# A Guide to Clinical Management and Public Health Response for Hand, Foot and Mouth Disease (HFMD)

World Health Organization
Western Pacific Region

REDI Centre
Regional Emerging Diseases Intervention

# A Guide to Clinical Management and Public Health Response for Hand, Foot and Mouth Disease (HFMD)

World Health Organization
Western Pacific Region

REDI Centre
Regional Emerging Diseases Intervention

**WHO Library Cataloguing in Publication Data**

**A Guide to clinical management and public health response for hand, foot and mouth disease (HFMD)**

1. Hand, foot and mouth disease – epidemiology. 2. Hand, foot and mouth disease – prevention and control. 3. Disease outbreaks. 4. Enterovirus A, Human. I. Regional Emerging Disease Intervention Center.

ISBN 978 92 9061 525 5 (NLM Classification: WC 500)

# Contents

# Acknowledgements

This document was jointly developed by the World Health Organization Regional Office for the Western Pacific and the Regional Emerging Diseases Intervention (REDI) Centre.

Its development was coordinated by Dr Satoko Otsu, (WHO Regional Office for the Western Pacific) and Dr Zarifah Hussain Reed (REDI Centre) with support from Dr Chin Kei Lee (WHO China), Dr Le Van Tuan (WHO Viet Nam), Dr Harpal Singh (WHO Malaysia), and Dr Weigong Zhou (Centers for Disease Control and Prevention, United States of America).

The following individuals contributed to chapters as lead writers, advisers or peer reviewers:

Dr Jane Cardosa, Dr Jeremy Farrar, Dr Feng Zijian, Dr Wakaba Fukushima, Dr Gao Zifen, Dr Truong Huu Khanh, Ms Keiko Kumatani, Dr Raymond Lin Tzer Pin, Dr Tzou-Yien Lin, Dr Ching-Chuan Liu, Dr Peter Charles McMinn, Dr Lam Yen Minh, Dr Revathy Nallusamy, Dr Nguyen Thi Hien Thanh, Dr Ooi Mong How, Dr Hiroyuki Shimizu, Dr Tom Solomon, Ms Maria Takechi, Dr Phan Van Tu, Dr Wong Kum Thong, Dr Dustin Chen-Fu Yang.

Thanks are also due to Dr Ruth Foxwell for providing technical and editorial oversight, and Ms Rhiannon Cook for providing editorial advice and contributing to the finalization of the publication.

# Acronyms

| | |
|---|---|
| ANS | Autonomic nervous system |
| BSL | Biosafety level |
| CA | Coxsackievirus A |
| CNS | Central nervous system |
| CODEHOP | Consensus degenerate hybrid oligonucleotide primer |
| CPE | Cytopathic effect |
| CSF | Cerebrospinal fluid |
| CT | Computed tomography |
| cAMP | cyclic adenosine monophosphate |
| ECMO | Extra-corporeal membrane oxygenation |
| EV | Enterovirus |
| HA | Herpangina |
| HEV | Human enterovirus |
| H&E | Haematoxylin and eosin stain |
| HFMD | Hand, foot and mouth disease |
| HLA | Human leukocyte antigen |
| IgM | Immunoglobulin M |
| IFA | Indirect immunofluorescence assay |
| IL | Interleukin |
| IVIG | Intravenous immunoglobulin |
| MCP | Monocyte chemoattractant protein |
| MIG | Monokine induced by interferon gamma |
| PDE | Phosphodiesterase |
| PSGL | Human P-selectin glycoprotein ligand |
| RD | Human rhabdomyosarcoma cells |
| RNA | Ribonucleic acid |
| RT-LAMP | Reverse transcription loop-mediated isothermal amplification |
| RT-PCR | Reverse transcription polymerase chain reaction |
| SCARB | Human scavenger receptor class B |
| SD | Standard deviation |
| UTR | Untranslated region |
| VTM | Virus transportation medium |

# Introduction

Hand, foot and mouth disease (HFMD) is a common infectious disease caused by a group of enteroviruses, including Coxsackievirus A16 (CA16) and Enterovirus 71 (EV71). Infection with EV71 is of particular concern as it can cause severe disease in children, sometimes resulting in death.

Over the last decade, many outbreaks of HFMD have been reported in countries of the Western Pacific Region, including Japan, Malaysia and Singapore, and across China. The incidence of HFMD, particularly that caused by EV71 infection, appears to be increasing across the Region. This has prompted concerns that, without intervention, the public health impact and spread of the disease will continue to intensify.

This publication has been developed to support the treatment, prevention and control of HFMD. It is intended as a resource for clinicians working with HFMD cases on a regular basis, as well as public health personnel who are responsible for preventing and responding to outbreaks of HFMD. It draws on the most recent scientific literature and captures the current understanding and experiences of international experts working on HFMD.



## Developing the guide

In 2008 and 2009, epidemiological, diagnostic and clinical issues relating to HFMD were reviewed and discussed at three international meetings. Those meetings emphasized the importance of establishing standardized surveillance systems that are supported by laboratory diagnosis, developing investigation and response strategies for HFMD outbreaks, and furthering research into the best clinical management for HFMD. In particular, it was suggested that standardized case definitions and guidelines for clinical management of severe cases were needed to assist in the overall control and management of EV71-associated HFMD.

The World Health Organization (WHO) Western Pacific Regional Office, in coordination with the Regional Emergency Diseases Intervention (REDI) Centre, subsequently organized an informal consultative meeting on HFMD in March 2010 in Kuala Lumpur, Malaysia. Seventeen regional and international experts attended the meeting. Findings were summarized and recommendations developed in the areas of: surveillance, epidemiology and burden of disease; characterization of etiological agents and

transmission; pathogenesis; laboratory diagnosis; clinical features and management; and prevention and control.

In July 2010, the REDI Centre invited 10 clinical management experts to a further meeting on HFMD in Singapore to review the draft guidance document and consolidate up-to-date knowledge and experiences on the clinical management of HFMD caused by EV71.

The resulting document from the above two meetings has been reviewed by experts within and outside the Western Pacific Region, and consensus reached on the content of each chapter.

The support of all those who contributed to development of this guide is gratefully acknowledged.

# Section 1: Epidemiology

## 1.1 Overview

Many small and large outbreaks associated with EV71 infection have been reported throughout the world since the early 1970s (1, 2). Children have been most commonly affected in those outbreaks, and clinical manifestation of cases has been mostly typical of HFMD, with fever, skin eruptions on hands and feet, and vesicles in the mouth. However, cases involving the central nervous system (CNS) and / or pulmonary oedema have also been observed (3).

In the Western Pacific Region, widespread epidemics have been reported in many countries, including Australia, Brunei Darussalam, China, Japan, Malaysia, Mongolia, the Republic of Korea, Singapore, and Viet Nam. Several countries have also reported fatal cases, with severe CNS disease or pulmonary oedema. In 2009, for example, an outbreak in mainland China involved 1 155 525 cases, 13 810 severe cases and 353 deaths.



Less is known regarding the descriptive epidemiology of HFMD or EV71 infection in countries outside the Western Pacific Region. Although dozens of fatalities with CNS involvement were reported during EV71 outbreaks in Bulgaria in 1975 and Hungary in 1978, there have been few fatal cases reported over the last three decades. A recent longitudinal study from Norway suggested asymptomatic circulation of EV71 in the community.

Information regarding the recent seroepidemiology of EV71 in the Western Pacific Region is limited. A cross-sectional study in Singapore indicated that, following the decline of maternal antibodies, the seroprevalence for EV71 increased at an average rate of 12% per year in children from two to five years of age, and reached a steady state of approximately 50% in those aged five years or older. Similar results using three groups of stored sera were found in a cross-sectional study performed in Taiwan (China). Two other seroepidemiological studies in Taiwan (China) indicated that, following a decrease in the circulation of EV71 in the community, an accumulation of susceptible young children may have contributed to the large-scale epidemic that occurred there in 1998.

## 1.2 Descriptive epidemiology

Disease associated with EV71 infection was first described by Schmidt and colleagues in 1974, who reported on 20 patients with CNS disease, including one fatality in California, United States of America, between 1969 and 1972 (1). Subsequent outbreaks associated with EV71 infection were reported in New York, United States of America, in 1972 and 1977 (4-6), Australia in 1972-1973 and 1986 (7, 8), Sweden in 1973 (9), Japan in 1973 and 1978 (10-13), Bulgaria in 1975 (14, 15), Hungary in 1978 (16, 17), France in 1979 (18), Hong Kong (China) in 1985 (19), and Philadelphia, United States of America in 1987 (20). During those outbreaks, EV71 caused a wide spectrum of diseases, including HFMD, aseptic meningitis, encephalitis, paralysis, acute respiratory symptoms and myocarditis.

Those outbreaks reported between 1974 and the mid-1990s may be classified as either "benign" or "severe" in nature (21). For the former, typical examples include the large outbreaks in Japan in 1973 and 1978, involving 3 296 and 36 301 cases, respectively. Although cases involving CNS were observed during those outbreaks, including a number of fatalities, clinical manifestation of cases was mostly typical of HFMD (10-13). That was also the case for the outbreak in Australia in 1986, although no fatalities were reported (8). Reports of other outbreaks, however, have contained significant components of CNS disease. A large epidemic in Bulgaria in 1975, involving 705 cases and including a large number of fatalities, was initially thought to represent poliomyelitis or encephalitis and was not characterized as HFMD (14). A similar epidemic of acute CNS disease in Hungary in 1978 showed that only four cases were classified as HFMD among 323 cases with EV71 infection (17)



In the late 1990s, two widespread community outbreaks associated with EV71 infection occurred; the first in Sarawak, Malaysia, in 1997, and the second in Taiwan (China) in 1998, with 2628 and 129 106 cases reported, respectively (22, 23). Although clinical manifestations during those outbreaks were mostly typical of HFMD, a cluster of deaths among young children was identified. Cases with rapidly progressive and fatal pulmonary oedema/haemorrhage were also observed for the first time

Numerous Member States in the Western Pacific Region have since experienced large HFMD epidemics associated with EV71 infection. Several countries have also reported substantial numbers of deaths. The following section presents the descriptive epidemiology of a number of those epidemics. In addition, findings from the surveillance data of selected countries in the Western Pacific Region since 1997 are summarized in Annex 1.

### *Australia*

Between February and September 1999, 14 cases of EV71-associated neurological disease were identified at a hospital during a community-wide outbreak of HFMD in

Perth, Western Australia. Twelve (86%) of the 14 children were less than four years of age (24).

An outbreak of HFMD due to EV71 occurred in Sydney in the summer of 2000–2001. Approximately 200 children presented to hospital, including nine patients with CNS disease and five with pulmonary oedema. EV71 was identified in all patients with pulmonary oedema (25).

### *Brunei Darussalam*

Brunei Darussalam experienced its first major reported outbreak of EV71 between February and August 2006. More than 1681 children were reportedly affected, with three deaths resulting from severe neurological disease. EV71 was isolated in samples from 34 of at least 100 patients diagnosed with HFMD or herpangina (HA), including two patients who died as a result of severe neurological complications (26).

### *China*

Between March and May 2007, an outbreak of HFMD occurred in Linyi City, Shandong Province, China. By 22 May 2007, 1149 cases had been reported through a countrywide disease-reporting system in mainland China. The majority of those patients (84.4%) were younger than five years of age. Eleven (0.9%) of the HFMD cases were classified as severe, presenting with neurological complications. Three (0.3%) children (aged three years or younger) died during the outbreak. A total of 233 clinical specimens were collected from 105 hospitalized patients, including 11 patients with severe HFMD. Among those, 55 (52.4%), including six severe cases, were confirmed to be EV71 infections (27).



Between 1 January and 9 May 2008, 61 459 HFMD cases and 36 deaths were reported through China's disease reporting system. However, prior to 2 May 2008, HFMD was not categorized as a notifiable disease and reporting of HFMD relied on voluntary reports submitted by clinicians. The number of reported cases increased sharply after the disease was designated as a class "C" notifiable disease, with cases being reported from nearly all provinces. The five provinces with the highest numbers of reported cases were Guangdong (11 374), Anhui (9235), Zhejiang (6134), Shandong (4566) and Henan (3230). Children younger than five years of age accounted for 92% of reported HFMD cases. Among 582 samples tested, EV71 accounted for 54.5% of cases (28).

More detailed studies of the 2008 outbreak were reported from Fuyang City, Anhui Province, where 6049 cases were reported between 1 March and 9 May 2008. Of those, 353 (5.8%) were severe and 22 were fatal (case fatality rate: 0.4%). Among the reported cases, the male-to-female ratio was 1.9:1 and the age ranged from 28 days to 18 years, with 78% of the cases being three years of age or younger. Epidemiological investigation revealed no contact between the 22 fatal cases, but environmental investigation of the households of the fatal cases revealed poor hygiene and sanitary

conditions. The initially high case-fatality rate (2.9% (18/610) between 1 March and 23 April 2008) was attributed to: rapid disease progression; late clinical presentation; and limited local medical capacity. The case-fatality rate decreased considerably (to 0.07% (4/5439) for the period between 24 April and 9 May 2008) once the etiology of the disease was known and early treatment was provided to severe patients. That was attributed to enhanced surveillance and implementation of prevention and control measures (28). It should be noted that, during the outbreak (as of 2 May 2008), HFMD was designated as a class "C" notifiable disease.

In 2009, the number of HFMD cases notified in mainland China amounted to 1 155 525, including 13 810 (1.2%) severe cases and 353 (0.03%) deaths. The male-to-female ratio was 1.8:1. Of those cases, 93% were five years of age or younger, and 75% were three years of age or younger. The cases were widely distributed across China and included both clinically diagnosed and laboratory-confirmed cases. For the laboratory-confirmed cases, EV71 was responsible for 41% of the cases, 81% of the severe cases and 93% of the deaths.

### *Taiwan (China)*

In Taiwan (China), HFMD/HA has been included in the national sentinel-physician reporting system since 3 March 1998 due to the prevalence of HFMD/HA cases. In 1998, 129 106 HFMD/HA cases were reported in two waves between 29 March and the end of the year. They included 405 (0.3%) patients with severe disease, most of whom were five years of age or younger. Seventy-eight (19.6%) patients with severe disease died, 71 (91%) of them five years of age or younger. Of the patients who died, 65 (83%) had pulmonary oedema or pulmonary haemorrhage. EV71 was found in 44 of 59 (75%) isolates from patients with severe infections who survived, while 34 of 37 (92%) isolates from patients who died were positive for EV71 (23). Further smaller outbreaks occurred in 2000 and 2001, involving 291 and 389 severe cases and 41 and 55 fatalities, respectively (41). Sentinel physician surveillance data for 2000 and 2001 indicated similar levels of disease were caused by CA16 and EV71 (17.1% and 18.2% versus 15.5% and 15%, respectively). EV71 was associated with 47.3% (26/545) of cases in 2000 and was the dominant strain associated with fatalities in 2001 (25/41, 61%).



Between 1998 and 2005, the number of severe HFMD/HA cases per year ranged from 35 to 405. Of the 1548 severe cases identified during the eight-year period, 93% were four years of age or younger, and 75% were two years of age or younger. The male-to-female ratio was 1.5:1. A total of 245 fatal cases were reported during the same period. EV71 positivity rates among the fatal cases ranged from 11% to 100% in each year (42-44). The number of severe cases and deaths, respectively, were: 11 and 0 in 2006; 12 and 2 in 2007; 373 and 14 in 2008; and 29 and 2 in 2009.

### *Japan*

Approximately 2400 paediatric clinics participated in the sentinel surveillance system in Japan between 1993 and 1998, with an average of 36.5 cases of HFMD reported per sentinel site annually (29). Since 1999, approximately 3000 paediatric clinics have participated in the sentinel surveillance network, with an average of 42.7 cases of HFMD reported per sentinel site between 1999 and 2005. Large-scale outbreaks occurred in 2000 and 2003, with the numbers of reported cases (cases per sentinel site) 205 365 (68.96) and 172 659 (56.78), respectively. Approximately 90% of the HFMD cases were aged five years of age or younger (30).

HFMD is also included in the Infectious Agents Surveillance system in Japan. The Infectious Agents Surveillance reports are derived from approximately 10% of the sentinel clinics and all the sentinel hospitals in the network. EV71 was found to be the primary causative agent of HFMD epidemics in both 2000 and 2003 (30). Although there is no surveillance system for severe or fatal cases of HFMD in Japan, such cases have been identified through case-series from hospitals during epidemic periods in the community. In 1997, three deaths of young children from HFMD or EV71 infection were identified in Osaka prefecture (30). In the summer of 1997, 12 patients, aged two weeks to six years, with serologically confirmed EV71 infections, were hospitalized in Otsu city as a result of CNS involvement (31). Between June and August 2000, 30 cases with HFMD complicated by CNS involvement were hospitalized in Hyogo prefecture. One patient aged two years died as a result of pulmonary oedema caused by brainstem encephalitis. EV71 was isolated from nine (69%) of 13 faecal samples, including a sample from the fatal case (32). A nationwide questionnaire survey found 272 complicated cases with HFMD during the period 2000-2002. Of these, 226 cases occurred in 2000, 32 in 2001, and 14 in 2002. There were four cases involving sequelae and one fatal case reported in 2000 (52).



