Journal of Infectious Diseases and Therapy
ISSN: 2332-0877

Lin et al., J Infect Dis Ther 2018, 6:3
DOI: 10.4172/2332-0877.1000370

Review Article Open Access

Update on Enterovirus 71 Infections: Epidemiology, Molecular Epidemiology, and Vaccine Development

Che-Liang Lin[1], **Kou-Huang Chen**[2] **and Kow-Tong Chen**[3,4*]

[1]*Internal Medicine Chest Division, Chi-Mei Medical Center, Liouying, Tainan, Taiwan*

[2]*School of Mechanical & Electronic Engineering, Sanming University, Sanming, Fujian Province, China*

[3]*Department of Occupational Medicine, Tainan Municipal Hospital (Managed by Show Chwan Medical Care Corporation), Tainan, Taiwan*

[4]*Department of Public Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

[*]**Corresponding author:** Dr. Kow-Tong Chen, Department of Occupational Medicine, Tainan Municipal Hospital (Managed by Show Chwan Medical Care Corporation), No. 670, Chongde Road, East District, Tainan, Taiwan, Tel: +886-6-2609926; Fax: +886-6-2606351; E-mail: ktchen@mail.ncku.edu.tw

Received date: June 5, 2018; **Accepted date:** June 21, 2018; **Published date:** June 27, 2018

Abstract

Enterovirus 71 (EV71) infections are one of the main etiological agents of hand, foot and mouth disease (HFMD), or herpangina, worldwide. The purpose of this study was to explore the epidemiology and molecular epidemiology of EV71 infection as well as the prospects for the development of an EV71 vaccine. We performed a search for "enterovirus 71" and "epidemiology" or "molecular epidemiology" or "vaccine" in Medline and PubMed to search through previous studies. Only articles that were published in the English language were included for review in this study. The morbidity of EV71 infection was different among different countries; seasonal variations in its incidence were also observed. Most patients with EV71 infection were children below 5 years of age. The organ most likely involved in EV71 infection is the brainstem. The infection's genetic lineages are undergoing rapid evolutionary changes. The association between the occurrence of EV71 infection and the circulation of different genetic strains of EV71 virus (genotypes B3, B4, C1, C2, C4, and C4a) has been identified around the world. EV71 infection is an important life-threatening communicable disease, and there is an urgent global need for vaccine development for the prevention and control of EV71 epidemics. To establish a global surveillance system of EV71 infection for the identification and detection of the potential emergence of new EV71 variants is needed in the future.

Keywords: Epidemiology; Herpangina; Enterovirus 71; Vaccine

Introduction

There are four species of human enteroviruses: human enterovirus A (HEV-A), HEV-B, HEV-C, and HEV-D. The classification of human enteroviruses is based on each strain's homology within the RNA region coding for the VP1 capsid protein [1]. Enterovirus 71 (EV71) is classified into the human enterovirus A (HEV-A) species of the *Picornaviridae* family [1].

Enterovirus 71 is one of the important causative agents of hand, foot, mouth disease (HFMD) and herpangina [1-3]. The clinical spectrum of EV71 infection is wide. It includes skin eruptions, internal organ, and neurological manifestations, and even death. In recent years, there are several large-scale epidemics of EV71 infection with severe neurological complications involving the central nervous system (CNS) in children reported. Although the EV71 virus is isolated in many countries, epidemics of the EV71 infection have been predominantly found in the Asia-Pacific region [2-8]. However, the reasons for this phenomenon are uncertain. For the reason of the potential of the virus to cause severe neurologic disorder and death, we need to know the features of and control measures for EV71 infection. The purpose of this study was to explore the epidemiology and molecular epidemiology of EV71 as well as the prospects for the development of an EV71 vaccine.

Data sources

All papers published between January 1965 and August 2017 related to EV71 infection were extracted by searching Medline (National Library of Medicine, Bethesda, Maryland, USA) and PubMed using the phrase "enterovirus 71" and "epidemiology" or "molecular epidemiology" or "vaccine." The results were limited to articles available in English. Additional citations were identified from the references of relevant literature. Data collection was performed in April and May of 2018.

Epidemiology

In 1969, EV71 was first isolated from a child with encephalitis in US [1]. Several EV71 epidemics were then reported in the 1970s by various countries in the Americas, Europe, Australia, and Asia [2,4,7-38].

