxsackievirus A6, Japan, 2011. Emerg Infect Dis 18: 337–339.
10. Gopalkrishna V, Patil PR, Patil GP, Chitambar SD (2012) Circulation of multiple enterovirus serotypes causing hand, foot and mouth disease in India. J Med Microbiol 61: 420–425.
11. Blomqvista S, Klemola P, Kaijalainen S, Paananen A, Simonen M-L, et al. (2010) Co-circulation of coxsackieviruses A6 and A10 in hand, foot and mouth disease outbreak in Finland. J Clin Virol 48: 49–54.

12. Mirand A, Henguel C, Archimbaud C, Ughetto S, Antonia D, et al. (2012) 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 18: E110–118.

13. Zhu B, Zhong JY, Xia HM, Gong ST, Xiao MS, et al. (2010) Etiology of hand, foot and mouth disease in Guangzhou in 2008. Zhonghua Er Ke Za Zhi 48: 127–130.

14. Wang ZG, Liu XL, Yang TT, Yi Y (2011) Etiology of hand, foot and mouth disease in Qingdao during 2009–2009. Bing Du Xue Bao 27: 439–441.

15. Yang F, Zhang T, Hu Y, Wang X, Dù J, et al. (2011) Survey of enterovirus infections from hand, foot and mouth disease outbreak in China, 2009. Virol J 8: 508.

16. Yang H, Tao Z-X, Wang H-Y, Li Y, Fan Q-Y, et al. (2010) The genetic characterization of VP1 region of coxsackie virus A10 isolated from hand, foot and mouth disease cases in Shandong Province of China. Chin J Infect Dis 20: 385–389.

17. Wang Y-Q, Ji Y-L, Qu M. (2011) Molecular characterization of hand, foot and mouth disease related non-EV71 non-CoxA16 enterovirus in Beijing. Int J Virol 18: 75–79.

18. Verstrepen WA, Bruynseels P, Mertens AH (2002) Evaluation of a rapid real-time RT-PCR assay for detection of enterovirus RNA in cerebrospinal fluid specimens. J Clin Virol 25: 2002.

19. Zhang CM, Liu YJ, Wang XC (2009) Detection of enteroviruses form water samples using optimized RT-PCR with universal primers. Int J Environment and Pollution 38: 151–165.

20. Nix WA, Oberste MS, Parish MA (2006) Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol 44: 2698–2704.

21. Ang LW, Koh BK, Chan KP, Chua LT, James L, et al. (2009) Epidemiology and control of hand, foot and mouth disease in Singapore, 2001–2007. Ann Acad Med Singapore 38: 106–112.

22. Chen S-P, Li Y-CHW-C, Chiu C-H, Huang C-G, Tsao K-C, et al. (2010) Comparison of clinical features between coxsackievirus A2 and enterovirus 71 during the enterovirus outbreak in Taiwan, 2008: a children’s hospital experience. J Microbiol Immunol Infect 43: 99–104.

23. Ma E, Chan KC, Cheng P, Wong C, Chuang SK (2010) The enterovirus 71 epidemic in 2008: public health implications for Hong Kong. Int J Infect Dis 14: e775–780.

24. Ryu W-S, Kang B, Hong J, Hwang S, Kim J, et al. (2010) Clinical and epidemiological characteristics of enterovirus 71-related diseases during a recent 2-year period in Korea. J Clin Microbiol 48: 2490–2494.

25. Li X-F, Wang Q-Y, Huang F, Li J, Qu M, et al. (2011) Epidemiological analysis of hand-foot-mouth disease in Beijing from 2007–2010. Int J Virol 18: 5–10.

26. Zhang J, Sun J-L, Chang Z-R, Zhang W-D, Wang Z-J, et al. (2011) Characterization of hand, foot, and mouth disease in China between 2008 and 2009. Biomed Environ Sci 24: 214–221.

27. Hu YF, Yang F, Du J, Dong J, Zhang T, et al. (2011) Complete Genome Analysis of Coxackievirus A2, A4, A5, and A10 Strains Isolated from Hand, Foot, and Mouth Disease Patients in China Revealing Frequent Recombination of Human Enterovirus A. J Clin Microbiol 49: 2426–2434.

28. Yozwiak NL, Skewes-Cox P, Gordon A, Saborio S, Kuan G, et al. (2010) Human enterovirus 109: a novel interspecies recombinant enterovirus isolated from a case of acute pediatric respiratory illness in Nicaragua. J Virol 84: 9047–9058.

29. Lewis-Rogers N, Bendall ML, Crandall KA (2009) Phylogenetic relationships and molecular adaptation dynamics of human rhinoviruses. Mol Biol Evol 26: 969–981.