### *Malaysia*

In Sarawak, Malaysia, a widespread community outbreak of HFMD, primarily caused by EV71 infection, began in early April 1997. From 1 June to 30 August 1997, a total of 2628 cases were reported to the Sarawak State Department of Health. During the outbreak, 889 children were hospitalized, including 39 patients with aseptic meningitis or acute flaccid paralysis. A total of 29 previously healthy children younger than six years of age (median, 1.5 years; range, 0.5–5.9 years; male-to-female ratio of 1.9:1) died of rapidly progressive cardiorespiratory failure. EV71 was isolated in samples from six of the fatal cases (22). Later in 1997, an outbreak involving 4625 hospital admissions occurred in peninsular Malaysia, resulting in 11 fatal cases (35).

A sentinel surveillance programme for HFMD was subsequently established in Sarawak in March 1998. Between March 1998 and June 2005, 4290 specimens were collected from 2950 children, with a male-to-female ratio of 1.4:1. During that period, two

large outbreaks were identified in Sarawak, in 2000 and 2003. EV71 was the dominant enterovirus serotype of the isolates in both years (36). The sentinel surveillance programme in Sarawak is ongoing, with two more outbreaks identified: the first in 2006 and the second in 2008/2009.

### *Mongolia*

Official reporting of HFMD in Mongolia began in 2008, with 3210 cases reported during that year. Cases were equally distributed between the capital city and the provinces. Among the 245 samples investigated, 102 (41.6%) were positive for EV71 (37).

### *Republic of Korea*

The incidence of HFMD/HA increased in the Republic of Korea during 2000, with an outbreak of HFMD/HA in Cheju Province. While the actual number of cases of HFMD/HA is unknown, no fatality was associated with the outbreak (33).

A sentinel surveillance system for EV infection was initiated in 2005. Between January 2008 and 30 October 2009, 719 suspected cases of HFMD or HA (200 cases in 2008 and 519 cases in 2009) were identified, including one fatal case resulting from severe neurological complications and two cases resulting in a comatose state. Enterovirus was detected in 447 (62.2%) cases. Of those, enteroviral genotype was identified in 218 cases (53 cases in 2008 and 165 cases in 2009). In 2008, the most common pathogen detected was CA10 (18 cases; 34.0%), while, in 2009, EV71 was the most common pathogen detected (91 cases; 55.2%) (34).



### *Singapore*

A large epidemic of HFMD caused by EV71 infection occurred in Singapore between September and October 2000, with 3790 cases reported and three deaths. Of the 104 patients who were clinically diagnosed with HFMD and whose samples yielded at least one virus, EV71 was the most commonly isolated virus (73%) (38).

Reporting HFMD subsequently became mandatory in October 2000. In the seven-year period from 2001 through 2007, nationwide epidemics of HFMD were observed in 2002 (16 228 reported cases), 2005 (15 256 reported cases), 2006 (15 282 reported cases) and 2007 (20 003 reported cases). The age-specific annual incidence rate was highest in those aged 0–4 years, ranging from 1640.5 to 5975.5 per 100 000 population and accounting for 62.2% to 74.5% of reported cases. During the EV71-associated HFMD epidemic in March and April 2006, 1.8% of the cases were hospitalized. That rate was more than twice as high as in those epidemics caused by CA16 (between March and April 2005, 0.8% of cases were hospitalized, and between April and May 2007, 0.7% of cases were hospitalized).

In a smaller outbreak in January and February 2001, 5187 cases were reported, including three HFMD-associated deaths. Of the fatal cases, one was aged four years, while the other two were both 11 months of age (39).

Between late March and May 2008, a nationwide epidemic of EV71-associated HFMD occurred. In the first 24 weeks of 2008, the number of reported HFMD cases was 15 030, a two-fold increase compared with the same period in 2006 (the most recent EV71-associated epidemic prior to 2008). For the epidemic period from week 8 to week 24 in 2008, EV71 constituted 33.2% of the samples tested. EV71 positivity was significantly higher during the 2008 epidemic period than in 2006 (40).

### *Viet Nam*

In southern Viet Nam, an outbreak of acute encephalitis associated with HFMD was reported in Ho Chi Minh City in 2003. In 2005, 764 children were diagnosed with HFMD in Ho Chi Minh City through sentinel surveillance at the largest paediatric hospital, with most cases (96.2%) being five years of age or younger. All patients provided specimens and HEV was isolated from 411 patients. Of those, 173 (42.1%) were identified as EV71, and 214 (52.1%) as CA16. Of those patients with EV71 infections, 51 (29.3%) were complicated by acute neurological disease and three (1.7%) were fatal (45).

In 2006–2007, sentinel surveillance at the same hospital reported 305 cases diagnosed as neurological disease, of which 36 cases (11%), and three deaths (0.01%), were associated with EV71.



In 2007, 2008 and 2009, the numbers of reported and fatal cases were: 5719 and 23, 10 958 and 25, and 10 632 and 23, respectively. The majority of the cases were in the southern part of the country.

In northern Viet Nam, EV71/C4 has only been identified in one patient with acute encephalitis since 2003. Between 2005 and 2007, EV71/C5 was identified in seven patients with acute flaccid paralysis. All cases were under five years of age. During 2008, 88 cases of HFMD were reported from 13 provinces. The results of virus isolation from the 88 cases confirmed that 33 (37.5%) isolates were enterovirus-positive, including nine (27.3%) with EV71, 23 (69.7%) with CA16, and one with CA10. No severe or fatal cases were reported. The majority of cases were under five years of age.

### *Countries outside the Western Pacific Region*

Less is known regarding the descriptive epidemiology of HFMD or EV71 infection in countries outside the Western Pacific Region. In The Netherlands, only severe, hospitalized cases of EV71 infection are reported as part of the national surveillance system. While between 1963 and 2008 there was no indication of EV71-related fatalities, 58 cases of EV71 infection requiring hospitalization were reported in 2007 after a 21-year period of low endemicity (46).

In the United Kingdom, there is evidence of continuous circulation of EV71, with EV71 isolated each year from 1998 to 2006, except for 2003. Of 32 patients with EV71 infection accompanied by neurological complications and/or cutaneous manifestations during that eight-year period, one had fatal encephalitis (47).

A longitudinal study from Norway, conducted between September 2001 and November 2003, indicated asymptomatic circulation of EV71. A total of 113 healthy infants from the age of three months were recruited to provide monthly stool samples and clinical data up until the age of 28 months. Prevalence of EV71 in stool samples showed that EV71 was circulating widely between October 2002 and October 2003. However, data from a surveillance/register system showed no corresponding increase in the number of hospitalized patients with encephalitis, HFMD or HA within the age group during the same period (48).

## 1.3 Seroepidemiological study

Recent data regarding the seroepidemiology of EV71 in the Western Pacific Region are limited. In Singapore, a serological survey was conducted at a paediatric clinic at the National University Hospital that included all children born at the hospital or those aged 12 years or younger brought for routine visits and vaccinations between July 1996 and December 1997, giving a total of 856 children. Antibody prevalence in cord blood suggested that 44% of mothers had antibodies to EV71. None of the children tested had maternal antibodies to EV71 after one month, and antibodies were found in only one of the 124 samples from children aged 1-23 months. In children aged from two to five years, the seropositive rate increased at an average of 12% per year. In samples from children five years of age and older, the age-specific seroprevalence steadied at approximately 50% (49).



Another cross-sectional study carried out in Taiwan (China) examined seroprevalence rates for EV71 by using a micro-neutralizing assay to test three groups of stored sera collected in 1994, 1997 and 1999. Regardless of the period from which the specimens were taken, the seropositive rates were relatively high (38%-44%) in infants younger than six months of age. The rates declined to 0%-15% in infants age 7-11 months, increased gradually thereafter until the age of six, and reached a plateau at about 50% in children older than six years of age (50).

Two seroepidemiological studies have contributed to the understanding of factors underlying the HFMD outbreak in Taiwan (China) in 1998. In a cross-sectional study, neutralizing antibodies to EV71 were assayed for 539 subjects who provided serum samples for vaccine trials or health examinations in two hospitals between July and December 1997. Age-specific EV71-seropositive rates before the outbreak were inversely related to age-specific mortality rates and severe-case rates in the community during the outbreak ($r = -0.82$ and $-0.93$, respectively) (51). A longitudinal study was also conducted using serum samples from a birth cohort study of 81 healthy children

who provided yearly blood samples between 1988 and 1998. The study found that the yearly incidence of EV71 seroconversion was 3%-11% between 1989 and 1997, and that 68% of children had serological evidence of EV71 infection by 1997. However, compared with previous years, the seroconversion rate for EV71 was relatively low between 1994 and 1997. The rarity of EV71 infection between 1994 and 1997 suggests decreased circulation of EV71 in the community. An accumulation of susceptible young children may therefore have contributed to the large-scale epidemic that occurred in 1998 (44, 50).

## References


1. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. Journal of Infectious Diseases, 1974, Mar,129(3):304–309.

2. Solomon T, et al. Virology, epidemiology, pathogenesis and control of enterovirus 71. Lancet Infectious Diseases, 2010, 10(11): 778–790.

3. Ooi MH, et al. Clinical features, diagnosis and management of human enterovirus 71 infection. Lancet Neurology, 2010, 9(11):1097–1105.

4. Deibel R, Gross LL, Collins DN. Isolation of a new enterovirus (38506). Proceedings of the Society for Experimental Biology and Medicine, 1975 Jan, 148(1):203–207.

5. Chonmaitree T, et al. Enterovirus 71 infection: report of an outbreak with two cases of paralysis and a review of the literature. Pediatrics, 1981, Apr, 67(4):489-493.

6. Goldberg F, Weiner LB. Cerebrospinal fluid white blood cell counts and lactic acid dehydrogenase in Enterovirus type 71 meningitis. Clinical Pediatrics (Philadelphia), 1981, May, 20(5):327-330.

7. Kennett ML, et al. Enterovirus type 71 infection in Melbourne. Bulletin of the World Health Organization, 1974, 51(6):609-615.

8. Gilbert GL, et al. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. Pediatric Infectious Disease Journal, 1988, Jul,7(7):484-488.

9. Blomberg J, et al. Letter: New enterovirus type associated with epidemic of aseptic meningitis and-or hand, foot, and mouth disease. Lancet, 1974, Jul 13,2(7872):112.

10. Tagaya I, Tachibana K. Epidemic of hand, foot and mouth disease in Japan, 1972–1973: difference in epidemiologic and virologic features from the previous one. Japanese Journal of Medical Science and Biology, 1975, Aug, 28(4):231–234.

11. Hagiwara A, Tagaya I, Yoneyama T. Epidemic of hand, foot and mouth disease associated with enterovirus 71 infection. Intervirology, 1978,9(1):60–63.

12. Ishimaru Y, et al. Outbreaks of hand, foot, and mouth disease by enterovirus 71. High incidence of complication disorders of central nervous system. Archives of Disease in Childhood, 1980, Aug, 55(8):583–588.

13. Tagaya I, Takayama R, Hagiwara A. A large-scale epidemic of hand, foot and mouth disease associated with enterovirus 71 infection in Japan in 1978. Japanese Journal of Medical Science and Biology, 1981, Jun, 34(3):191–196.

14. Shindarov LM, et al. Epidemiological, clinical, and pathomorphological characteristics of epidemic poliomyelitis-like disease caused by enterovirus 71. Journal of Hygiene, Epidemiology, Microbiology and Immunology, 1979, 23(3):284–295.

15. Chumakov M, et al. Enterovirus 71 isolated from cases of epidemic poliomyelitis-like disease in Bulgaria. Archives of Virology, 1979, 60(3-4):329–340.

16. World Health Organization. Virus diseases surveillance, 1979.

17. Nagy G, et al. Virological diagnosis of enterovirus type 71 infections: experiences gained during an epidemic of acute CNS diseases in Hungary in 1978. Archives of Virology, 1982, 71(3):217–227.

18. World Health Organization. Enterovirus type 71 surveillance, 1979.

19. Samuda GM, et al. Monoplegia caused by Enterovirus 71: an outbreak in Hong Kong. Pediatric Infectious Disease Journal, 1987, Feb, 6(2):206-208.

20. Hayward JC, et al. Outbreak of poliomyelitis-like paralysis associated with enterovirus 71. Pediatric Infectious Disease Journal, 1989, Sep, 8(9):611–616.

21. Ho M. Enterovirus 71: the virus, its infections and outbreaks. Journal of Microbiology, Immunology and Infection, 2000, Dec,33(4):205–216.

22. Chan LG, et al. Deaths of children during an outbreak of hand, foot, and mouth disease in Sarawak, Malaysia: clinical and pathological characteristics of the disease. For the Outbreak Study Group. Clinical Infectious Diseases, 2000, Sep, 31(3):678–683.

23. Ho M, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. New England Journal of Medicine, 1999, Sep 23,341(13):929–935.

24. McMinn P, et al. Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. Clinical Infectious Diseases, 2001, Jan 15, 32(2):236–242.

25. Nolan MA, et al. Survival after pulmonary edema due to enterovirus 71 encephalitis. Neurology, 2003, May 27, 60(10):1651–1656.

26. AbuBakar S, et al. Enterovirus 71 outbreak, Brunei. Emerging Infectious Diseases, 2009, Jan, 15(1):79–82.

27. Zhang Y, et al. An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. Journal of Clinical Virology, 2009, Apr, 44(4):262-267.

28. Report on the hand, foot, and mouth disease in Fuyang City, Anhui Province and the prevention and control in China. Beijing, Chinese Center for Disease Control and Prevention and the office of the World Health Organization in China 2008.

29. Taniguchi K, et al. Overview of infectious disease surveillance system in Japan, 1999-2005. Journal of Epidemiology, 2007, Dec, 17 Suppl:S3–13.

30. Hand, foot and mouth disease, 2000-2003, Japan. Infectious Agents Surveillance Report. 2004 25(9):224–225.

31. Komatsu H, et al. Outbreak of severe neurologic involvement associated with Enterovirus 71 infection. Pediatric Neurology, 1999, Jan, 20(1):17–23.

32. Fujimoto T, et al. Outbreak of central nervous system disease associated with hand, foot, and mouth disease in Japan during the summer of 2000: detection and molecular epidemiology of enterovirus 71. Microbiology and Immunology, 2002, 46(9):621–627.

33. Jee YM, et al. Genetic analysis of the VP1 region of human enterovirus 71 strains isolated in Korea during 2000. Archives of Virology, 2003, Sep, 148(9):1735–1746.

34. Ryu WS, et al. Clinical and etiological characteristics of enterovirus 71-related diseases during a recent 2-year period in Korea. Journal of Clinical Microbiology, 2010, Jul, 48(7):2490–2494.

35. Shekhar K, et al. Deaths in children during an outbreak of hand, foot and mouth disease in Peninsular Malaysia-clinical and pathological characteristics. Medical Journal of Malaysia, 2005, Aug, 60(3):297–304.

36. Podin Y, et al. Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: lessons from the first 7 years. BMC Public Health, 2006, 6:180.

37. Report of the Second Meeting on Vaccine Preventable Diseases Reference Laboratory Networks in the Western Pacific Region. 22-26 February 2010, Manila, Philippines.Manila, WHO Regional Office for the Western Pacific, 2010

38. Chan KP, et al. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerging Infectious Diseases, 2003, Jan, 9(1):78–85.

39. Ang LW, et al. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. Annals, Academy of Medicine, Singapore, 2009, Feb, 38(2):106–112.

40. Epidemiological news bulletin, Ministry of Health, Singapore. 2008, 34(4).

41. Lin TY, et al. Enterovirus 71 outbreaks, Taiwan: occurrence and recognition. Emerging Infectious Diseases, 2003, Mar, 9(3):291–293.

42. Chen KT, et al. Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. Pediatrics, 2007, Aug, 120(2):e244–252.

43. Chen SC, et al. An eight-year study of epidemiologic features of enterovirus 71 infection in Taiwan. American Journal of Tropical Medicine and Hygiene, 2007, Jul, 77(1):188–191.

44. Chang LY. Enterovirus 71 in Taiwan. Pediatrics and Neonatology, 2008, Aug, 49(4):103–112.

45. Tu PV, et al. Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005. Emerging Infectious Diseases, 2007, Nov, 13(11):1733–1741.

46. van der Sanden S, et al. Epidemiology of enterovirus 71 in the Netherlands, 1963 to 2008. Journal of Clinical Microbiology, 2009, Sep, 47(9):2826–2833.

47. Bible JM, et al. Molecular epidemiology of human enterovirus 71 in the United Kingdom from 1998 to 2006. Journal of Clinical Microbiology, 2008, Oct, 46(10):3192–3200.

48. Witso E, et al. Asymptomatic circulation of HEV71 in Norway. Virus Research, 2007, Jan, 123(1):19–29.

49. Ooi EE, et al. Seroepidemiology of human enterovirus 71, Singapore. Emerging Infectious Diseases, 2002, Sep, 8(9):995–997.

50. Lu CY, et al. Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. Journal of Medical Virology, 2002, Jun, 67(2):217–223.

51. Chang LY, et al. Risk factors of enterovirus 71 infection and associated hand, foot, and mouth disease/herpangina in children during an epidemic in Taiwan. Pediatrics, 2002, Jun, 109(6):e88.



52. Suzuki Y, et al. Risk factors for severe hand foot and mouth disease. Pediatrics International 2010 Apr;52(2):203–7

# Section 2: Virology

## 2.1 Overview

The major etiological agents that cause HFMD are the human enteroviruses species A (HEV-A), particularly coxsackievirus A16 (CA16) and enterovirus 71 (EV71). These belong to the genus Enterovirus within the family Picornaviridae. Other HEV-A serotypes, such as Coxsackievirus A6 and Coxsackievirus A10, are also associated with HFMD and herpangina. While all these viruses can cause mild disease in children, EV71 has been associated with neurological disease and mortality in large outbreaks in the Asia Pacific region over the last decade (1–4).