In the Asia-Pacific region, a large EV71 epidemic occurred in Sarawak, Malaysia in 1997 [12]. During the same period, there was an EV71 outbreak with four fatal cases reported in Malaysia [12], and a handful of cases with severe neurological disorder were reported in Japan [7]. In 1998, a huge outbreak of EV71 infection occurred in Taiwan [2]. In this outbreak, 405 hospitalized children with serious neurological complications, and 78 died were reported. In 2008, a large-scale outbreak occurred in China, in which approximately 490,000 children with EV71 infections and 126 deaths were reported [8]. In addition to these epidemics, a pattern of 2- to 3-year cyclic epidemics has been observed in various countries or regions, including Japan [13], Sarawak [4,16], and Taiwan [2].

Outside of the Asia-Pacific region, small outbreaks or sporadic cases have occurred in North America, Europe, and Africa in recent decades [25-35]. A low level of the EV71 virus circulates in these regions [25-35]. In 1975, there was a large EV71 outbreak in Bulgaria, in which 140 cases were complicated with paralysis and 27 cases died [27]. In 1978, there was an outbreak of EV71 infection in Hungary, in which the main clinical spectrum was meningitis and encephalitis [34]. In 1998, 20 children experienced severe EV71 infections in Canada [28]; among these cases, 50% of them involved neurological organ with aseptic meningitis, and one-third had clinical manifestations of respiratory tract. However, most of the cases recovered rapidly. In the USA, several small outbreaks of EV71 infection with neurological involvement occurred in 2003 and 2005 [29]. Among these outbreaks, 16 children aged younger than 9 years old were affected, and one child died. One study in Austria found that 9% of children who were infected with EV71 were hospitalized with aseptic meningitis between 2001 and 2004 [20]. In the year of 1998 and 2006, a number of 32 severe cases of EV71 infection with neurological involvement or clinical symptoms related to EV71 infection including cutaneous, visceral, or neurological manifestations was identified in the UK [31]. In 2007, after 21 years of a low level of endemicity, there were 58 patients of EV71 infection with CNS involvement reported in the Netherlands [32]. Widespread circulation of EV71 was reported in Norway between 2002 and 2003 [33]. In this outbreak, EV71 virus was isolated from 19 (17%) of 113 healthy children. In addition, between 1999 and 2000, there were two outbreaks of EV71 infection occurred in HIV treatment centers in Nairobi, Kenya [34].

Seasonal epidemic patterns of EV71 infection were observed in some countries. In Asia, a higher incidence was observed during the summer months [35,39,40]. Some studies have showed a variation in the peak season between different years [13,41]. Most cases of EV71 infection occurred in children five years of age and younger, and males had a higher risk of EV71 infection than females [4].

It is postulated that the reasons for the seasonal patterns of EV71 infection are due to host immune competencies related to fluctuation changes which influenced by seasonal factors. These could include the levels of melatonin or vitamin D; seasonal variation, behavioral risk factors (e.g., school attendance, indoor crowding), or environmental factors (e.g., meteorological factors) [1,17,34,36]; however, human behavioral risk factors alone cannot explain the reason of seasonal variation observed for certain cases of EV71 infection, for example, cases that occur in school-aged children or in association with household crowding [35]. The relationship between climatic factors and the occurrence of EV71 infection has been studied. The previous study [36] conducted in Taiwan showed that the higher incidence rate of EV71 infection was significantly related to summer season (from April to June); the occurrence of EV71 infections started to increase once the temperature rose above 13°C; above a temperature of approximately 26°C, the number of cases started to decrease, producing an inverted V-shaped relationship. This study demonstrated that higher temperatures and humidity lead to an increasing the number of EV71 infection in Taiwan.

Clinical characteristics of EV71 infection

Most EV71 infections result in mild clinical symptoms, including herpangina and hand, foot and mouth disease (HFMD. However, some patients progress to severe or fatal illness [1]. Viremia occurred more frequently in children aged one year or younger [41,42]. Most of patients with viremia did not reflex a significant manifestation on the clinical severity of EV71 infection. In addition, the occurrence of patients with CNS involvement was not different statistically between patients with or without viremia [42,43].

EV71 is a highly neurotropic virus [44]. The most common organ of EV71 involvement is brain stem [44,45]. It has hypothesized that there are two likely routes by which the EV71 virus involves the CNS: the EV71 virus is either transmitted to the CNS from the blood across the blood-brain barrier (BBB) or enters the CNS through peripheral nerves *via* retrograde axonal transport [46,47].