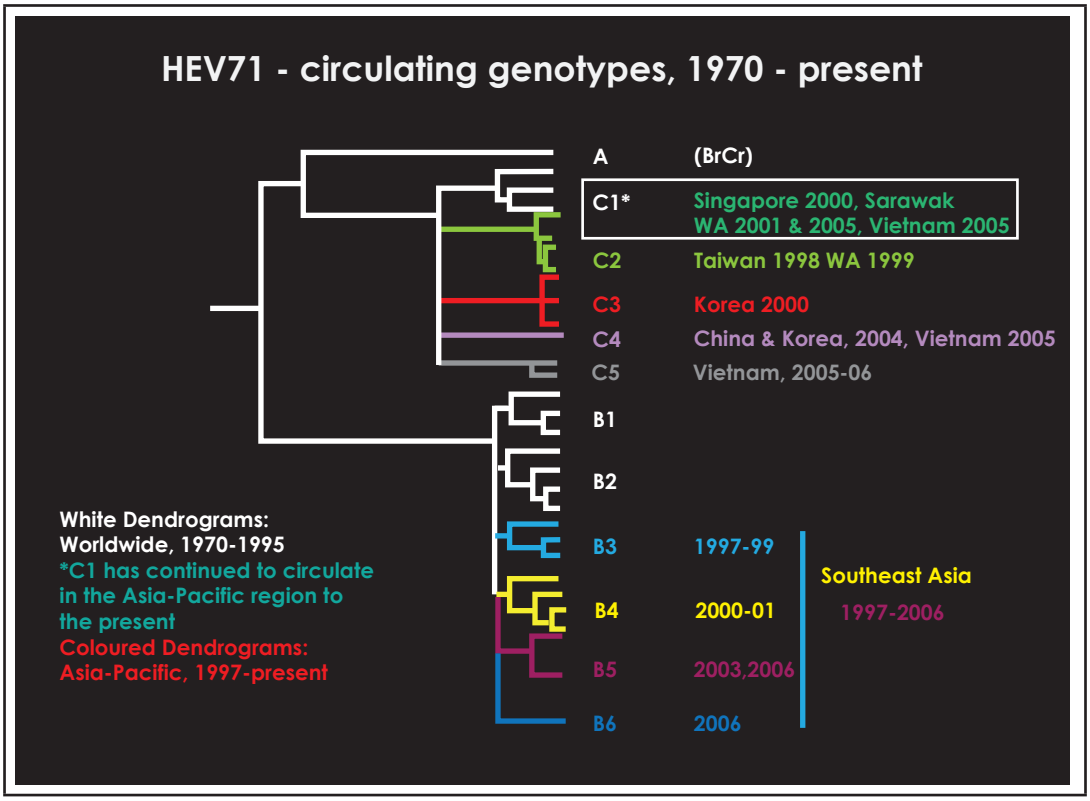
Enteroviruses are small viruses with virions that are about 30 nm in diameter and composed of four structural proteins called VP1, VP2, VP3 and VP4. VP1 is the major capsid protein on the surface of the virion, while VP4 is not exposed on the surface. Serotyping of human enteroviruses has traditionally been based on neutralization tests using specific antiserum pools: as such they are directed particularly at serological responses to the VP1 protein. More recently, due in part to limited access to serotyping antisera and improved accessibility to molecular technology, there has been a move towards using molecular typing methods. The gene encoding VP1 is the target gene most often used in molecular typing methods for enteroviruses. It is for this reason that a wealth of genetic sequence data on EV71 is available, enabling the genetic classification of virus strains in common circulation (5–7).



Ribonucleic acid (RNA) viruses, such as the enteroviruses, generate evolutionary changes fairly rapidly. Labels (genogroups) can be applied to clusters of epidemiologically related EV71 strains, generally those with up to a 5% nucleotide difference in the VP1 region. These genogroup divisions have no known significance for any enterovirus other than being a convenient label to reflect viral sequence clustering within a sea of genetic diversity. It is important to note that there is not yet evidence of any association between virulence and particular genogroups or subgenogroups of EV71.

Figure 1 on the succeeding page shows a phylogenetic tree containing the three genogroups of EV71: genogroups A, B and C. The prototype EV71 virus is BrCr, isolated in California in 1969. This is the only sampled virus of genogroup A (8).

**Figure 1: Circulating genotypes of HEV71 between 1970 and 2010**






Subgenogroup B1 was circulating in the United States of America, Europe, Japan and Australia in the 1970s, while subgenogroup B2 was sampled mainly in the United States in the 1980s. In 1997, when the first of several recent and large Asia Pacific outbreaks of EV71 occurred in Sarawak, Malaysia, the major circulating virus was a B genogroup virus that was clearly distinct from the genogroup B viruses that had been sampled in the 1970s and 1980s. This was then named subgenogroup B3. The virus was also found in the Malaysian peninsula and in Singapore (1, 2).

In 1998, another large outbreak of EV71 HFMD occurred in the Asia Pacific region, this time in Taiwan (China). The major circulating virus was from subgenogroup C2. This subgenogroup had also been found in Japan and was associated with an outbreak in Perth, Western Australia, in 1999, where genogroup B3 viruses were also circulating. In Taiwan (China) in 1998, subgenogroup B4 viruses were also sampled and, by 2000, those viruses had been associated with large outbreaks throughout the region, including in Japan, Malaysia and Singapore, as well as in Taiwan (China), where subgenogroup B4 viruses replaced the C2 viruses. In Sarawak, Malaysia, subgenogroup B4 was replaced by subgenogroup B5 in 2003, and this has since remained the dominant subgenogroup. Subgenogroup B5 viruses are also important circulating viruses in Japan, Singapore and Taiwan (China) (1–3, 9–21).

Subgenogroup C1 viruses were sampled in North America in the 1970s and 1980s. Throughout the 1990s, and up to the present day, they have been consistently identified in many parts of the world, including in Australia, Japan, Malaysia, New Zealand, Norway, Thailand and the United Kingdom, Despite being found in circulation in many countries, however, the subgenogroup C1 viruses have not caused large outbreaks during the last two decades. The subgenogroup C2 viruses that were replaced by B4 viruses in Taiwan (China) by 2000 have continued to be identified in Japan, Singapore and Thailand over the last few years.

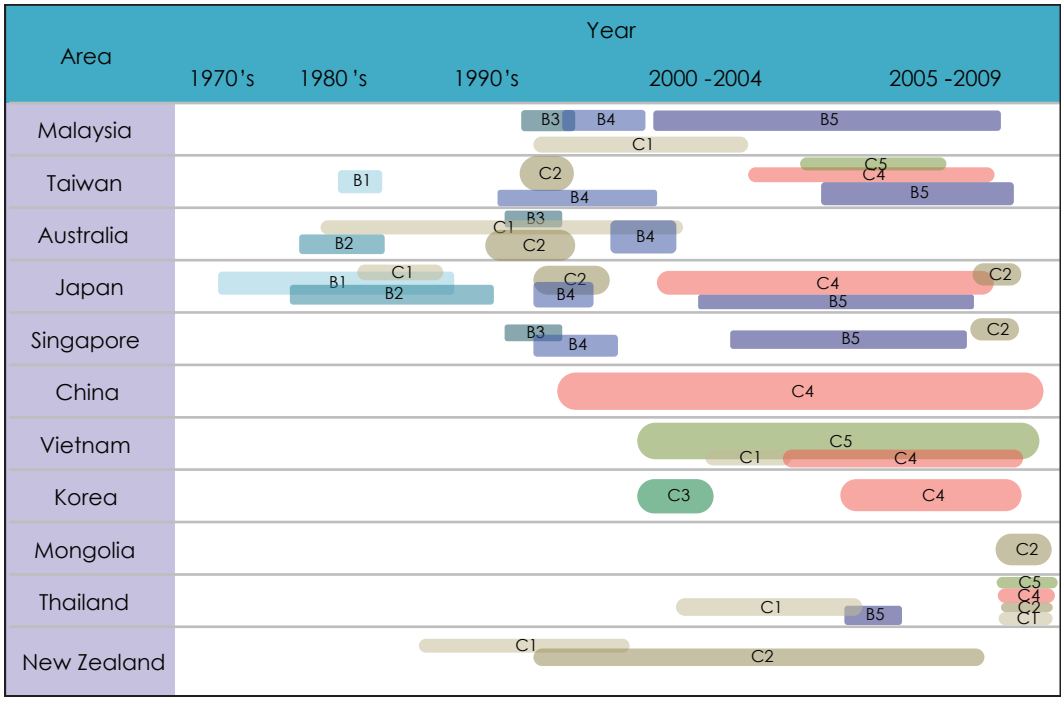
Viet Nam began investigating EV71 in 2005. It is interesting to note that, although C1 and C4 viruses do circulate in Viet Nam, the dominant subgenogroup has been, and remains, C5 (22). That subgenogroup emerged in Taiwan (China) in 2006.

A similar situation exists in China, where the single dominant subgenogroup C4 caused major outbreaks in 2008 and 2009 (23, 24). That subgenogroup was sampled in China and Japan in the late 1990s and early 2000s and was dominant in Taiwan (China) in 2004 and 2005. It is currently circulating in Japan, the Republic of Korea, Taiwan (China), Thailand and Viet Nam. The Republic of Korea recorded a subgenogroup C3 cluster of cases in 2000, when B4 viruses were dominant elsewhere in the region (25).

Figure 2 illustrates the temporal distribution of the different subgenogroups of EV71 circulating in various countries in the region. The figure includes data generated following the enhanced surveillance activities in operation in many countries since 1997. It is therefore important to note that there are gaps in the knowledge regarding circulating strains prior to that period.



**Figure 2: Recorded prevalence of EV71 subgenogroups in the Asia-Pacific region**



## 2.2 Virus receptor

Nishimura (26), Yamayoshi (27) and Yang (28), with their colleagues, recently independently demonstrated that human P-selectin glyprotein ligand-1 (PSGL-1), human scavenger receptor class B, member 2 (SCARB2), and sialic-acid-linked glycans act as functional receptors for EV71 by using different human cell lines and different cloning strategies. Identification of three structurally and functionally different receptors for EV71 may provide valuable insights into the molecular basis of EV71 infection, including HFMD and various neurological diseases.

## 2.3 Recombination

As with other enteroviruses, recombination has been observed in several EV71 field isolates (29–32). It has been observed most frequently in the 5'UTR (untranslated region) and 3'UTR regions; it has rarely been identified in the structural protein gene region. Although the effect of recombination on the virulence or transmissibility of EV71 is unknown, laboratory studies have shown that replacement of the 3' half of the genome of a non-recombinant EV71 field isolate with one derived from a HEV-B species virus significantly improves growth in cell culture compared with a non-recombinant strain (33).

## 2.4 Reservoir of EV71



EV71 replicates in the intestinal tract and is typically shed for between two and four weeks, and sometimes for as long as 12 weeks post-infection. Replication also occurs in the upper respiratory tract and the virus has been recovered from throat swabs for up to two weeks post-infection. Thus transmission can include faecal-oral and respiratory secretions through direct person-to-person contact, droplets or fomites. Factors that affect the transmission include level of hygiene, water quality, and the extent of crowding (34).

In general, the enteroviruses have a distinct seasonal pattern of circulation that varies by geographic area. In tropical and subtropical countries, circulation tends to be year round, with more outbreaks in the rainy season.

## References

1. McMinn P, et al. Phylogenetic analysis of enterovirus 71 strains isolated during linked epidemics in Malaysia, Singapore, and Western Australia. Journal of Virology, 2001, Aug, 75(16):7732–7738.

2. Cardosa MJ, et al. Molecular epidemiology of human enterovirus 71 strains and recent outbreaks in the Asia-Pacific region: comparative analysis of the VP1 and VP4 genes. Emerging Infectious Diseases, 2003, Apr, 9(4):461–468.

3. Shimizu H, et al. Molecular epidemiology of enterovirus 71 infection in the Western Pacific Region. Pediatrics International, 2004, Apr, 46(2):231–235.

4. Bible JM, et al. Genetic evolution of enterovirus 71: epidemiological and pathological implications. Reviews in Medical Virology, 2007, Nov–Dec, 17(6):371–379.

5. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. Journal of Clinical Microbiology, 2006, Aug, 44(8):2698–2704.

6. Oberste MS, et al. Improved molecular identification of enteroviruses by RT-PCR and amplicon sequencing. Journal of Clinical Virology, 2003, Apr, 26(3):375–377.

7. Oberste MS, et al. Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. Journal of Clinical Microbiology, 2000, Mar, 38(3):1170–1174.

8. Brown BA, et al. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. Journal of Virology, 1999, Dec, 73(12):9969–9975.

9. Tee KK, et al. Evolutionary genetics of human enterovirus 71: origin, population dynamics, natural selection, and seasonal periodicity of the VP1 gene. Journal of Virology, Apr, 84(7):3339–3350.

10. Huang SW, et al. Reemergence of enterovirus 71 in 2008 in taiwan: dynamics of genetic and antigenic evolution from 1998 to 2008. Journal of Clinical Microbiology, 2009, Nov, 47(11):3653–3662.

11. Iwai M, et al. Genetic changes of coxsackievirus A16 and enterovirus 71 isolated from hand, foot, and mouth disease patients in Toyama, Japan between 1981 and 2007. Japanese Journal of Infectious Diseases, 2009, Jul, 62(4):254–259.

12. Mizuta K, et al. Cross-antigenicity among EV71 strains from different genogroups isolated in Yamagata, Japan, between 1990 and 2007. Vaccine, 2009, May 21, 27(24):3153–3158.

13. Kung SH, et al. Genetic and antigenic analyses of enterovirus 71 isolates in Taiwan during 1998-2005. Clinical Microbiology and Infection, 2007, Aug, 13(8):782–787.

14. Hosoya M, et al. Genetic diversity of enterovirus 71 associated with hand, foot and mouth disease epidemics in Japan from 1983 to 2003. Pediatric Infectious Disease Journal, 2006, Aug, 25(8):691–694.

15. Podin Y, et al. Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: lessons from the first 7 years. BMC Public Health, 2006, 6:180.

16. Lin KH, et al. Evolution of EV71 genogroup in Taiwan from 1998 to 2005: an emerging of subgenogroup C4 of EV71. Journal of Medical Virology, 2006, Feb, 78(2):254–262.

17. Sanders SA, et al. Molecular epidemiology of enterovirus 71 over two decades in an Australian urban community. Archives of Virology, 2006, May, 151(5):1003–1013.

18. Mizuta K, et al. Frequent importation of enterovirus 71 from surrounding countries into the local community of Yamagata, Japan, between 1998 and 2003. Journal of Clinical Microbiology, 2005, Dec, 43(12):6171–6175.

19. Fujimoto T, et al. Outbreak of central nervous system disease associated with hand, foot, and mouth disease in Japan during the summer of 2000: detection and molecular epidemiology of enterovirus 71. Microbiology and Immunology, 2002, 46(9):621–627.

20. Chu PY, et al. Molecular epidemiology of enterovirus 71 in Taiwan. Archives of Virology, 2001, 146(3):589–600.

21. Shimizu H, et al. Enterovirus 71 from fatal and nonfatal cases of hand, foot and mouth disease epidemics in Malaysia, Japan and Taiwan in 1997-1998. Japanese Journal of Infectious Disease, 1999, Feb, 52(1):12–5.

22. Tu PV, et al. Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005. Emerging Infectious Diseases, 2007, Nov, 13(11):1733–1741.

23. Yang F, et al. Enterovirus 71 outbreak in the People's Republic of China in 2008. Journal of Clinical Microbiology, 2009, Jul, 47(7):2351–2352.

24. Zhang Y, et al. An outbreak of hand, foot, and mouth disease associated with subgenotype C4 of human enterovirus 71 in Shandong, China. Journal of Clinical Virology, 2009, Apr, 44(4):262–267.

25. Jee YM, et al. Genetic analysis of the VP1 region of human enterovirus 71 strains isolated in Korea during 2000. Archives of Virology, 2003, Sep, 148(9):1735–1746.

26. Nishimura Y, et al. Human P-selectin glycoprotein ligand-1 is a functional receptor for enterovirus 71. Nature Medicine, 2009, Jul, 15(7):794–797.



27. Yamayoshi S, et al. Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nature Medicine, 2009, Jul, 15(7):798–801.

28. Yang B, Chuang H, Yang KD. Sialylated glycans as receptor and inhibitor of enterovirus 71 infection to DLD-1 intestinal cells. Virology Journal, 2009, 6:141.

29. Chen X, et al. Analysis of recombination and natural selection in human enterovirus 71. Virology, 2010, Mar 15, 398(2):251–261.

30. Ding NZ, et al. Appearance of mosaic enterovirus 71 in the 2008 outbreak of China. Virus Research, 2009, Oct, 145(1):157–161.

31. Huang SC, et al. Appearance of intratypic recombination of enterovirus 71 in Taiwan from 2002 to 2005. Virus Research, 2008, Feb, 131(2):250–259.

32. Yoke-Fun C, AbuBakar S. Phylogenetic evidence for inter-typic recombination in the emergence of human enterovirus 71 subgenotypes. BMC Microbiology, 2006, 6:74.

33. Phuektes P. Development of a reverse genetics system for Human enterovirus 71 (HEV71) and the molecular basis of its growth phenotype and adaptation to mice. PhD Thesis, Murdoch University, Australia. 2009.

34. Ooi EE, et al. Seroepidemiology of human enterovirus 71, Singapore. Emerging Infectious Diseases, 2002, Sep, 8(9):995–997.

# Section 3: Laboratory Diagnosis

## 3.1 Overview

Rapid identification of the causative agent of HFMD, particularly whether there is EV71 infection, can help clinical management by focusing attention on the possible complications. When patients present with other manifestations of EV71, such as CNS disease or cardiovascular collapse, rapid virological diagnosis is even more helpful because of the broad differential for those conditions and the specific treatments available (1). Identification of the agents responsible for outbreaks of HFMD is also important with regards to predicting the severity of the outbreak and initiating appropriate public health interventions. As with other enteroviruses, confirmatory diagnosis has traditionally been based on cell culture and virus isolation and identification, both of which are time-consuming and resource-intensive. Modern molecular techniques have been developed and are now in use in some laboratories. Effective and accurate virological diagnosis depends on the correct timing and collection of appropriate clinical specimens, and their transport to the laboratory under optimal conditions. This requires close cooperation between laboratory experts, epidemiologists and clinicians.



## 3.2 Laboratory safety

To ensure safe handling of clinical samples and infectious materials, laboratories should follow the necessary biosafety, chemical, fire and electrical safety requirements to protect staff, the community and the environment (2). The directors and all staff of diagnostic laboratories should be familiar with international and local biosafety requirements, including safe handling and transportation of clinical samples and infectious materials, including good microbiological techniques and basic biosafety level of the facilities, normally BSL-2 for primary isolation (3).

## 3.3 Clinical samples

Throat and vesicle (if available) swab samples in virus transportation medium (VTM) are considered to be the most useful specimens in terms of the rate of virus detection of HFMD (4) and in terms of both inpatient and outpatient availability. EV71 can be shed in the stool for several weeks and stool (rectal swab) samples are also appropriate clinical specimens for virus detection and/or isolation.

The risk of recent coincidental infection, rather than casual infection, should be considered for some clinical samples, particularly for stool samples. Vesicle isolates are the most useful, because they always represent current systemic infection (4). Given the potential number of samples, from large numbers of patients, laboratories can become overwhelmed.

To increase the possibility of enterovirus detection, collecting throat swab samples for all patients plus swabs from at least two vesicles or from the rectum for patients with no vesicles is recommended. The virus detection rates of EV71 and CA16 from cerebrospinal fluid (CSF) samples are rather low (less than 5%) (4, 5). However, CSF samples are useful for virus detection of other enteroviruses, particularly for HEV-B-associated aseptic meningitis. Serum samples from inpatients may be useful for serological diagnosis, but the reliability of serological tests for EV71 infection requires careful evaluation.

## 3.4 Laboratory diagnosis methods

As for other enterovirus infections, including poliomyelitis, confirmed diagnoses based on cell culture, virus isolation and identification of enteroviruses is still the standard method for laboratory diagnosis. While virus isolates are needed for further molecular characterizations of EV71, virus isolation and identification are generally laborious and time-consuming and such methods are not practical for clinical decision-making. Although rapid laboratory diagnostic methods using clinical specimens are available, standardization of the methods and comparative evaluation of the reliability of the rapid tests are still needed.



### *Virus isolation*

Some clinical specimens require appropriate pre-treatment (complete mixing, filtration, chloroform treatment) before inoculation. A number of human and non-human primate cell lines are available for virus isolation and identification of enteroviruses. Of these cell lines, RD (human rhabdomyosarcoma cells) and Vero (African green monkey kidney cells) cells have been widely used for virus isolation of EV71 and CA16 because of their relatively high sensitivity and the apparent cytopathic effect (CPE) induced by EV71 and CA16. RD cells are available from the Global Polio Laboratory Network and the quality control of the cells is routinely carried out according to the Polio Laboratory Manual (3) to ensure sensitivity against polioviruses. The quality of cell cultures used in virological investigation is important for the standardization of enterovirus isolation and its characterization.

It is important to use several different human and non-human primate cell lines to increase the possibility of virus isolation in cell cultures. Additional cell lines (including MRC-5, HEL, HeLa, L20B) can also be used for virus isolation of EV71 and other

enteroviruses, including polioviruses. Although the receptor-specificities of EV71 isolates and the other HEV-A strains would need to be characterized, mouse cell lines expressing functional cellular receptors for EV71 (human P-selectin glycoprotein ligand-1 and human scavenger receptor class B, member 2) would be useful for the selective isolation of EV71 and/or CA16 from clinical specimens (6, 7). Suckling mice (less than 48 hours old) are still useful for virus isolation of some HEV-A isolates, particularly for clinical samples from herpangina cases (8). However, virus isolation and identification using suckling mice is time-consuming and requires specific human and equipment resources.

### *Identification of virus isolates*

#### Neutralization test

Following virus isolation in cultured cells, the serotype of EV71 and CA16 isolates can be identified by a conventional neutralization test with a qualified type-specific antiserum. The type-specific antisera against HEV-A strains, including EV71 and CA16, are not commercially available and the supply of in-house antisera is therefore generally limited. The neutralization test is still a reliable method for enterovirus identification, but it can take up to a week (5–7 days) to complete the assay.

#### Reverse transcription – polymerase chain reaction (RT-PCR) and sequencing

Following virus isolation in cultured cells, reverse transcription polymerase chain reaction (RT-PCR) amplification of viral RNA and sequencing of the deoxyribonucleic acid (DNA) amplicons, using various gene targets (5' untranslated region [5'UTR], VP1, VP4/VP2 genes), have been widely used for molecular identification of enterovirus isolates. The advantage of the RT-PCR and sequencing strategy is the universal detection and amplification of enterovirus gene targets, regardless of the serotypes and genotypes of enteroviruses, possibly including newly emerged variants and new serotypes of enteroviruses (9–11). While sequencing of the shorter VP4 region (or VP4 plus partial VP2) is sufficient for regular surveillance, confirmation of the data for molecular epidemiological research should use the VP1 region of the genome. This is also important as the VP1 gene is the most exposed and immunodominant of the capsid proteins and is therefore most likely to change in response to immunogenic pressure (12, 13). The RT-PCR and sequencing strategy may depend on routine access to the sequencing facility at diagnostic laboratories and requires higher installation and running costs. Specific training activities for laboratory experts and technicians on molecular identification of enteroviruses are needed to minimize the risk of cross-contamination and to ensure the reliability of identification, including sequence analysis and data management.



#### EV71-specific RT-PCR

Following virus isolation in cultured cells, RT-PCR amplification of viral RNA using EV71-specific primers is useful for the molecular identification of EV71 (14). The

EV71-specific RT-PCR amplification is a rapid and simple identification method and requires lower installation and running costs. EV71-specific primers should be revised according to the sequences of newly emerging EV71 genogroups and variants to maintain the reliability of the EV71-specific RT-PCR test (14). General training activities for laboratory technicians on RT-PCR are needed to minimize the risk of cross-contamination.

#### Indirect immunofluorescence assay

Indirect immunofluorescence assay (IFA) tests using anti-EV71 monoclonal antibodies can provide rapid, presumptive EV71 identification. The IFA method is technically simple and rapid, and the antibodies are commercially available (15), although relatively expensive to purchase.

### *Rapid diagnosis directly from clinical samples*

#### Nested RT-PCR

Targeting of conserved 5'UTR has been widely used for the universal detection of enteroviruses. However, 5'UTR RT-PCR methods are inappropriate for serotype-specific detection and identification of EV71. Technical improvement of RT-PCR methods based on the VP1 region has enabled partial VP1 sequencing using consensus-degenerate hybrid oligonucleotide primer (CODEHOP) with a high sensitivity and broader specificity for all known enterovirus serotypes (16). Enteroviruses can therefore be identified by VP1 sequences derived from the CODEHOP PCR products directly from clinical samples. Multiplex RT-PCR methods have been developed for the identification of EV71 and CA16 (17) and for the specific detection of EV71 and CA16 directly from clinical samples (18).



#### Real-time PCR

Real-time RT-PCR systems have recently become available to diagnostic laboratories for rapid and specific detection of a number of viral infectious diseases. One-tube real-time RT-PCR systems can reduce the risk of cross-contamination when compared with conventional RT-PCR, particularly nested PCR systems. While several different EV71-specific real-time RT-PCR systems have been reported (19–21) the reliability of such systems needs to be addressed using different genogroups of EV71, CA16, HEV-A strains, and clinical samples. In general, EV71-specific real-time RT-PCR primers and probes should be revised according to the sequences of newly identified EV71 genogroups and variants to maintain the reliability of EV71-specific real-time RT-PCR.

#### New diagnostic approaches

In addition to EV71-specific real-time RT-PCR systems, several other promising options for rapid and convenient (bedside) diagnosis are under investigation. Although rapid viral antigen-detection kits using immunochromatography are commercially available for a number of viral infectious diseases, EV71 antigen-detection kits have not yet

been developed, mainly due to the low sensitivity from clinical samples (22). While the reverse transcription loop-mediated isothermal amplification (RT-LAMP) method can be used as a rapid and sensitive isothermal detection method for enteroviruses, it is not specific to certain serotypes (genotypes) of enterovirus (23).

**Evaluation of the reliability of new laboratory diagnostic methods**

To encourage the development of rapid and convenient (bedside) diagnostic kits for EV71 infection in the region, a standardized evaluation system to compare the sensitivity and specificity of diagnostic methods is critical. For the evaluation of molecular identification methods, establishment of an EV71 strain bank, containing all available EV71 subgenogroups (A, B1-B5, C1-C5, plus newly emerging genogroups), should be considered. Linking this to a quality assurance system and networking of laboratories would ensure standards for identification of enteroviruses and their subgroups can be applied in the future.

### *Molecular epidemiological analysis (genotyping) of EV71 strains*

A powerful tool for tracking the circulation of EV71 strains is the molecular characterization of EV71 genomes (genotyping). By comparing the extent of genetic changes that are observed between EV71 strains, the geographic and evolutional origin of a virus can be determined. Based upon a capsid VP1 sequence database of EV71 strains, it is possible to develop rapid approaches to tracking the regional transmission and current prevalence of EV71 strains (see the section on Virology). For detailed molecular epidemiological analysis of EV71 strains, the entire VP1 sequence should be determined and analysed, although partial VP1 sequencing would be sufficient to genotype each EV71 isolate.



### *Serological analysis*

Testing for neutralizing antibodies against enteroviruses is not recommended for routine use in the diagnosis of enterovirus infections. Paired serum samples are collected for serological diagnosis of certain serotypes of enterovirus, but interpretation of serum antibody titers is sometimes difficult. On the other hand, the neutralizing test against EV71 would be useful for evaluating of immunity levels for EV71 infection within communities (23, 24), as well as for monitoring the cross-reactivity of serum among different genogroups of EV71 (25).

Serum samples from inpatients could be useful for the rapid immunoglobulin M (IgM) detection of EV71 (22, 26), but the specificity and sensitivity of serological tests for EV71 infection remains an area where careful evaluation is needed.

## References


1. Ooi MH, et al. Clinical features, diagnosis and management of human enterovirus 71 infection. Lancet Neurology, 2010, 9(11):1097–1105

2. Laboratory biosafety manual, 3rd edition. Geneva, World Health Organization, 2004.

3. Polio laboratory manual, 4th edition. Geneva, World Health Organization, 2004.

4. Ooi MH, et al. Evaluation of different clinical sample types in diagnosis of human enterovirus 71-associated hand-foot-and-mouth disease. Journal of Clinical Microbiology, 2007, Jun, 45(6):1858–1866.

5. Chan KP, et al. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerging Infectious Diseases, 2003, Jan, 9(1):78–85.

6. Nishimura Y, et al. Human P-selectin glycoprotein ligand-1 is a functional receptor for enterovirus 71. Nature Medicine, 2009, Jul, 15(7):794–797.

7. Yamayoshi S, et al. Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nature Medicine, 2009, Jul, 15(7):798–801.

8. Shima T, et al. Enterovirus detection status from patients with herpangina and hand, foot and mouth disease in Kanagawa Prefecture, Japan. Japanese Journal of Infectious Disease, 2007, Feb, 60(1):63–64.

9. Oberste MS, et al. Typing of human enteroviruses by partial sequencing of VP1. Journal of Clinical Microbiology, 1999, May, 37(5):1288–1293.

10. Oberste MS, et al. Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. Journal of Virology, 1999, Mar, 73(3):1941–1948.

11. Brown BA, et al. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. Journal of Virology, 1999, Dec, 73(12):9969–9975.

12. Cardosa MJ, et al. Molecular epidemiology of human enterovirus 71 strains and recent outbreaks in the Asia-Pacific region: comparative analysis of the VP1 and VP4 genes. Emerging Infectious Diseases, 2003, Apr, 9(4):461–468.

13. Perera D, et al. A comparison of the VP1, VP2, and VP4 regions for molecular typing of human enteroviruses. Journal of Medical Virology, 2010, Apr, 82(4):649–657.

14. Perera D, et al. Incorrect identification of recent Asian strains of Coxsackievirus A16 as human enterovirus 71: improved primers for the specific detection of human enterovirus 71 by RT PCR. BMC Infectious Diseases, 2004, May 4;4:11.

15. APNET. [cited 2010 July 30]; Available from: http://sydney.edu.au/medicine/apnet/diagnostics/index.php.

16. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. Journal of Clinical Microbiology, 2006, Aug, 44(8):2698–2704.

17. Chiueh TS, et al. Multiplex Reverse Transcription-semi-nested PCR for Differentiating Enterovirus 71, Coxsackievirus A16, and polioviruses from other enteroviruses. Journal of Medical Sciences, 2001, 21(6):277–282.

18. Chen TC, et al. Combining multiplex reverse transcription-PCR and a diagnostic microarray to detect and differentiate enterovirus 71 and coxsackievirus A16. Journal of Clinical Microbiology, 2006, Jun, 44(6):2212–2219.

19. Tan EL, et al. Specific detection of enterovirus 71 directly from clinical specimens using real-time RT-PCR hybridization probe assay. Molecular and Cellular Probes, 2006, Apr, 20(2):135–140.

20. Tan EL, et al. Rapid detection of enterovirus 71 by real-time TaqMan RT-PCR. Journal of Clinical Virology, 2008, Jun, 42(2):203–206.

21. Xiao XL, et al. Simultaneous detection of human enterovirus 71 and coxsackievirus A16 in clinical specimens by multiplex real-time PCR with an internal amplification control. Archives of Virology, 2009, 154(1):121–125.

22. Wu H-S (Centers for Disease Control, Taiwan[China]). Personal communication to H. Shimizu,2010.

23. Arita M, et al. Development of a reverse transcription-loop-mediated isothermal amplification (RT-LAMP) system for a highly sensitive detection of enterovirus in the stool samples of acute flaccid paralysis cases. BMC Infectious Diseases, 2009, 9:208.

24. Ooi EE, et al. Seroepidemiology of human enterovirus 71, Singapore. Emerging Infectious Diseases, 2002, Sep, 8(9):995–997.

25. Mizuta K, et al. Cross-antigenicity among EV71 strains from different genogroups isolated in Yamagata, Japan, between 1990 and 2007. Vaccine, 2009, May 21, 27(24):3153–3158.

26. Wang SY, et al. Early and rapid detection of enterovirus 71 infection by an IgM-capture ELISA. Journal of Virological Methods, 2004, Jul, 119(1):37–43.

# Section 4: Pathogenesis in EV71 Infection

## 4.1 Overview

There is very little information concerning the pathology of uncomplicated EV71-associated HFMD and herpangina. In a mouse model, the virus was shown to infect skin, suggesting that human skin or oral mucosa lesions could also be the result of direct infection of squamous cells (1). While it is possible that pathological changes are confined to skin rashes and oral lesions, there is a lack of information available from skin or oral mucosa biopsies.

The neurovirulence factors of EV71 in human infections are still unknown. So far, there is no convincing evidence of specific virus genome mutations that may facilitate infection of the human central nervous system (CNS). Other factors, such as higher initial infective doses leading to severe disease, are also possible. Certain cytokines, chemokines, alterations of cell mediated immunity and human leukocyte antigen (HLA) subtypes appear to be associated with a higher incidence of cardiopulmonary complications. The relationship between pathogenesis and distribution of viral entry receptors (scavenger receptor B2, P-selectin glycoprotein ligand-1 and sialic acid-linked glycans) and host factors, such as gender and age group, is unknown.



The pathological findings in the CNS in fatal cases of EV71 are stereotyped and consist of neuronophagia, perivascular cuffing, focal oedema and infiltration of inflammatory cells. Viral cytolysis appears to be an important mechanism for neuronal damage. The most severe inflammation is found in the hypothalamus, brain stem, spinal cord and cerebellar dentate nucleus. Medullary inflammation and findings in the lungs and heart are consistent with neurogenic pulmonary oedema as a cause of death. Animal studies confirm some of the human autopsy findings and suggest that motor pathways may play an important role in viral transmission in the CNS.

## 4.2 Virus neuro-virulence factors

### *Viral genome mutations*

To date, no convincing evidence of specific virus genome mutations that confer neurovirulence in humans has been reported.

However, clinical epidemiological evidence from outbreaks in Perth, Australia, and Sarawak, Malaysia, suggests that there may be differences between genogroups in terms of biological behaviour and virulence (2). In Perth, in 1999, subgroups B3 and C2 were both circulating (3,4).