According to the data in mice study, the strong neurotropism of EV71 and retrograde axonal transport in neurons might represent the major transmission route of EV71 [46,48]. In previous studies [46-49], that mice were infected *via* the oral and parenteral routes with a murine-adapted virus strain that originated from a fatal human case, and the results showed that the EV71 virus entered the CNS *via* peripheral motor nerves after a skeletal muscle infection and spread within the CNS through motor and other neural pathways. Inflammation was found to be the most marked in the spinal cord gray matter, brainstem, hypothalamus, and subthalamic and dentate nuclei in an autopsy sample in Malaysia [49]. Previous studies [50,51] found that the EV71 infection process was primarily in the respiratory tract epithelium and then able to enter a pre-conventional dendritic cell population of dendritic cells at the infection site; these cells could potentially transmit the virus from local sites to other organs through blood stream during the infection process.

EV71 infection can lead to severe or fatal disease due to pulmonary edema. It is unclear the mechanism of pulmonary edema among severe case with EV71 infection. The previous evidences [52,53] indicate that brainstem involvement of EV71 infection is an important etiology of neurogenic pulmonary edema. The combination of the interactive effect of the virus and the host is vital for viral replication, virulence, and pathogenicity during the viral life cycle [54]. However, tissue-specific viral virulence remains unclear in both cell-based systems and animal models. It needs further study in the future.

Molecular epidemiology

Using the VP1 protein for analysis, EV71 can be divided into four distinct genotypes (A, B, C, and D) [37]. Genogroup A comprises the prototype EV71 strain (BrCr-CA-70) was first isolated in 1969 in the United States [40], but not identified until 2008 [8]. Genotype B can be divided into genotypes B1-B5 and genotype C can be divided into C1-C4 furtherly [17]. Genotype D was identified in India, and genotypes E and F were identified in Africa [28,39].

The phylogenetic origins of the EV71 strains recently circulating in the Asia-Pacific region are showed in Table 1 [2-18,38]. Genotype A is not identified in Asia-Pacific region until 2008 [8]. In contrast, genogroup B and C viruses have been leading to several large-scale outbreaks in Asian region since 1997 [18]. The four distinct genogroups (B3, B4, C1, and C2) have been found to co-circulate in the Asia-Pacific region from 1990-2016 (Table 1). Genotype C4, particularly C4a, has emerged in the Asia-Pacific region [39,40,55-71]. The evolutionary branch C4a has crucial nucleotide and amino acid of mutations relative to branch C4b, and these changes may be the reasons for the increasing of its neurovirulence and risk of outbreaks of EV71 infection in China [61,63,66]. Through genetic and antigenic analysis, genotype C4a viruses of EV71 has been confirmed to spread from China to Vietnam and caused a large-scale epidemic in Ho Chi Minh City and southern Vietnam in 2011 [71].

	Years					
Region/Countries	**1960-1969**	**1970-1979**	**1980-1989**	**1990-1999**	**2000-2009**	**2010-2016**
Asia-Pacific region						
Singapore	-	-	-	B3, B4	B4, B5, C1	-
Malaysia	-	-	-	B3, B4	B4, B5, C1,	-
Australia	-	-	-	B3, C2	C1	-
Japan	-	-	-	B3, B4, C2	B4, B5, C2, C4a	C2
Korea	-	-	-	B4, C2	C2, C3, C4a, C4b	C4a
Taiwan	-	-	-	B4, C2	B4, B5, C4, C5	B5, C4
China	-	-	-	-	C4	C4, C4a
Cambodia	-	-	-	--	-	C4
Vietnam	-	-	-	-	-	C4, B5
Other countries						
France	-	-	-	-	C1, C2, C4	C4
UK	-	-	-	C1	C1, C2	-
Germany	-	-	-	-	C1, C2	-
Austria	-	-	-	-	C1, C4	-
Norway	-	-	-	-	C1	-
Netherlands	B0	B1	B2	C1	C1, C2	-
Hungary	-	B1	-	-	C1, C4	-
Bulgaria	-	B1	-	-	-	-
USA	A	B1	B2	C1, C2	C2	-
Canada	-	-	-	-	-	-
Peru	-	-	-	-	C1	-
Bold type indicates predominant strain; - indicates no data available.						

Table 1: A summary of human enterovirus 71 genotypes circulating in different countries worldwide, 1960-2016 [2-57].