C2 viruses were linked to the 1998 epidemic in Taiwan (China) and were almost exclusively isolated from children with severe neurological disease; only one isolate came from a case of uncomplicated HFMD (3, 4). By contrast, B3 viruses that were similar to those from the 1997 Sarawak epidemic, were isolated mainly from children with uncomplicated HFMD or aseptic meningitis, or those with neurological complications, none of whom died (5). In two discrete epidemics in Sarawak in which either B4 or B5 viruses were predominant, a study of 277 children with EV71-associated HFMD showed that B4 viruses were less likely than B5 viruses to cause CNS infection or be part of a family cluster (6).

More extensive virological surveillance and genetic analysis from the region may provide further evidence of potential genetic determinants of virulence. Animal studies, however, have demonstrated that there are four co-expressed mutations derived from the Sabin poliovirus type 1 attenuated EV71 in both cynomolgus monkeys (7) and in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice (8). A 3D polymerase gene mutation has been shown to introduce a temperature-sensitive phenotype in cell culture and an attenuation of virulence in newborn mice (9).



### *Other factors*

Other factors, such as higher infective virus doses leading to more severe illness, are possible and should be investigated further.

## 4.3 Host factors

### *Immunopathology*

There have been relatively few studies of the human immune response to EV71 infection. Induction of specific cytokines and chemokines has been associated with certain adverse outcomes of EV71 infection, most notably pulmonary oedema. Abnormal elevation of cytokines may be one mechanism for pulmonary oedema in fatal cases. In at least two studies, cerebrospinal fluid levels of interleukin (IL)-6 were found to be raised in cases with pulmonary oedema (10, 11). Higher levels of interferon gamma were also found in cerebrospinal fluid in similar cases (11). Levels of IL-6, tumour necrosis factor alpha, IL-1 beta (12) and IL-10 (11) were also found to be higher in the serum of pulmonary oedema cases. Furthermore, levels of chemokines, IL-8, interferon induced protein (IP-10), monocyte chemoattractant protein (MCP-1) and monokine induced by interferon gamma (MIG) were found to be higher in serum, and MIG in both serum and cerebrospinal fluid in pulmonary oedema (13).

With regards to cell-mediated immunity, a reduction of T lymphocytes and NK cells has been reported in patients with pulmonary oedema (14). Another study demonstrated reduced production of interferon gamma, IL-1 beta, IL-6 and macrophage inflammatory protein-1 alpha in stimulated peripheral mononuclear cells in patients with pulmonary oedema (15).

HLA-A33 is reported to be associated with susceptibility to EV71 infection, particularly in Asian populations where the HLA prevalence (17%-35%) is higher than in Caucasian populations. In addition, HLA-A2 may possibly be linked to increased risk of cardiopulmonary complications (16).

### *Virus receptors on host target cells*

In 2009, three receptors were identified that facilitate virus entry into host cells: scavenger receptor B2 (17) and P-selectin glycoprotein ligand-1 (18). Respectively, those receptors have been reported to be ubiquitously expressed or limited to leukocytes. Whether the presence or absence of those receptors in human tissues might impact on viral pathogenesis has not been investigated. Similarly, viral replication sites and viral transmission routes within the body may be related to the presence or absence of receptors in susceptible tissues.

### *Other host factors*



Other host factors that may be important for susceptibility to infection could include gender, as a higher incidence of severe disease has been reported in male children. Moreover, the relative immaturity of the innate and adaptive immune system in children aged between one and five years could account for the higher incidence of neurological complications in that age group. Evidence for this is still lacking.

## 4.4 Pathological findings

### *Human autopsies*

Autopsies conducted in mainland China, Malaysia, Singapore and Taiwan (China) have been useful in improving understanding of the pathogenesis of severe disease and the underlying pathological insult leading to death. The inflammatory response seen in EV71 encephalomyelitis is typical of viral encephalitides, the features of which include neuronophagia, perivascular cuffing, focal oedema and macrophage/microglia infiltration (Figure 3) (19-22). The main areas of inflammation appear to be localized to the hypothalamus, brain stem, spinal cord and cerebellar dentate nucleus. Although mild inflammation can be seen in the cerebral cortex (especially the motor cortex), it is entirely absent from the anterior pontine nuclei and cerebellar hemisphere. The topographic distribution of inflammation and virus suggests retrograde peripheral motor nerve viral spread into the CNS. Within the CNS, other neural pathways, including motor pathways, could be involved (19). Viral antigens/RNA have been demonstrated

**Figure 3:**

Perivascular cuffing and parenchymal infiltration by inflammatory cells in the human medulla in Enterovirus 71 encephalomyelitis (Haematoxylin and eosin stain (H&E), magnification x 10 objective)

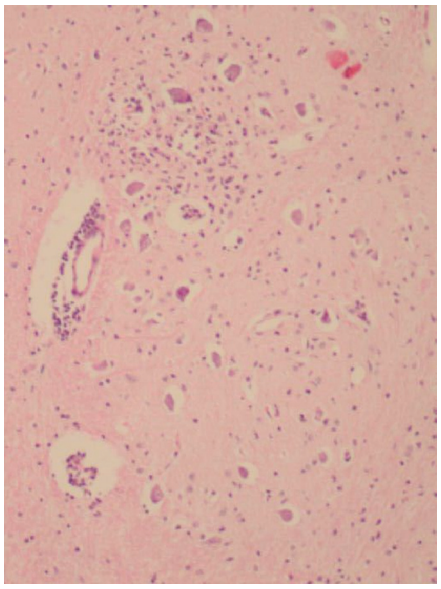
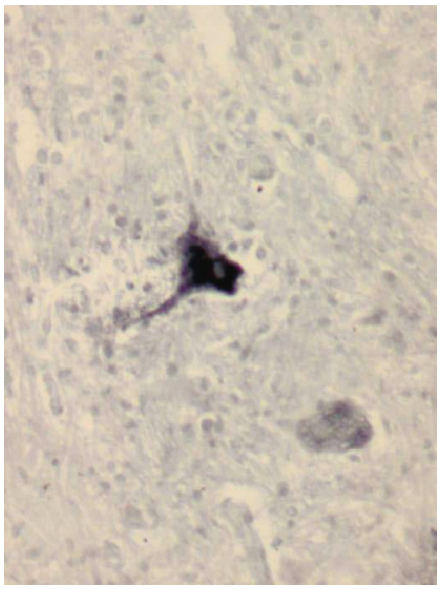


**Figure 4:**

Enterovirus 71-infected neuron showing cytoplasmic viral RNA (in situ hybridization, magnification x 40 objective)





in neuronal body and processes (Figure 4), and in macrophages participating in the neuronophagia seen in inflamed areas. This provides evidence that neurons are the main viral targets and that viral cytolysis is an important mechanism for neuronal injury. The mechanism could involve apoptosis, as the virus is able to induce apoptosis in neuronal and glial cell cultures (23, 24). Pathological evidence of medullary inflammation and destruction is consistent with neurogenic pulmonary oedema as the primary cause of death.

Lung findings at autopsy were found to be consistent with interstitial pneumonitis, pulmonary oedema and haemorrhage, and myocardial congestion (20). Other studies have found that, apart from pulmonary oedema, there was no evidence of pulmonary inflammation or viral antigen in the lung parenchyma (25). Myocarditis and myocardial infection are generally absent. The pathogenesis of death resulting from pulmonary oedema alone or pulmonary oedema with concurrent pulmonary haemorrhage during EV71 infection is not completely understood. Based on the findings above, however, myocarditis is unlikely to be the cause of death.

### *Animal models*

Monkey (26, 27) and mouse models (1, 28-30) have been used to study the neurovirulence of EV71. In such models, the CNS pathology has, so far, confirmed that EV71 is neuronotropic. A major drawback of the monkey models is that monkeys are refractory to infection by the oral route.

In one mouse model of EV71 encephalomyelitis, the distribution of virus infiltration and inflammation was similar to that seen in human encephalomyelitis, suggesting that motor pathways are important for viral transmission into and within the CNS (30). Viral antigens were found in the skeletal muscle and brown fat, but this has not been reported in human EV71 infections. Infectivity studies show that the skeletal muscles are major primary virus replication sites for EV71 in mice and that myositis is a primary cause of death (29, 30). The currently available animal models could be used for further investigation of disease pathogenesis and for anti-viral drug and vaccine testing.

## References


1. Chen YC, et al. A murine oral enterovirus 71 infection model with central nervous system involvement. Journal of General Virology, 2004, Jan, 85(Pt 1):69–77.

2. Solomon T, et al. Virology, epidemiology, pathogenesis and control of entervirus 71. Lancet infectious diseases, 2010: 10(11):778–790.

3. McMinn P, et al. Phylogenetic analysis of enterovirus 71 strains isolated during linked epidemics in Malaysia, Singapore, and Western Australia. Journal of Virology, 2001, Aug, 75(16):7732–7738.

4. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. FEMS Microbiology Reviews, 2002, Mar, 26(1):91–107.

5. McMinn P, et al. Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. Clinical Infectious Diseases, 2001, Jan 15, 32(2):236–242.

6. Ooi MH, et al. Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. Clinical Infectious Diseases, 2007, Mar 1, 44(5):646–656.

7. Arita M, et al. Temperature-sensitive mutants of enterovirus 71 show attenuation in cynomolgus monkeys. Journal of General Virology, 2005, May, 86(Pt 5):1391–1401.

8. Arita M, et al. Cooperative effect of the attenuation determinants derived from poliovirus sabin 1 strain is essential for attenuation of enterovirus 71 in the NOD/SCID mouse infection model. Journal of Virology, 2008, Feb, 82(4):1787–1797.

9. Kung YH, et al. Introduction of a strong temperature-sensitive phenotype into enterovirus 71 by altering an amino acid of virus 3D polymerase. Virology, 2010, Jan 5, 396(1):1–9.

10. Lin TY, et al. Different proinflammatory reactions in fatal and non-fatal enterovirus 71 infections: implications for early recognition and therapy. Acta Paediatrica, 2002, 91(6):632–635.

11. Wang SM, et al. Cerebrospinal fluid cytokines in enterovirus 71 brain stem encephalitis and echovirus meningitis infections of varying severity. Clinical Microbiology and Infection, 2007, Jul, 13(7):677–682.

12. Lin TY, et al. Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. Clinical Infectious Diseases, 2003, Feb, 36(3):269–274.

13. Wang SM, et al. Acute chemokine response in the blood and cerebrospinal fluid of children with enterovirus 71-associated brainstem encephalitis. Journal of Infectious Diseases, 2008, Oct 1, 198(7):1002–1006.

14. Wang SM, et al. Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. Journal of Infectious Diseases, 2003, Aug 15, 188(4):564–570.

15. Chang LY, et al. Status of cellular rather than humoral immunity is correlated with clinical outcome of enterovirus 71. Pediatric Research, 2006, Oct, 60(4):466–471.

16. Chang LY, et al. HLA-A33 is associated with susceptibility to enterovirus 71 infection. Pediatrics, 2008, Dec, 122(6):1271–1276.

17. Yamayoshi S, et al. Scavenger receptor B2 is a cellular receptor for enterovirus 71. Nature Medicine, 2009, Jul, 15(7):798–801.

18. Nishimura Y, et al. Human P-selectin glycoprotein ligand-1 is a functional receptor for enterovirus 71. Nature Medicine, 2009, Jul, 15(7):794–797.

19. Wong KT, et al. The distribution of inflammation and virus in human enterovirus 71 encephalomyelitis suggests possible viral spread by neural pathways. Journal of Neuropathology and Experimental Neurology, 2008, Feb, 67(2):162–169.

20. Chong CY, et al. Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases. Acta Paediatrica, 2003, Oct, 92(10):1163–1169.

21. Yang Y, et al. Neuropathology in 2 cases of fatal enterovirus type 71 infection from a recent epidemic in the People's Republic of China: a histopathologic, immunohistochemical, and reverse transcription polymerase chain reaction study. Human Pathology, 2009, Sep, 40(9):1288–1295.

22. Shieh WJ, et al. Pathologic studies of fatal cases in outbreak of hand, foot, and mouth disease, Taiwan. Emerging Infectious Diseases, 2001, Jan-Feb, 7(1):146–148.

23. Li ML, et al. The 3C protease activity of enterovirus 71 induces human neural cell apoptosis. Virology, 2002, Feb 15, 293(2):386–395.

24. Shih SR, et al. Viral protein synthesis is required for Enterovirus 71 to induce apoptosis in human glioblastoma cells. Journal for Neurovirology, 2008, Jan, 14(1):53–61.

25. Lum LC, et al. Fatal enterovirus 71 encephalomyelitis. Journal of Pediatrics, 1998, Dec, 133(6):795–798.

26. Nagata N, et al. Differential localization of neurons susceptible to enterovirus 71 and poliovirus type 1 in the central nervous system of cynomolgus monkeys after intravenous inoculation. Journal of General Virology, 2004, Oct, 85(Pt 10):2981–2989.

27. Nagata N, et al. Pyramidal and extrapyramidal involvement in experimental infection of cynomolgus monkeys with enterovirus 71. Journal of Medical Virology, 2002, Jun, 67(2):207–216.

28. Wang YF, et al. A mouse-adapted enterovirus 71 strain causes neurological disease in mice after oral infection. Journal of Virology, 2004, Aug, 78(15):7916–7924.

29. Chua BH, et al. The molecular basis of mouse adaptation by human enterovirus 71. Journal of General Virology, 2008, Jul, 89(Pt 7):1622–1632.

30. Ong KC, et al. Pathologic characterization of a murine model of human enterovirus 71 encephalomyelitis. Journal of Neuropathology and Experimental Neurology, 2008, Jun, 67(6):532–542.

# Section 5: Clinical Features and Case Management

## 5.1 Case definitions

Hand, foot and mouth disease is characterized by a brief febrile illness in children and typical skin rash, with or without mouth ulcers. Typically, the rash is papulovesicular and affects the palms or soles of the feet, or both. In some cases the rash may be maculopapular without vesicles, and may also involve the buttocks, knees or elbows, particularly in younger children and infants.

Herpangina is also characterized by fever and multiple, painful mouth ulcers, predominantly affecting the posterior oral cavity, including the anterior pharyngeal folds, uvula, tonsils and soft palate. In some children, the mouth ulcers can affect other parts of the mouth, including the buccal mucosa and tongue, with relative sparing of the posterior aspect of the oral cavity.



In practice it is not uncommon for children to complain first of painful oral ulcers before typical skin lesions appear over the palms and soles a day or two later. From a clinical perspective, HFMD and HA could be considered to represent both ends of a spectrum of mucocutaneous manifestations in a childhood febrile rash syndrome, where herpangina with isolated oral mucosal involvement is at one end, and HFMD with a combination of oral lesions and skin changes affecting palms and soles at the other.

Both HFMD and HA are caused by HEV-A, which includes CA (serotypes 2-8, 10, 12, 14, 16) and EV71. The enteroviruses often co-circulate during outbreaks of HFMD/HA and result in clinically indistinguishable mucocutaneous lesions. In most instances, the acute enteroviral infection is a benign, self-limiting illness. The skin lesions heal spontaneously without scarring. Secondary bacterial skin infection is very unusual. The most common clinical problem associated with HFMD/HA is dehydration, a result of inadequate intake of fluid secondary to odynophagia caused by painful mouth ulcers. However, recent epidemics of HFMD in Asia have shown that infection caused by EV71, in contrast to that caused by CA viruses, may involve the CNS and result in severe, and sometimes fatal, systemic complications in a small proportion of children, particularly those aged five years or younger (1-3).

Approximately 10%-30% of hospitalized cases during EV71-associated HFMD epidemics in Asia have developed a spectrum of CNS complications, including aseptic meningitis, encephalitis and acute flaccid paralysis (3-7). Brainstem encephalitis, a distinctive form of encephalitis with stereotypic neuropathological characteristics (8, 9), has become the hallmark of severe EV71-associated HFMD in the recent recurrent EV71 epidemics in Asia, which began in the late 1990s. A detailed study of clinical features of cases of HFMD with CNS involvement during the 1998 epidemic in Taiwan (China) showed that recurrent myoclonus was the most common neurological sign. Tremors, ataxia and cranial nerve palsies may also be present. The most severely affected children can develop fulminant cardiorespiratory failure, which is often fatal and causes a high incidence of severe neurological and possible psychobehavioural sequelae among survivors, despite intensive care support (10).

Fatal-case children typically experience a brief febrile illness and present with only subtle neurological signs before succumbing dramatically to acute refractory myocardial dysfunction and fulminant pulmonary oedema within hours of developing tachycardia, poor peripheral perfusion and tachypnea. Laboratory abnormalities include a raised white cell count with relative neutrophilia, hyperglycaemia (11, 12) and elevated cerebrospinal fluid (CSF) lactate(12). Elevated creatine kinase is sometimes seen in patients with cardiac involvement (13). Unlike in acute viral myocarditis or congenital heart disease, the heart size on chest X-ray is always normal despite the severity of pulmonary oedema and cardiac dysfunction, although a globular, poorly contractile heart, particularly the left ventricle, is consistently shown on echocardiography (9, 14). Computed tomography (CT) scans of the brain are not useful because the primary site of CNS pathology is located at the brainstem. Magnetic resonance imaging is therefore the diagnostic imaging of choice in such cases, and shows characteristic high signal intensities on T2 weighted images in the dorsal pons and medulla, most of the midbrain, and the dentate nuclei of the cerebellum. Similar high signal lesions may also be found in the anterior horn cells of the cervical spinal cord (10, 15).

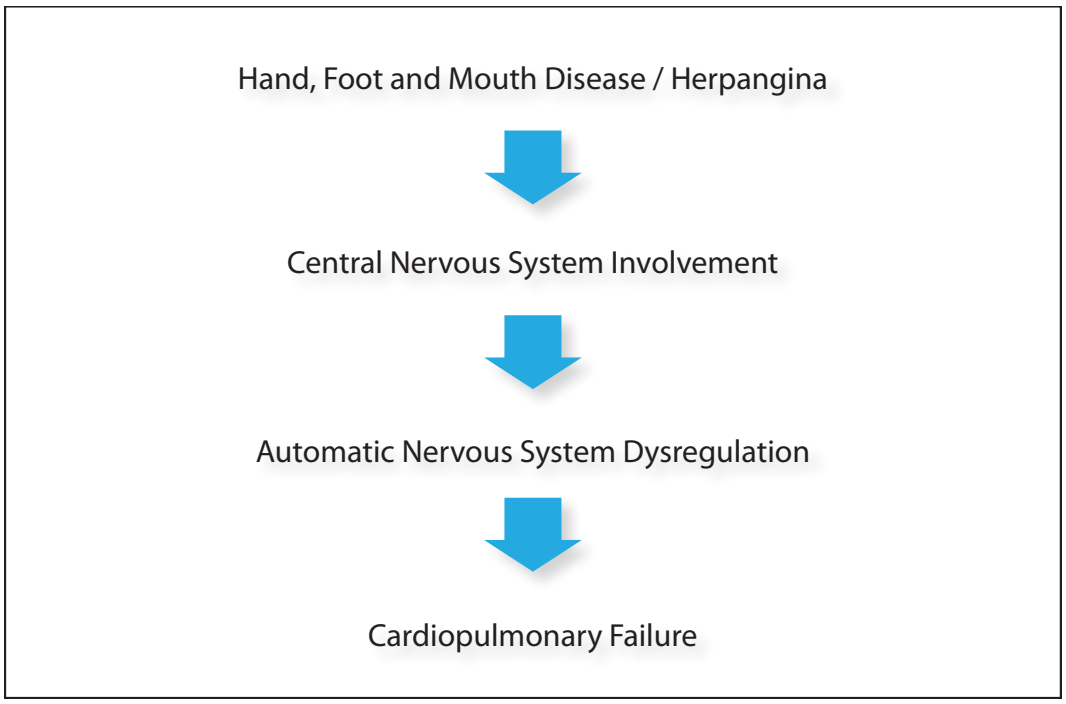


The exact disease mechanism of cardiopulmonary failure is still not well defined, although it has been linked to brainstem encephalitis following a number of clinical and pathological studies (11, 16).

CSF pleocytosis, an objective marker for CNS involvement, has been universally observed among fatal-case children, despite the absence of obvious neurological signs before sudden cardiopulmonary collapse, indicating that CNS involvement precedes the onset of cardiopulmonary failure (Figure 5). CNS involvement may therefore be considered a harbinger of acute systemic complication in HFMD. Early recognition of children with CNS involvement will enable doctors to focus special attention on those children and provide timely intervention before the onset of fulminant, intractable cardiopulmonary failure. In resource-limited settings, the diagnosis of brainstem encephalitis can be made in children with frequent myoclonic jerks and CSF pleocytosis. Other clinical features associated with, and predictive of, CNS involvement include body

temperature of 38.5 °C or higher, duration of fever longer than three days, lethargy, recurrent vomiting, limb weakness and myoclonic jerks (3, 11, 17).

**Figure 5: Clinical course of fulminant EV71-associated hand, foot and mouth disease**






However, not all children with CNS involvement will develop acute fulminant cardiac dysfunction and pulmonary oedema. Children presenting with features of autonomic nervous system (ANS) dysregulation, including cold sweating, mottled skin, tachycardia, tachypnea, hypertension and hyperglycemia, are at risk of rapid progression to cardiopulmonary failure (13, 18). Abnormal heart rate variability (19) (an indicator for ANS dysregulation) and elevated cardiac Troponin I have been shown in two small studies (20, 21) to be useful laboratory markers that, in some settings, may help doctors identify children at risk of systemic complication several hours before the onset of overt signs of disease progression. However, these two laboratory tests are expensive and are not widely available in primary care settings (16).

**Table 1. Proposed clinical case definitions for HFMD/herpangina and associated complications**

| Disease | Proposed Case Definition |
|---|---|
| HFMD | Febrile illness with papulovesicular rash on palms and soles, with or without vesicles/ulcers in the mouth. Rash may occasionally be maculopapular without vesicular lesion, and may also involve the buttocks, knees or elbows, particularly in younger children and infants. |
| Herpangina | Febrile illness with multiple oral ulcers on the posterior parts of the oral cavity. |
| Aseptic meningitis | Febrile illness with headache, vomiting and meningism associated with presence of more than 5 – 10 white cells per cubic millimeter in cerebrospinal (CSF) fluid, and negative results on CSF bacterial culture. |
| Brainstem encephalitis | Myoclonus, ataxia, nystagmus, oculomotor palsies, and bulbar palsy in various combinations, with or without MRI.<br>In resource-limited settings, the diagnosis of brainstem encephalitis can be made in children with frequent myoclonic jerks and CSF pleocytosis. |
| Encephalitis | Impaired consciousness, including lethargy, drowsiness or coma, or seizures or myoclonus. |
| Encephalomyelitis | Acute onset of hyporeflexic flaccid muscle weakness with myoclonus, ataxia, nystagmus, oculomotor palsies and bulbar palsy in various combinations. |
| Acute flaccid paralysis | Acute onset of flaccid muscle weakness and lack of reflexes. |
| Autonomic nervous system (ANS) dysregulation | Presence of cold sweating, mottled skin, tachycardia, tachypnea, and hypertension. |
| Pulmonary oedema/ haemorrhage | Respiratory distress with tachycardia, tachypnea, rales, and pink frothy secretion that develops after ANS dysregulation, together with a chest radiograph that shows bilateral pulmonary infiltrates without cardiomegaly. |
| Cardiorespiratory failure | Cardiorespiratory failure is defined by the presence of tachycardia, respiratory distress, pulmonary oedema, poor peripheral perfusion requiring inotropes, pulmonary congestion on chest radiography and reduced cardiac contractility on echocardiography. |

## 5.2 Differential diagnosis

The differential diagnoses for HFMD include herpetic gingivostomatitis, aphthous stomatitis, scabies infestation, chickenpox (varicella), measles and rubella. In herpetic gingivostomatitis, patients are usually febrile and look toxic. They may have gingival erythema, swelling or bleeding, and associated cervical lymphadenopathy. There may be circumoral ulcers or vesicles without extremity involvement. Aphthous stomatitis is characterized by larger, ulcerative lesions of the lips, tongue and buccal mucosa that are exquisitely painful. It most commonly affects older children and adults, can have multiple recurrences, and is generally not associated with constitutional symptoms. Scabies infestation may sometimes be confused with HFMD because it also causes pustules, vesicles or nodular lesions over the hands and feet. An intense itch and inter-digital space involvement are useful clinical clues to parasitic infestation. In contrast to HFMD, varicellar lesions are centrifugal in distribution and involve a larger skin area, including the scalp, but spare the palms and soles. The varicellar lesions heal by formation of crusts, while vesicles of HFMD resolve by reabsorption of vesicular fluid. Besides generalized maculopapular rash, children with a typical measles infection often present with cough, coryza and conjunctivitis, and koplik spots may be found on examination of the mouth. The skin rash in rubella has centripedal distribution and occipital lymphadenopathy.

## 5.3 Clinical assessment and management



In most cases, HFMD is a self-limiting illness, with the majority of children recovering spontaneously with symptomatic treatment. Only a small proportion of children with HFMD develop neurological involvement, which may further progress to potentially fatal cardiopulmonary failure (see Figure 1). Since children at risk of severe systemic complications often present with subtle clinical features during the early phase of the illness, yet later deteriorate very rapidly with a fulminant disease course, early recognition and timely intervention is the key to reducing acute morbidity and mortality associated with severe presentation of this clinical syndrome. The main aim of primary care doctors observing cases of HFMD/herpangina is to identify cases that are likely to develop a severe form of the disease, and to admit them to hospital for close observation, investigation and early treatment.

Clinical management of HFMD is largely supportive in nature and there are no specific antivirals. Several treatment approaches have been used in Malaysia and Viet Nam and, while more recent clinical practices appear to reduce acute morbidity and mortality rates, no randomized controlled trials have been conducted. An approach to the clinical management of HFMD/herpangina is proposed in the case management algorithm in Figure 6. Assessment should begin with a good history and physical examination, paying particular attention to eliciting the warning signs indicative of CNS involvement. Fever of brief duration is typical of the condition, however, the severity and duration of

fever have been shown to be important independent risk factors for CNS involvement in several studies. Other signs, such as vomiting, lethargy, agitation or irritability, have similarly been shown to be associated with CNS involvement (3, 11). More specific neurological signs, such as myoclonic jerk (usually observed during the early stage of sleep, but also seen in severe cases when patients are awake), truncal ataxia and "wandering eyes" (rotary eye movement without fixation), are commonly observed in children in the early stage of severe disease (17). With disease progression or increased severity affecting the autonomic nervous system and leading to cardiopulmonary failure, signs such as mottled skin and dyspnea/tachypnea may also be evident in those presenting at a later stage.

Based on the above assessment, the clinician should be able to determine if a child has HFMD/herpangina and to assess disease severity. Children with mucucutaneous involvement only (i.e. uncomplicated HFMD) may be treated at home, as long as there are no social circumstances of concern, such as anxious parents or poor access to health care facilities. The family should be adequately counselled on how to care for the patient and what warning signs to look for, with clear instructions to return to the clinic immediately if such signs are observed. Daily follow-up by the clinic may be advisable for at least seven days after the onset of illness.

If one or more of the warning signs is present, the clinician must carefully assess the neurological and haemodynamic status of the patient in order to assess disease severity and decide on a treatment and monitoring strategy. Observational studies of the spectrum of disease during previous outbreaks suggest that severe disease is characterized by three distinct stages: those with CNS involvement, those with ANS dysregulation and, later, those with frank cardiopulmonary failure, including pulmonary oedema or haemorrhage (10, 16). Those presenting with neurological signs indicative of CNS involvement but without signs of autonomic system dysregulation, such as persistent resting tachycardia, hypertension or profuse sweating, can be considered to be in the CNS involvement stage (early stage of severe disease). This can manifest as aseptic meningitis, brainstem encephalitis and encephalomyelitis (including acute flaccid paralysis).



CSF examination should be carried out to confirm CNS involvement. Laboratory abnormalities, such as leucocytosis, thrombocytosis (platelet $> 4 \times 10^5/mm^3$) and hyperglycemia, may be present. Imaging studies, such as echocardiography and MRI, can be considered to support the diagnosis, as well as to support assessment of further progression.

Patients with aseptic meningitis generally have a good prognosis. They can be managed symptomatically and generally recover without further intervention. On the other hand, patients with brainstem encephalitis or encephalomyelitis are at a higher risk of progression to ANS dysregulation. It is important to monitor children with CNS

involvement closely in order to detect signs of ANS dysregulation early. Although such patients can be managed in the general ward setting, they should be monitored closely for signs of ANS dysregulation and the development of cardiopulmonary failure, which would require their transfer to the critical care unit.

In many countries affected by outbreaks, intravenous immunoglobulin (IVIG) has been used on a presumptive basis (9, 16, 22). One assumption is that neutralizing antibody in the pooled immunoglobulin preparation may help to neutralize the enterovirus. Historically, IVIG has been used for other severe enteroviral infections, such as neonatal enteroviral sepsis or persistent enteroviral meningoencephalitis among children with primary immunodeficiency (23). Another assumption is based on observations that EV71-associated ANS dysregulation and pulmonary oedema/haemorrhage is associated with production of pro-inflammatory cytokines, such as Interleukin (IL)-6, IL-10 and IL-13 and chemokines IL-8, IP-10, MCP-1, and possible MIG (12, 24). A retrospective analysis of cases in Taiwan (China) suggests that IVIG may have immunomodulatory effects in children with brainstem encephalitis and ANS dysregulation (25).

Anecdotal experience in Asia indicates that IVIG, if administered early, could halt disease progression to ANS, and subsequently to devastating pulmonary oedema. Retrospective analysis of IVIG-treated cases in Taiwan (China) and Sarawak, Malaysia, seen during earlier outbreaks, appear to demonstrate a better outcome (3, 25, 26). However, the use of IVIG has yet to be supported by evidence from randomized clinical trials. IVIG is not without risk (use of human blood products and significant infusion volume required) and it is very expensive. Before its use can be recommended, randomized —preferably double-blind —controlled trials are required.



Seizures are uncommon in children with HFMD. If they occur, routine anti-convulsants may be considered. Frequent myoclonic jerks, particularly if distressing to the child and parent, may be managed with sedation (e.g. midazolam) and/or anticonvulsants (e.g. phenytoin).

As the brainstem becomes progressively affected, signs of autonomic system dysregulation become evident, such as profuse sweating and respiratory abnormalities. At this stage, despite persistent resting tachycardia and hypertension, serial echocardiographic studies of these children show rapid, progressive reduction in cardiac function (13, 18). Such patients require critical care management with continuous monitoring of their haemodynamic status (heart rate, arterial pressure, arterial blood gases, echocardiography). Assessment of haemodynamic and hydration status should guide intravenous fluid therapy, as well as the use of inotropes to support cardiac function during this critical stage. Overzealous fluid resuscitation should be avoided as it may precipitate the development of pulmonary oedema/haemorrhage.

Early intubation is recommended, particularly for children who are restless and agitated due to frequent myoclonic jerks. Other indicators for intubation include altered sensorium, respiratory abnormalities, persistent tachycardia, poor tissue perfusion, hypoxemia and fluctuating oxygen-saturation levels (27).

Anecdotal experience suggests that administration of milrinone at this stage may be useful in halting progression into established cardiopulmonary failure. A study in Taiwan (China) found that a milrinone-treated group was associated with reduced mortality corresponding to attenuated sympathetic activity and cytokine production in comparison with a non-treated group (28). Milrinone is a bipyridine derivative that specifically inhibits phosphodiesterase (PDE) subtype III and elevates intracellular cyclic adenosine monophosphate (cAMP). Specific PDE inhibitors were found to have a variety of therapeutic anti-inflammatory effects in cellular and animal models of inflammation. Anecdotal experience indicates that IVIG could also be useful at this stage.

Established cardiopulmonary failure represents the last and most severe stage of the disease and is associated with very poor outcomes. In addition to evidence of ANS dysregulation, it is characterized by hypotension, shock, pulmonary oedema, pulmonary haemorrhage and heart failure. One of the most important differential diagnoses is septicaemic shock and a blood culture should be conducted.



Children at this stage invariably require mechanical ventilation and multiple inotropes (milrinone, dobutamine, dopamine, epinephrine) (27). IVIG may still be used if it has not previously been administered. A novel rescue approach for such critically ill children attempted in some centres is extracorporeal membrane oxygenation (ECMO) (29–31). However, further data are needed to determine if such measures can truly improve the overall outcome. Finally, there is a need to carry out clinical studies to gather more evidence and to improve the clinical management of emerging neurological infection.