The phylogenetic origins of the EV71 strains circulating outside the Asia-Pacific region are also presented in Table 1 [19-30]. From 1963 to 1986, EV71 infections were caused by genotypes B0, B1 and B2 in outside the Asia-Pacific region. In contrast, after 1987, the genotype B strain was replaced by genotype C strains of lineages C1 and C2 [19-30]. According to the epidemiological study, the EV71 subgenotypes B1, B2, C1 and C2 appeared to have a global spread.

Vaccine development

It is believed that close person-to-person contact is the most common route of transmission of EV71 virus. However, the persistent presence of EV71 circulation in the community and the majority of EV71 infections are mild or asymptomatic. These factors results the lower efficacy of public health interventions, such as hand washing. In the absence of effective treatment, to develop an effective vaccine is the best way to prevent and eliminate EV71 infection. Several EV71 vaccines have been studied, including an inactivated virus vaccine [72-74], a virus-like particle vaccine [75], DNA vaccines [76], a subunit vaccine [77], and a live attenuated vaccine [78-80]. All these vaccines are still in the initial stages of development. Stability, purity, and cost of production are the future challenge of these vaccines.

Due to their stability, the research and development of inactivated EV71 vaccines have progressed further than other types of vaccines. In the recent years, five inactivated EV71 vaccines have been developed. Some studies in China shows that Phase III clinical trials of inactivated EV71 vaccines have been conducted [81,82]. These studies involved more than 30,000 infants and children and shows that the safety of the EV71 vaccine is satisfactory in infants and children and that the vaccine can prevent over 90% of EV71-associated HFMD and 80% of EV71-associated disease [72,73,82]. The cost-effectiveness of a national enterovirus 71 vaccination has been done in China [83,84]. They showed the vaccination would be cost-saving or cost-effective to

prevent EV71 related morbidity, mortality, and use of health service among children aged five or younger, compared to no vaccination. This bodes well for the introduction of a safe and effective EV71 vaccine in the near future. In December 2015, the Food and Drug Administration (FDA) of China approved the first EV71 vaccine [83]. Similar vaccines are being investigated in Taiwan [85] and Singapore [86], both of which have entered Phase I clinical trials. In China, inactivated EV71 vaccines are currently in commercial production.

It still has big challenge of inactivated EV71 vaccine. Although both C4-based and B4-based antibodies cross-neutralized the current circulating EV71 isolates [87], the B4 vaccine poorly neutralized an atypical C2 strain [88]. In addition, no formalin-inactivated EV71 vaccines protected against CAV16, which is primary cause of annual HFMD outbreaks, and humoral immunity correlated with protection but waned after the first 6 months of vaccination [73,87]. However, inactivated EV71 vaccines have an advantage over live attenuated vaccines for safety reasons because of their inability to replicate.

Conclusions

EV71 has been an important etiological agent of infection and death in children in the world. It causes severe neurological symptoms in younger children who become infected. In the absence of effective treatment, to develop effective vaccines is a priority for the prevention and control of EV71 infection. Although inactivated EV71 vaccines have been developed in the last few years, the vaccines have some limitations. At this time, inactivated EV71 vaccines can protect against EV71 but not CAV16 infection. In addition, due to the diversity of enterovirus strains, a sustainable EV71 infection surveillance system should be established in order to identify and detect the potential emergence of new EV71 variants.

Acknowledgements

None.

Funding

No funding or sponsorship was received for this study or the publication of this article.

Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures

The authors declare no conflict of interest.

Compliance with Ethics Guidelines

This article is based on previously conducted studies and does not contain any studies with human participants or animals or that were performed by any of the authors.

References

1. Pallansch M, Roos R (2007) Enterovirus: Polioviruses, Coxsackieviruses, Echoviruses and Newer Enteroviruses. In: Virology (Knipe DM, Howley PM, Griffin DE, et al. eds) pp. 839-893.
2. Chen KT, Chang HL, Wang ST, Cheng YT, Yang JY (2007) Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. Pediatrics 120: 244-252.
3. Chan K, Goh K, Chong C, Teo E, Lau G, et al. (2005) Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerg Infect Dis 9: 78-85.
4. Chan LG, Parashar UD, Lye MS, Ong FG, Zaki SR, et al. (2000) Deaths of children during an outbreak of hand, foot, and mouth disease in Sarawak, Malaysia: clinical and pathological characteristics of the disease. Clin Infect Dis 31: 678-83.
5. McMinn P, Stratov I, Nagarajan L, Davis S (2001) Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. Clin Infect Dis 32: 236-242.
6. Tu PV, Thao NT, Perera D, Huu TK, Tien NT, et al. (2005) Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005 Emerg Infect Dis 13: 1733-1741.
7. Komatsu H, Shimizu Y, Takeuchi Y, Ishiko H, Takada H (1999) Outbreak of severe neurologic involvement associated with Enterovirus 71 infection. Pediatr Neurol 20: 17-23.
8. Zhang Y, Zhu Z, Yang W, Ren J, Tan X, et al. (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.
9. Kennett ML, Birch CJ, Lewis FA, Yung AP, Locarnini SA, et al. (1974) Enterovirus type 71 in Melbourne. Bull World Health Organ 51: 609-615.
10. Hagiwara A, Tagaya I, Yoneyama T (1978) Epidemic of hand, foot and mouth disease associated with enterovirus 71 infection. Intervirology 9: 60-63.
11. Samuda GM, Chang WK, Yeug CY, Tang PS (1987) Monoplegia caused by enterovirus 71 an outbreak in Hong Kong. Pediatr Infect Dis J 6: 206-208.
12. Lum LC, Wong KT, Lam SK, Chua KB, Goh AY, et al. (1998) Fatal enterovirus 71 encephalomyelitis. J Pediatr 133: 795-798.
13. Mizuta K, Abiko C, Murata T, Matsuzaki Y, Itagaki T, et al. (2005) Frequent importation of enterovirus 71 from surrounding countries into the local community of Yamagata, Japan, between ,1998 and 2003. J Clin Microbiol 43: 6171-6175.
14. Lin KH, Hwang KP, Ke GM, Wang CF, Ke LY, et al. (2006) Evolution of EV71 genogroup in Taiwan from 1998 to 2005 an emerging of subgenogroup C4 of EV71. J Med Virol 78: 254-262.
15. Hosoya M, Kawasaki Y, Sato M, Honzumi K, Kato A, et al. (2006) Genetic diversity of enterovirus 71 associated with hand, foot and mouth disease epidemics in Japan from 1983 to 2003. Pediatr Infect Dis J 25: 691-694.
16. Podin Y, Gias EL, Ong F, Leong YW, Yee SF, et al. (2006) Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia lessons from the first 7 years. BMC Public Health 6: 180.
17. 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.
18. Chia MY, Chiang PS, Chung WY, Luo ST, Lee MS (2014) Epidemiology of Enterovirus 71 Infections in Taiwan. Pediatr Neonatol 55: 243-249.
19. Chumakov M, Voroshilova M, Shindarov L, Lavrova I, Gracheva L, et al. (1979) Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Arch Virol 60: 329-340.
20. Nagy G, Takátsy S, Kukán E, Mihály I, Dömök I (1982) Virological diagnosis of enterovirus type 71 infections experiences gained during an epidemic of acute CNS disease in Hungary in 1978. Arch Virol 71: 217-27.
21. Merovitz L, Demers AM, Newby D, McDonald J (2000) Enterovirus 71 infections at a Canadian center. Pediatr Infect DisJ 19: 755-757.
22. Yip CC, Lau SK, Woo PC, Yuen KY (2013) Human enterovirus 71 epidemics: what's next? Emerg Health Threats J 6: 19780.