**Table 2. Diagnostic and management pitfalls**

| Diagnostic and Clinical Management pitfalls |
|---|
| • Failure to make a diagnosis of HFMD/Herpangina<br>• Failure to recognize warning signs of CNS involvement<br>• Failure to detect signs of autonomic nervous system dysregulation<br>• Failure to closely monitor HR and BP in children with CNS involvement<br>• Over reliance on laboratory results and imaging tests instead of clinical judgment to assess and manage patients(e.g. Chest X-ray for pulmonary oedema)<br>• Use of rapid fluid boluses when resuscitating children with cardiac dysfunction<br>• Inappropriate use of IVIG in children with aseptic meningitis |

## MANAGEMENT ALGORITHM OF HAND, FOOT AND MOUTH DISEASE (HFMD)/Herpangina

**Assessment**

Presumptive Diagnosis:
- HFMD
  - Fever or history of fever
  - Papulovesicular rash on hand and foot with or without oral ulcers
- Herpangina
  - Fever or history of fever
  - Oral ulcers

Warning Signs of CNS Involvement: *(one or more of the following)*

- Fever ≥ 39°C or ≥ 48 hours
- Vomiting
- Lethargy
- Agitation/irritability
- Myoclonic jerks
- Limb weakness
- Truncal ataxia
- "Wandering eyes"
- Dyspnea/tachypnea
- Mottled skin

Special Consideration:
- Anxious parents
- From remote area/poor access to healthcare

Absent → Uncomplicated HFMD/Herpangina Stage (May be sent home)

Present → HFMD with CNS Involvement Stage (Aseptic Meningitis/Brainstem Encephalitis/Encephalomyelitis)* (Pediatric Ward)

Present → HFMD with Autonomic Nervous System (ANS) Dysregulation Stage (Pediatric ICU)

Present → HFMD with Cardiopulmonary Failure Stage (Pediatric ICU)

**Diagnosis**

**Uncomplicated HFMD/Herpangina Stage**

Criteria:
Patients with any of the following and no warning signs:
- Skin rash
- Oral ulcers

Laboratory Test:
- Optional

**Treatment**

Treatment:
- Paracetamol
- Adequate fluid intake

Monitoring:
- Educate parents to watch out for warning signs
- Clinic follow up every 1 – 2 days for the next 7 days (if possible)

**Monitoring and Reassessment**

Laboratory:
Samples for virological investigation (for CNS Involvement Stage, Autonomic Nervous System Dysregulation Stage and Cardiopulmonary Failure Stage) :
- Throat Swab
- Vesicles
- Rectal Swab/Stool
- CSF

**HFMD with CNS Involvement Stage (Aseptic Meningitis/Brainstem Encephalitis/Encephalomyelitis)* (Pediatric Ward)**

Criteria
Patients with HFMD/Herpangina and any of the following:
- Meningism
- Myoclonic jerks
- Ataxia, tremors
- Lethargy
- Limb weakness

Laboratory Test:
- Full blood count
- Blood glucose
- CSF examination
- Echocardiography (May be considered)
- MRI, if needed (CT scan is not recommended)

Treatment:
- Paracetamol
- Oxygen
- Intravenous immunoglobulin (IVIG) * (Recommended in patients with encephalitis plus acute flaccid paralysis; may be considered in patients with brainstem encephalitis)

Monitoring
- Vital signs
  - To transfer to ICU if resting heart rate >150/min and/or hypertension
- Myoclonic jerks

* Patients with aseptic meningitis generally have a good prognosis, IVIG is not indicated

**HFMD with Autonomic Nervous System (ANS) Dysregulation Stage (Pediatric ICU)**

Criteria:
Patients with CNS Involvement and any of the following:
- Resting Heart rate 150- 170/min
- Hypertension
- Profuse sweating
- Respiratory abnormalities (tachypnea, labored breathing)

Laboratory Test:
- Full Blood Count
- Blood glucose
- CSF examination
- Arterial blood gas
- Echocardiography
- Chest X-ray

Treatment:
- Judicious intravenous fluid therapy
- Consider early intubation ∞
- IVIG
- Inotropes:
  - Dobutamine
  - Milrinone

Monitoring
- Vital signs
- Central venous pressure
- Arterial blood gases
- Echocardiography

∞ *Indications: Persistent and frequent myoclonus, persistent tachycardia, respiratory abnormalities, hypoxemia, fluctuating oxygen saturation level, poor tissue perfusion, altered sensorium*

**HFMD with Cardiopulmonary Failure Stage (Pediatric ICU)**

Criteria:
Patients with ANS Dysregulation and any of the following:
- Hypotension/Shock
- Pulmonary edema/hemorrhage
- Heart failure

Laboratory Test:
- Full blood count
- Blood glucose
- Arterial blood gas
- Echocardiography
- Chest X-ray
- Blood culture (if septicemic shock cannot be excluded)

Treatment:
- Judicious intravenous fluid therapy
- Mechanical ventilation
- Inotropes: Milrinone, Dobutamine, (Dopamine or epinephrine is not recommended)
- IVIG may be considered if not previously used

Monitoring
- Vital signs
- Central venous pressure
- Arterial blood gases
- Echocardiography

## References


1. Ang LW, et al. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. Annals of the Academy of Medicine Singapore, 2009, Feb, 38(2):106–112.

2. Chen KT, et al. Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. Pediatrics, 2007, Aug, 120(2):e244–252.

3. Ooi MH, et al. Identification and validation of clinical predictors for the risk of neurological involvement in children with hand, foot, and mouth disease in Sarawak. BMC Infectious Diseases, 2009, 9:3.

4. Ooi MH, et al. Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. Clinical Infectious Diseases, 2007, Mar 1, 44(5):646–656.

5. Ho M, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. New England Journal of Medicine, 1999, Sep 23, 341(13):929–935.

6. Chong CY, et al. Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases. Acta Paediatrica, 2003, Oct, 92(10):1163–1169.

7. Tu PV, et al. Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005. Emerging Infectious Diseases, 2007, Nov, 13(11):1733–1741.

8. Ong KC, et al. Pathologic characterization of a murine model of human enterovirus 71 encephalomyelitis. Journal of Neuropathology and Experimental Neurology, 2008, Jun, 67(6):532–542.

9. Wang SM, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. Clinical Infectious Diseases, 1999, Jul, 29(1):184–190.

10. Huang CC, et al. Neurologic complications in children with enterovirus 71 infection. New England Journal of Medicine, 1999, Sep 23, 341(13):936–942.

11. Chang LY, et al. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. Lancet, 1999, Nov 13, 354(9191):1682–1686.

12. Wang SM, et al. Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. Journal of Infectious Diseases, 2003, Aug 15, 188(4):564–570.

13. Fu YC, et al. Cardiac complications of enterovirus rhombencephalitis. Archives of Disease in Childhood, 2004, Apr, 89(4):368–373.

14. Wu JM, et al. Cardiopulmonary manifestations of fulminant enterovirus 71 infection. Pediatrics, 2002, Feb, 109(2):E26–.

15. Shen WC, et al. MR imaging findings of enteroviral encephaloymelitis: an outbreak in Taiwan. American Journal of Neuroradiology, 1999, Nov-Dec, 20(10):1889–1895.

16. Lin TY, et al. The 1998 enterovirus 71 outbreak in Taiwan: pathogenesis and management. Clinical Infectious Diseases, 2002, May 1, 34 Suppl 2:S52–S57.

17. Lu HK, et al. Prognostic implications of myoclonic jerk in children with enterovirus infection. Journal of Microbiology, Immunology and Infection, 2004, Apr, 37(2):82–87.

18. Fu YC, et al. Comparison of heart failure in children with enterovirus 71 rhombencephalitis and cats with norepinephrine cardiotoxicity. Pediatric Cardiology, 2006, Sep-Oct, 27(5):577–584.

19. Lin MT, et al. Heart rate variability monitoring in the detection of central nervous system complications in children with enterovirus infection. Journal of Critical Care, 2006, Sep, 21(3):280–286.

20. Huang YF, et al. Cardiac troponin I: a reliable marker and early myocardial involvement with meningoencephalitis after fatal enterovirus-71 infection. Journal of Infection, 2003, May, 46(4):238–243.

21. Hsia SH, et al. Predictors of unfavorable outcomes in enterovirus 71-related cardiopulmonary failure in children. Pediatric Infectious Disease Journal, 2005, Apr, 24(4):331–334.

22. Wang JN, et al. Critical management in patients with severe enterovirus 71 infection. Pediatrics International, 2006, Jun, 48(3):250–256.

23. McKinney RE, Jr., Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. Reviews of Infectious Diseases, 1987, Mar-Apr, 9(2):334–356.

24. Wang SM, et al. Acute chemokine response in the blood and cerebrospinal fluid of children with enterovirus 71-associated brainstem encephalitis. Journal of Infectious Diseases, 2008, Oct 1, 198(7):1002–1006.

25. Wang SM, et al. Modulation of cytokine production by intravenous immunoglobulin in patients with enterovirus 71-associated brainstem encephalitis. Journal of Clinical Virology, 2006, Sep, 37(1):47–52.

26. Chang LY, et al. Outcome of enterovirus 71 infections with or without stage-based management: 1998 to 2002. Pediatric Infectious Disease Journal, 2004, Apr, 23(4):327–332.

27. Wang SM, Liu CC. Enterovirus 71: epidemiology, pathogenesis and management. Expert Review of Anti-Infective Therapy, 2009, Aug, 7(6):735–742.

28. Wang SM, et al. Therapeutic efficacy of milrinone in the management of enterovirus 71-induced pulmonary edema. Pediatric Pulmonology, 2005, Mar, 39(3):219–223.

29. Huang FL, et al. Left ventricular dysfunction in children with fulminant enterovirus 71 infection: an evaluation of the clinical course. Clinical Infectious Diseases, 2002, Apr 1, 34(7):1020–1024.

30. Matsubayashi T, et al. Percutaneous cardiopulmonary support in a child with enterovirus 71 encephalitis. Pediatrics International, 2006, Jun, 48(3):327–329.

31. Fu YC, et al. Pulmonary edema of enterovirus 71 encephalomyelitis is associated with left ventricular failure: implications for treatment. Pediatric Pulmonology, 2003, Apr, 35(4):263–268.

# Section 6: Prevention and Control Measures

## 6.1 Overview

No pharmacological intervention has been proven to prevent or control human HFMD/EV71. The prevention and control measures currently being used are primarily non-pharmaceutical, and for the most part are meant to interrupt the chain of virus transmission, thus preventing severe disease and death. Early detection of outbreaks and early recognition and intervention in cases at high risk of developing the rare but severe forms of the disease are therefore among the key principles applied to minimize the impact of the disease.

Decisions on public health interventions to prevent and control HFMD must be made despite the lack of definitive scientific and technical evidence. They must also balance technical expertise, prior experience and perceived risk-benefit. Factors such as public perception and political pressure can also impact the success of the interventions applied. Such factors may differ between countries, as well as for different population groups within a country. For this purpose, risk assessment frameworks can be used to systematically characterize the hazard, exposure and vulnerabilities, and to determine appropriate intervention measures that will best fit the local context (1).



The main measures currently being taken to manage HFMD outbreaks include:

1) establishing and strengthening surveillance;
2) conducting information and education campaigns on good hygiene and basic sanitation;
3) providing assistance to kindergartens, day-care facilities and schools during outbreaks;
4) strengthening infection-control measures in both health care facilities and the community;
5) improving clinical case-management services, particularly for severe manifestations requiring intensive medical care;
6) exchanging information and disseminating best practices related to the preparedness, response and management of HFMD, particularly during outbreaks;

7) providing an administrative framework to national agencies/bodies to implement prevention and control options, including the:
   - delegation of powers to act to key ministries, including through supportive legislation;
   - development of mechanisms to establish and support interagency/ intersectoral collaboration; and
   - strengthening of coordinated risk communication; and
8) monitoring and evaluation.

It is highly likely that a combination of interventions will be needed to effectively reduce transmission. Each of these measures is described in more detail below, while the benefits and limitations of specific approaches are summarized at Appendix 2.

**Outbreak definition:** Although the criteria used to define an outbreak of HFMD varies from country to country, it is important to note that such definitions frequently act as triggers for the implementation of specific public health measures. A commonly used threshold for an outbreak is when the number of cases reaches two standard deviations (2SD) above the normal baseline. A cluster is when two or more cases occur within an institution, indicating transmission within a cohort.

For example, in Malaysia;

- An outbreak is defined as two or more cases occurring within one locality.
- The epidemic threshold is the occurrence of cases 2SD above the normal rate.
- Sporadic cases are defined as single cases in the absence of previous known close contact with another case.



## 6.2 Prevention Measures: Recommendations and Rationale

### *(1) Establishing and strengthening surveillance*

Adequate and functioning surveillance systems are needed to provide timely data and information for risk assessments and subsequent decision-making about appropriate public health interventions. They are also essential for monitoring and evaluating the impact of such interventions.

The type of surveillance conducted should be driven by the information required to make decisions in the public health context. Societal, economic and political circumstances also influence the surveillance approaches taken. In jurisdictions where a structured surveillance system is lacking, the occurrence of severe cases or deaths associated with EV71 is usually the trigger for action. This implies that there is a need to ensure

that countries collect baseline data to inform risk assessments and facilitate earlier interventions.

Given the endemicity of this common childhood illness, it is unnecessary to detect and report each case of HFMD. Rather, the focus should be on the detection of events that indicate an outbreak situation; clusters; serious disease manifestations; or deaths.

Data on rates of asymptomatic or subclinical infections are limited. A study conducted in Singapore found that only one out of 124 (0.8%) samples from children aged 1-23 months had anti-EV71 antibodies. In children age 2-5 years the seropositive rate increased by 12% per year, suggesting that most infections occur in preschool-aged children (2).

Seroepidemiological studies in Taiwan (China) show an inverse relationship between pre-epidemic seroprevalence and severe disease mortality, suggesting age-related disease susceptibility or a protective effect from specific antibodies (3). Knowing the pre-epidemic seroprevalence of EV71 could help in determining the risk factors associated with infection, severe disease and mortality during epidemics. Such information is also important in identifying and examining the cost-effectiveness of appropriate local intervention measures for the prevention and control of HFMD.

Different enteroviral species responsible for HFMD circulate in communities. At times of outbreaks, specific virus types (such as CA16 or EV71) will dominate. While EV71 has been associated with more severe disease outcomes, as yet there is a lack of association between specific genogroups of EV71 and the severity of clinical outcomes. Monitoring changes at the subgenotype level for EV71 is therefore important for establishing potential severity links, for vaccine development and for detecting evidence of cross-protection and cross-antigenicity (4-7).



### *(2) Conducting information and education campaigns on good hygiene and basic sanitation*

National strategies on health education and the promotion of individual and public health behaviours, developed jointly by ministries of health and education in collaboration with relevant professional bodies, such as infection control societies, can provide a framework that is appropriate to individual country and cultural needs. Education campaigns directed at reducing the spread of disease, particularly in high-risk age groups, include disseminating information on good personal hygiene habits, such as frequent hand-washing and avoiding thumb-sucking and nail-chewing.

Using local health promotion boards to disseminate information, advertisements and resources may assist in filling gaps in parents' understanding of HFMD and its control. It can also encourage the general public to exercise greater social responsibility and take stronger ownership of hygiene and health issues in the child-care/pre-school setting.

### *(3) Providing assistance to kindergartens, daycare facilities and schools during outbreaks*

Despite the lack of concrete evidence on the effectiveness of child-care centre and school closures in controlling HFMD transmission, such measures are widely used on the assumption that they will reduce transmission or delay the spread of HFMD to the community. There are no standard thresholds in terms of the number of cases or events that should act as a trigger to closure, and options range from voluntary to mandatory and tiered school closures. While information regarding virus shedding is lacking, the average duration of closures ranges from one week to 10 days. In Singapore, for example, depending on the severity of the situation, actions taken have included issuing alert letters to affected institutions, conducting field investigations, and mobilizing multi-agency efforts to prevent further spread of the disease. Licensing authorities are also kept up to date on the local HFMD situation (number, size and attack rate, and transmission period of clusters) on a twice-weekly basis.

### *(4) Strengthening infection control measures both in health care facilities and in the community*

Infection control measures need to be implemented consistently and strengthened rapidly during outbreaks in both the community and health care facilities. In Singapore, for example, educational institutions are referred to infection-control guidelines for child-care centres, pre-schools and schools on practices recommended to reduce disease transmission in a school setting by the Infection Control Association (Singapore).



### *(5) Improving clinical case management services, particularly for severe manifestations requiring intensive medical care*

Ensuring that health care staff are aware of HFMD and its potentially severe manifestations requires disseminating information and providing training for those medical staff who are most likely to be the primary consult for the disease. In Taiwan (China), medical education, such as on the symptoms of EV71 and the timing of referral for severe cases to hospital, has been facilitated by the Digital Infectious Disease Learning Net, and linked to activities giving physicians continuing education credits. The medical service response has also been upgraded and guidelines developed for the treatment of HFMD and severe cases. In mainland China, medical services have been upgraded through:

- The establishment of a patient triage system.
- The designation of specific hospitals for treatment.
- Clinical monitoring for the early detection and intervention of severe cases.
- The establishment of a paediatric intensive care unit (PICU).
- Reimbursement of medical fees based on the new rural cooperative medical care regulation.

### *(6) Exchanging information and disseminating best practices related to preparedness, response and management of HFMD, particularly in outbreak conditions*

There is currently no network specific to HFMD. Mechanisms for information exchange should be promoted, including networks, meetings, exchange groups and multilateral or bilateral collaborations. In many countries, enterovirus laboratory capacity is built on to existing networks for polio and influenza. A regional network that builds on existing networks could be initiated. Regional clinical networks as well as subregional networks, such as ASEAN plus 3, have recently been formed, and could be utilized as a platform for information and knowledge exchange.

### *(7) Providing the necessary administrative framework to national agencies/bodies to support the implementation and management of prevention and control options, including:*

- **The delegation of powers to act to key ministries, including through supportive legislation**

  Legislation can facilitate the implementation of public health interventions. For example, legislation on "EV71 Control Policies" in Taiwan (China) enabled public health authorities to take action to improve public health control and upgrade the medical service preparedness and response to EV71.

  Many countries affected by HFMD have passed legislation on mandatory notification, requiring medical practitioners to notify the Ministry of Health of all clinical cases of HFMD within 24 hours of diagnosis.

- **Mechanisms to establish and support interagency/intersectoral collaboration**

  Effective control of HFMD outbreaks often requires the participation of multiple agencies, such as the health and education ministries. Pro-actively determining mechanisms for intersectoral discussion and action, such as a task force or crisis-management group, is important to facilitate such collaboration.

- **Coordinated risk communication**

  One challenge presented by HFMD has been the demand for greater and improved public health communication, primarily during outbreaks. There is growing awareness of the importance of accurate and consistent messages to the general public as well as to the media. Information resulting from clinical, laboratory and epidemiological investigations must be communicated to those directly involved in the outbreak response to further strengthen surveillance, control and preventive actions. Other stakeholders, such as public officials and health providers, also need timely information.

  The main objectives of risk communication during an emergency are:

  - to provide, accurate, timely and consistent information as well as essential coordination;

- to inform the public of potential risks and the steps being taken; and
- to assist individuals, stakeholders or communities to accept the imperfect nature of choices and to make the best possible decisions.