23. Ortner B, Huang CW, Schmid D, Mutz I, Wewalka G, et al. (2009) Epidemiology of enterovirus types causing neurological disease in Austria 1999-2007: detection of clusters of echovirus 30 and enterovirus 71 and analysis of prevalent genotypes. J Med Virol 81: 317-324.
24. Bible JM, Iturriza-Gomara M, Megson B, Brown D, Pantelidis P, et al. (2008) Molecular epidemiology of human enterovirus 71 in the United Kingdom from 1998 to 2006. J Clin Microbio 46:3192-3200.
25. Van der Sanden S, van Eek J, Martin DP, van der Avoort H, Vennema H, et al. (2011) Detection of recombination breakpoints in the genomes of human enterovirus 71 strains isolated in the Netherlands in epidemic and non-epidemic years, 1963-2010. Infect Genet Evol 11:886-894.
26. Witsø E, Palacios G, Rønningen KS, Cinek O, Janowitz D, et al. (2007) Asymptomatic circulation of HEV71 in Norway. Virus Res 123:19-29.
27. Schuffenecker I, Mirand A, Antona D, Henquell C, Chomel JJ, et al. (2011) Epidemiology of human enterovirus 71 infections in France, 2000-2009. J Clin Virol 50: 50-56.
28. Bessaud M, Razafindratsimandresy R, Nougairède A, Joffret ML, Deshpande JM, et al. (2014) Molecular comparison and evolutionary analyses of VP1 nucleotide sequences of new African human enterovirus 71 isolates reveal a wide genetic diversity. PLoS One 9: e90624.
29. Chakraborty R, Iturriza-Gomara M, Musoke R, Palakudy T, D'Agostino A, et al. (2004) An epidemic of enterovirus 71 infection among HIV-1-infected orphans in Nairobi. AIDS 18: 1968-1970.
30. Huaman JL, Carrion G, Ampuero JS, Ocaña V, Laguna-Torres VA, et al. (2016) Enterovirus-71 genotype C isolated in Peru between 2006 and 2009. J Clin Virol 85: 40-43.
31. Urashima M, Shindo N, Okabe N (2003) Seasonal models of herpangina and hand-foot-mouth disease to simulate annual fluctuations in urban warming in Tokyo. Jpn J Infect Dis 56: 48-53.
32. Park SK, Park B, Ki M, Kim H, Lee K, et al. (2010) Transmission of seasonal outbreak of childhood enteroviral aseptic meningitis and hand-foot-mouth disease. J Korean Med Sci 25: 677-683.
33. Ma E, Lam T, Wong C, Chuang SK (2010) Is hand, foot and mouth disease associated with meteorological parameters? Epidemiol Infect 138: 1779-1788.
34. Dowell SF. (2001) Seasonal variation in host susceptibility and cycles of certain infectious diseases. Emerg Infect Dis 7: 369-374.
35. Chang LY, Tsao KC, Hsia SH, Shih SR, Huang CG, et al. (2004) Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. JAMA 291: 222-227.
36. Chang HL, Chio CP, Su HJ, Liao CM, Lin CY (2012) The association between enterovirus 71 infections and meteorological parameters in Taiwan. PLoS One 7: e46845.
37. Brown BA, Oberste MS, Alexander JP Jr, Kennett ML, Pallansch MA (1999) Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. J Virol 73: 9969-9975.
38. Shih SR. Ho MS, Lin KH, Wu SL, Chen YT (2000) Genetic analysis of enterovirus 71 isolated from fatal and nonfatal cases of hand, foot and mouth disease during an epidemic in Taiwan. Virus Res 68: 127-136.
39. McWilliam Leitch EC, Cabrerizo M, Cardosa J, Harvala H, Ivanova OE, et al. (2012) The association of recombination events in the founding and emergence of subgenogroup evolutionary lineages of human enterovirus 71. J Virol 86: 2676-2685.
40. Schmidt NJ, Lennette EH, Ho HH (1974) An apparently new enterovirus isolated from patients with disease of the central nervous system. J Infect Dis 129: 304-309.
41. Dolin R. (1999) Enterovirus 71--emerging infections and emerging questions. N Engl J Med 341: 984-985.
42. Cheng HY, Huang YC, Yen TY, Hsia SH, Hsieh YC, et al. (2014) The correlation between the presence of viremia and clinical severity in patients with enterovirus 71 infection: a multi-center cohort study. BMC Infect Dis 14: 417.
43. Kung CM, King CC, Lee CN, Huang LM, Lee PI, et al. (2006) Differences in replication capacity between enterovirus 71 isolates obtained from patients with encephalitis and those obtained from patients with herpangina in Taiwan. J Med Virol 79: 60-68.
44. Lin TY, Chang LY, Hsia SH, Huang YC, Chiu CH, et al. (2002) The 1998 enterovirus 71 outbreak in Taiwan pathogenesis and management. Clin Infect Dis 34: s52-57.
45. Chang LY, Lin TY, Hsu KH, Huang YC, Lin KL, et al. (1999) Clinical features and risk factors of pulmonary oedema after enterovirus 71-related hand, foot, and mouth disease. Lancet 354: 1682-1686.
46. Chen CS, Yao YC, Lin SC, Lee YP, Wang YF, et al. (2007) Retrograde axonal transport a major transmission route of enterovirus 71 in mice. J Virol 81: 8996-9003.
47. Xing J, Liu D, Shen S, Su Z, Zhang L, et al. (2016) Pathologic Studies of Fatal Encephalomyelitis in Children Caused by Enterovirus 71. Am J Clin Pathol 146: 95-106.
48. Ong KC, Badmanathan M, Devi S, Leong KL, Cardosa MJ, et al. (2008) Pathologic characterization of a murine model of human enterovirus 71 encephalomyelitis. J Neuropathol Exp Neurol 67: 532-542.
49. Cordey S, Petty TJ, Schibler M, Martinez Y, Gerlach D, et al. (2012) Identification of site-specific adaptations conferring increased neural cell tropism during human enterovirus 71 infection. PLoS Pathog 8: e1002826.
50. Huang HI, Weng KF, Shih SR (2012) Viral and host factors that contribute to pathogenicity of enterovirus 71. Future Microbiol 7: 467-479.
51. Zhao T, Zhang Z, Zhang Y, Feng M, Fan S, et al. (2017) Dynamic Interaction of Enterovirus 71 and Dendritic Cells in Infected Neonatal Rhesus Macaques. Front Cell Infect Microbiol 7: 171.
52. Lin JY, Shih SR (2014) Cell and tissue tropism of enterovirus 71 and other enteroviruses infections. J Biomed Sci 21: 18.
53. Huang YF, Chiu PC, Chen CC, Chen YY, Hsieh KS, et al. (2003) Cardiac Troponin I a reliable marker and early myocardial involvement with meningoencephalitis after fatal enterovirus 71 infection. J Infect 46: 238-243.
54. Hsia SH, Wu CT, Cahng JJ, Lin TY, Chung HT, et al. (2005) Predictors of unfavorable outcomes in enterovirus 71 related cardiopulmonary failure in children. Pediatr Infect Dis J 24: 331-334.
55. Zhang B, Wu X, Huang K, Li L, Zheng L, et al. (2014) The variations of VP1 protein might be associated with nervous system symptoms caused by enterovirus 71 infection. BMC Infect Dis 14: 243.
56. Shimizu H, Utama A, Onnimala N, Li C, Li-Bi Z, et al. (2004) Molecular epidemiology of enterovirus 71 infection in the Western Pacific Region. Pediatr Int 46: 231-235.
57. Drake JW, Holland JJ (1999) Mutation rates among RNA viruses. Proc Natl Acad Sci USA 96: 13910-13913.
58. Santti J, Hyypia T, Kinnunen L, Salminen M (1999) Evidence of recombination among enteroviruses. J Viol 73: 8741-8749.
59. Halim S, Ramsingh AI (2000) A point mutation in VP1 of Coxsackievirus B4 alters antigenicity. Virology 269: 86-94.
60. Bible JM, Pantelidis P, Chan PKS, Tong CYW (2007) Genetic evolution of enterovirrus 71 epidemiological and pathological implications. Rev Med Virol 17: 371-379.
61. Chan YF, AbuBakar S (2006) Phylogenetic evidence for inter-typic recombination in the emergence of human enterovirus 71 subgenotypes. BMC Microbiol 6: 74.
62. Chen X, Zhang Q, Li J, Cao W, Zhang JX (2010) Analysis of recombination and natural selection in human eheterovirus 71. Virology 398: 251-261.
63. Fuijimoto T, Chikahira M, Yoshida S, Ebira H, Hasegawa A, et al. (2002) Outbreak of central nervous system disease associated with hand, foot, and mouth disease in Japan during the summer 2000 detection and molecular epidemiology of enterovirus 71. Microbiol Immunol 46: 621-627.
64. Li L, He Y, Yang H, Zhu J, Xu X, et al. (2005) Genetic characteristics of human enterovirus 71 and coxsackievirus A16 circulating from 1999 to 2004 in Shenzhen, People's Republic of China. J Clin Microbiol 43: 3835-3839.

65. Wang YF, Yu CK. (2014) Animal models of enterovirus 71 infection applications and limitations. J Biomed Sci 21: 31-41.
66. Rao CD, Yergolkar P, Shankarappa KS (2012) Antigenic diversity of enteroviruses associated with nonpolio acute flaccid paralysis, India, 2007-2009 Emerg Infect Dis 18: 1833-1840.
67. Zhang Y, Wang J, Guo W, Wang H, Zhu S, et al. (2011) Emergence and transmission pathways of rapidly evolving evolutionary branch C4a strains of human enterovirus 71 in the Central Plain of China. PLoS One 6: e27895.
68. Huang SW, Kiang D, Smith DJ, Wang JR (2011) Evolution of re-emergent virus and its impact on enterovirus 71 epidemics. Exp Biol Med (Maywood) 236: 899-908.
69. Kim HJ, Hyeon JY, Hwang S, Lee YP, Lee SW, et al. (2016) Epidemiology and virologic investigation of human enterovirus 71 infection in the Republic of Korea from 2007 to 2012 a nationwide cross-sectional study. BMC Infect Dis 16: 425.
70. Duong V, Mey C, Eloit M, Zhu H, Danet L, et al. (2016) Molecular epidemiology of human enterovirus 71 at the origin of an epidemic of fatal hand, foot and mouth disease cases in Cambodia. Emerg Microbes Infect 5: e104.
71. Geoghegan JL, Tan le V, Kühnert D, Kühnert D, Halpin RA, et al. (2015) Phylodynamics of Enterovirus A71-Associated Hand, Foot, and Mouth Disease in Viet Nam. J Virol 89: 8871-8879.
72. Zhu FC, Meng FY, Li JX, Li XL, Mao QY (2013) Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China a multicentre, randomized, double-blind, placebo-controlled, phase 3 trial. Lancet 381: 2024-2032.
73. Zhu F, Xu W, Xia J, Liang Z, Liu Y (2014) Efficacy, safety, and immunogenicity of an enterovirus 71 vaccine in China. N Engl J Med 370: 818-828.
74. Li R, Liu L, Mo Z, Wang X, Xia J, et al. (2014) An inactivated enterovirus 71 vaccine in healthy children. N Engl J Med 370: 829-837.
75. Chung YC, Ho MS, Wu JC, Chen WJ, Huang JH (2008) Immunization with virus-like particles of enterovirus 71 elicits potent immune responses and protects mice against lethal challenge. Vaccine 26: 1855-1862.
76. Tung WS, Bakar SA, Sekawi Z, Rosli R (2007) DNA vaccine constructs against enterovirus 71 elicit immune response in mice. Genet Vaccines Ther 5: 6.
77. Foo DG, Alonso S, Phoon MC, Ramachandran NP, Chow VT, et al. (2007) Identification of neutralizing linear epitopes from the VP1 capsid protein of Enterovirus 71 using synthetic peptides. Virus Res 125: 61-68.
78. Xu J, Qian Y, Wang S, Serrano JM, Li W (2010) EV71 an emerging infectious disease vaccine target in the Far East. Vaccine 28: 3516-3521.
79. Chong P, Liu CC, Chow YH, Chou AH, Klein M (2015) Review of enterovirus 71 vaccines. Clin Infect Dis 60: 797-803.
80. Cheng A, Fung CP, Liu CC, Lin YT, Tsai HY, et al. (2013) A Phase I, randomized, open-label study to evaluate the safety and immunogenicity of an enterovirus 71 vaccine. Vaccine 31: 2471-2476.
81. Hu Y, Zeng G, Chu K, Zhang J, Han W, et al. (2018) Five-year immunity persistence following immunization with inactivated enterovirus 71 type (EV71) vaccine in healthy children A further observation. Hum Vaccin Immunother 14: 1-7.
82. Chen YJ, Meng FY, Mao Q, Li JX, Wang H, et al. (2014) Clinical evaluation for batch consistency of an inactivated enterovirus 71 vaccine in a large-scale phase 3 clinical trial. Hum Vaccin Immunother 2014 10: 1366-1372.
83. Wang W, Song J, Wang J, Li Y, Deng H, et al. (2017) Cost-effectiveness of a national enterovirus 71 vaccination program in China. PLoS Negl Trop Dis 11: e0005899.
84. Wu JT, Jit M, Zheng Y, Leung K, Xing W, et al. (2016) Routine Pediatric Enterovirus 71 Vaccination in China a Cost-Effectiveness Analysis. PLoS Med 13: e1001975.
85. Chong P, Hsieh SY, Liu CC, Chou AH, Chang JY (2012) Production of EV71 vaccine candidates. Hum Vaccin Immunother 8: 1775-1783.
86. Reed Z, Cardosa MJ (2016) Status of research and development of vaccines for enterovirus 71. Vaccine 34: 2967-2970.
87. Chou AH, Liu CC, Chang JY, Jiang R, Hsieh YC, et al. (2013) Formalin-inactivated EV71 vaccine candidate induced cross-neutralizing antibody response against subgenotype B1, B4, B5 and CA4A in adult volunteers. PLoS One 8: e79783.
88. Li JX, Mao Qy, Liang ZI, Ji H, Zhu FC (2014) Development of enterovirus 71 vaccine from the lab bench to phase 3 clinical trials. Expert Rev Vaccine 13: 609-618.