During the 2008 HFMD outbreak in mainland China, health officials across the country who received risk communication training were found to use better risk communication strategies in post-training messages compared with their pre-training messages. The type of training and resulting messages resonated well with the Chinese population and could be easily reproduced and adapted to other similar situations.

The WHO guidelines on outbreak communication provide further information and can be downloaded from

http://www.who.int/infectious-disease-news/IDdocs/whocds200528/whocds200528en.pdf

### *(8) Monitoring and evaluation*

Despite the emphasis on personal hygiene in disease mitigation, the significance of such measures is not well understood. It has been suggested that cohort studies involving child-care centres with an expanded hygiene package would be required to better understand these measures.

## 6.3 Future Considerations



### Greater knowledge on asymptomatic transmission of HFMD

The significance of the contribution of asymptomatic cases in HFMD transmission in preschool centres is unknown, although the rate of asymptomatic cases of EV71 was found to be as high as 71% in a community setting in Taiwan (China). Enteroviruses can be excreted in the stools for up to six weeks and in throat secretions for up to two weeks. However the viral load and the exact significance of this to the transmission of disease in preschool centres is unknown.

### *Novel vaccines*

Several vaccines for HFMD are currently under development. Robust surveillance data and information on the impact of prevention measures are critical in determining how a vaccine should be applied and how it should be evaluated.

## References

1. Mitigating the impact of the new influenza A(H1N1): options for public health measures. Manila, WHO Regional Office for the Western Pacific. 2009.
2. Ooi EE, et al. Seroepidemiology of human enterovirus 71, Singapore. Emerging Infectious Diseases, 2002, Sep, 8(9):995-997.

3. Chang LY, et al. Risk factors of enterovirus 71 infection and associated hand, foot, and mouth disease/herpangina in children during an epidemic in Taiwan. Pediatrics, 2002, Jun, 109(6):e88.

4. Kung SH, et al. Genetic and antigenic analyses of enterovirus 71 isolates in Taiwan during 1998-2005. Clinical Microbiology and Infection, 2007, Aug, 13(8):782-787.

5. Mizuta K, et al. Cross-antigenicity among EV71 strains from different genogroups isolated in Yamagata, Japan, between 1990 and 2007. Vaccine, 2009, May 21, 27(24):3153-3158.

6. Wu TC, et al. Immunity to avirulent enterovirus 71 and coxsackie A16 virus protects against enterovirus 71 infection in mice. Journal of Virology, 2007, Oct, 81(19):10310-10315.

7. Arita M, et al. An attenuated strain of enterovirus 71 belonging to genotype a showed a broad spectrum of antigenicity with attenuated neurovirulence in cynomolgus monkeys. Journal of Virology, 2007, Sep, 81(17):9386-9395.

# Appendix 1 : Summary of epidemiologic findi surveillance data in Western Pacific Region (s

| Ref. No. | Type of Surveillance | Participatory Institutions | Year / Month | Case definition / Patients from whom samples were collected | Type of specimen | Number of cases / Number of specimens | |
|---|---|---|---|---|---|---|---|
| Serawak, Malaysia | | | | | | | |
| [1] | Sentinel Surveillance | 3 pediatric clinics + 2 government hospitals | Mar 1998 - Aug 1999 | A history of oral or other skin lesions, typical of HFMD | Required:<br>throat swabs<br>rectal swabs<br><br>Desirable:<br>vesicle swabs<br>ulcer swabs | 263 cases | |
| | Sentinel surveillance | 1998 -<br>3 paediatric clinics +<br>2 government hospitals<br><br>2000 - 4th paediatric clinic added<br><br>Sentinel clinics were located in the areas of Kuching and Sibu | Mar 1998 - June 2005 | A history of oral or other skin lesions, typical of HFMD | Required:<br>throat swabs<br><br>Desirable:<br>vesicle swabs | 2950 children and their 4290 specimens | |
| Singapore | | | | | | | |
| [2] | National surveillance | Medical institution | 2001 - 2007 | Clinical criteria<br>- fever<br>- rash over the palms, soles, dorsum of the feet, buttocks<br>- mouth ulcers over the soft/ hard palate, uvula, buccal mucosa, etc. (provided in a guidebook for medical practitioners) | NA | 2001: 5187<br>2002: 16 228<br>2003: 5603<br>2004: 6411<br>2005: 15 256<br>2006: 15 282<br>2007: 20 003 | |

# ngs of HFMD from since 1997)

| | Age | Sex | Incidence Rate / Attack rates | No. of deaths / Case fatality rates | Wave | Laboratory findings EV71 involvement |
|---|---|---|---|---|---|---|
| | | | | | | |
| | 6 mos. - 13 yrs. | Male: 58.2% Female: 41.8% | | | | 529 uncontaminated specimens from 259 cases were tested for EV, and 235 (44.4%) were EV positive<br><br>EV71 positivity rates<br>6.4% of EV (+) specimens (15/235)<br>3.9% of EV (+) children (6/153) |
| | | | | | 2 large outbreaks in 2000 and 2003<br><br>Disease trend in 2000<br>Rise in the number of cases: by week 7<br>Peak: between weeks 11 and 13<br>Decreasing trend: stretching to the end of the year<br><br>Disease trend in 2003<br>Rise in the number of cases: same as in 2000<br>Peak: at week 12<br>Decreasing trend: dropped sharply by week 19<br>Epidemic curves of total HFMD cases were dissimilar in 2000 and 2003 | EV71 was a predominant serotype during the 2 large outbreaks<br>EV71 activity in 2000<br>Decrease in the no. of EV71 cases: sharply by the end of June (week 27)<br><br>EV71 activity in 2003<br>Decrease in the no. of EV71 cases: by the end of April (week 18)<br><br>Epidemic curves of EV71 cases were similar in 2000 and 2003. |
| | | | | | | |
| | 0-4 yrs: 62.2 - 74.7%<br><br>5-9yrs: 17 - 26.2% | 1.3:1 ~ 1.6:1 | All / Male/Female / 0-4/5-9yrs)<br>2001: 125.5 / 142.5/107.3 / 1640.5/330.3<br>2002: 388.6 / 454.5/320.1 / 5256.8/1127.5<br>2003: 136.2 / 167.4/104.2 / 1830.2/447.1<br>2004: 153.9 / 174.2/133.1 / 2111.6/541.4<br>2005: 357.6 / 399.3/315.0 / 4807.7/1485.8<br>2006: 347.2 / 383.2/310.0 / 4649.0/1474.1<br>2007: 453.9 / 376.8/392.7 / 5975.5/2140.8<br>(per $10^5$ population) | No. of deaths<br>2000: 4 Sep - Oct<br>2001: 3 Jan & Feb<br>4 yr-old boy<br>11 mth-old boy<br>11 mth-old boy<br><br>Case fatality rate<br>2001: 0.06% | Single peak<br>2002: May<br><br>2 peaks<br>2005: March & Oct<br>2006: March & Aug<br>2007: May & Aug<br><br>Dips were observed during school holidays | |

| Ref. No. | Type of Surveillance | Participatory Institutions | Year / Month | Case definition / Patients from whom samples were collected | Type of specimen | Number of cases / Number of specimens | |
|---|---|---|---|---|---|---|---|
| [2] | Institutional outbreak surveillance | Child-care centres<br>preschools<br>primary schools | 2001-2007 | Outbreak definition:<br>2 or more cases of HFMD occurring within 10 days in the same institution | NA | No. of outbreaks<br>2001: 167<br>2002: 539<br>2003: 169<br>2007: 1723<br><br>Child-care centres (2-6yrs): 44.8~86.4%<br>Kindergartens (5-6yrs): 13.0~34.1% | |
| [2] | Laboratory notification | KK Women's and Children's Hospital and Singapore General Hospital's Virology Lab (tested samples included samples from childcare centres, kindergartens and school with parental consent).<br>From April 2006, selected stool samples, vesicle and throat swabs were also tested at the Microbiology Laboratory, KKH. Hospital | 2001-2007 | 1. and 2. were collated<br>1.Samples randomly collected from outpatients and inpatients with HFMD at KK Women's and Children's Hospital<br>2. Samples tested at Singapore General Hospital's Virology Lab. | Included:<br>stools<br>throat swabs<br>rectal swabs<br>vesicle swabs<br>ulcer swabs | No. tested<br>2001: 305<br>2002: 225<br>2003: 469<br>2004: 66<br>2005: 183<br>2006: 339<br>2007: 328 | |
| Taiwan (China) | | | | | | | |
| [3] | Sentinel Surveillance | An average of 800 (8.5 %) of physicians participated | Mar 1998 - Dec 2005 | HFMD: Vesicular lesions on the hands, feet, mouth and, frequently, buttocks. Lesions in the mouth were often ulcerated.<br>HA: Vesicular exanthema of the fauces and soft palate, often accompanied by fever, sore throat and pain on swallowing. | | 0.8 - 19.9 cases per sentinel doctor per week through 1998 - 2005 (highest: 1998) | |
| [3], [4] | Monitoring system for severe and fatal HFMD/HA | 23 academic medical centres, 80 regional hospitals, and 435 district hospitals | May 1998 - 2006 | Severe cases:<br>Presence of symptoms of HFMD/HA in addition to the occurrence of ≥1 of the following complications<br>- encephalitis<br>- aseptic meningitis<br>- acute flaccid paralysis<br>- pulmonary oedema/ hemorrhage<br>- myocarditis | | No. of severe cases<br>1998: 405<br>1999: 35<br>2000: 291<br>2001: 393<br>2002: 162<br>2003: 70<br>2004: 50<br>2005: 142<br>2006: 11<br>Total (~2006): 1559 | |

| | Age | Sex | Incidence Rate / Attack rates | No. of deaths / Case fatality rates | Wave | Laboratory findings EV71 involvement |
|---|---|---|---|---|---|---|
| | | | Majority of the outbreaks had less than 10% attack rates | | | |
| | | | | | | EV71 (+) rates among non Polio EV (+) samples<br>2001 45.6% among 178<br>2002 3.8% among 210<br>2003 68.0% among 50<br>2004 4.7% among 21<br>2005 52.7% among 76<br>2006 45.5% among 145<br>2007 8.5% among 94<br>EV71 predominance was observed in 2001 and 2003 |
| | | | | | Epidemic peaks seemed to occur during the summer every year<br><br>1st wave<br>Encompasses all 4 regions<br>2nd wave<br>Largely limited to southern region from Sept to Dec. | |
| | ~2005<br>Mean: 2.2 yrs (3 mnths – 14 yrs)<br><br>≤4 yrs: 93%<br><br>≤2 yrs: 75% | 2005<br>Ratio: 1.5:1 | Severe case incidence rate<br>Male /Female<br>1998: 9.02 / 7.11<br>1999: 0.85 / 0.61<br>2000: 7.37 / 4.86<br>2001: 9.59 / 7.00<br>2002: 4.10 / 2.75<br>2003: 1.82 / 1.20<br>2004: 1.30 / 0.94<br>2005: 4.21 / 2.27<br>(per $10^5$ under 15 years of age) | No. of deaths (case-fatality rate)<br>All / Male/Female<br>1998: 78(19%) (20.6%/17.4%)<br>1999: 9(26%) (23.8%/28.6%)<br>2000: 41(14%) (9.9%/20.9%)<br>2001: 58(15%) (15.3%/13.9%)<br>2002: 30(19%) (21.0%/14.5%)<br>2003: 8(11%) (4.7%/22.2%)<br>2004: 5(10%) (16.7%/0%)<br>2005: 16(11%) (10.5%/12.8%)<br>2006: 0 (NA)<br>Total (~2006) 245(15.7%)<br><1 year old had the highest fatality rate | | EV71 (+) rates among fatal cases<br>1998: 34 ( 44%)<br>1999: 1 ( 11%)<br>2000: 25 ( 61%)<br>2001: 27 ( 47%)<br>2002: 8 ( 27%)<br>2003: 4 ( 50%)<br>2004: 5 (100%)<br>2005: 7 ( 44%)<br>2006: 0<br>Total (~2006) 111 (45.3%)<br>EV71 was the most common circulating serotype in 1998, 2000, and 2001 |

| Ref. No. | Type of Surveillance | Participatory Institutions | Year / Month | Case definition / Patients from whom samples were collected | | Type of specimen | Number of cases / Number of specimens | |
|---|---|---|---|---|---|---|---|---|
| [5] | Reporting system for severe EV cases | Notifiable disease surveillance | 2000-2005 | Patients suspected of having serious complications due to EV infections<br><br>Cases were reported or referred when ≥1 of the following symptoms/diagnosis was suspected | | Definitive virology results were not required at the time of reporting, specimens were to be collected as soon as possible and submitted to Taiwan (China) CDC | No. of reported severe cases<br>2000: 457<br>2001: 628<br>2002: 314<br>2003: 139<br>2004: 162<br>2005: 274<br>Total: 1977<br><br>No. of confirmed* severe cases<br>2000: 291<br>2001: 388<br>2002: 156<br>2003: 70<br>2004: 50<br>2005: 141 | |
| | | | | Patients of all ages | Infants < 3 months | | | |
| | | | | - Myoclonic jerks<br>- encephalitis,<br>- encephalo-myelitis<br>- acute flaccid paralysis<br>- poliomyeli-tis-like syndrome<br>- myocarditis<br>- pericarditis<br>- pulmonary oedema/ haemorrhage<br>- acute cardio-pulmonary failure | -thrombocyto-penia<br>- sepsis<br>- hepatic failure<br>- multi-organ failure | | | |
| [3] | Virological surveillance | Reference laboratories in 11 hospitals and Taiwan (China) CDC | May 98-Dec 05 | Inpatients or outpatients suspected of having enteroviral infection | | Included:<br>throat swabs<br>stool<br>cerebro-spinal fluid<br>blood samples | 14 910 specimens | |
| [5] | Virological surveillance | Sentinel physicians were requested to collect clinical specimens matching the definition<br>(Currently, over 700 physicians from 75% of basic administrative units participated in the Sentinel Physician Surveillance.)<br><br>150 physicians were selected to collect specimens from at least two patients matching the definition each week<br><br>10 ~12 reference laboratories | 2000-2005 | Clinical samples from patients suspected of flu-like illness and HFMD/HA | | Throat swabs<br><br>In cases of HFMD/HA: Stool / rectal swabs;;Nasopha-ryngeal secretion / swabs; Cerebro-spinal fluid would be collected | No. of specimens<br>2000: 7798<br>2001: 12 512<br>2002: 15 671<br>2003: 15 864<br>2004: 14 801<br>2005: 16 383 | |
| Japan | | | | | | | | |

| | Age | Sex | Incidence Rate / Attack rates | No. of deaths / Case fatality rates | Wave | Laboratory findings EV71 involvement |
|---|---|---|---|---|---|---|
| | | | | | | No. of EV71(+) / no. of confirmed with etiology identified** |
| | | | | | | 2000: 152 / 196<br>2001: 182 / 238<br>2002: 57 / 84<br>2003: 44 / 52<br>2004: 20 / 29<br>2005: 82 / 119<br>Total: 537 / 721 |
| | | | | | | EV71 (+) rates among EV (+) specimens |
| | | | | | | 1998: 37% among 194<br>1999: 3% among 224<br>2000: 35% among 983<br>2001: 28% among 1569<br>2002: 16% among 1164<br>2003: 3% among 1134<br>2004: 30% among 759<br>2005: 20% among 2003<br>Total: 23% among 8030 |
| | | | | | | EV (+): 12,236<br>Median age 3 yrs<br>Predominant age <3 yrs: 63%<br>Sex Male: 57.6%<br>General trend 52% were identified between April – July, and the 2nd wave occurred between Sept – Nov |
| | | | | | | EV71 (+) rates among EV(+) specimens |
| | | | | | | 2000 18.2% (239/1310)<br>2001 15.0% (318/2114)<br>2002 7.9% (145/1839)<br>2003 13.0% (269/2066)<br>2004 8.5% (204/2389)<br>2005 13.9% (351/2518)<br>Total (1526/12 236) |

| Ref. No. | Type of Surveillance | Participatory Institutions | Year / Month | Case definition / Patients from whom samples were collected | Type of specimen | Number of cases / Number of specimens |
|---|---|---|---|---|---|---|
| [6], [7] | Sentinel surveillance | About 3000 paediatric clinics | 1999-2007 | Cases meeting both criteria as below<br>1. About 2-5mm vesicular lesions on palms, feet/soles, mucous membrane in the oral cavity cure<br>2. Vesicles healing without formation of crust | | No. of cases per sentinel (no. of sentinels)<br>1999: 17.67 (2875)<br>2000: 68.96 (2978)<br>2001: 42.32 (3019)<br>2002: 29.98 (3036)<br>2003: 56.78 (3041)<br>2004: 29.39 (3019)<br>2005: 28.84 (3065)<br>2006: 33.16 (3014)<br>2007: 31.11 (3012)<br><br>Between 2000 to 2007 number of cases per sentinel was highest in children age one year. |
| [8], [9], [10] | Virological surveillance | About 10% of sentinel paediatric clinics | 1997-2009 | HFMD patients | | No. of specimens with pathogen detected<br>1997: 308<br>1998: 644<br>1999: 210<br>2000: 749<br>2001: 390<br>2002: 447<br>2003: 710<br>2004: 276<br>2005: 408<br>2006: 515<br>2007: 518<br>2008: 546<br>2009: 264 |



* Detailed data from every suspected cases were discussed retrospectively by a panel of paediatric and infectious disease specialists who met bi-weekly during the EV peak season or monthly during the reminder of the year.

** Every specimen was screened for the following viruses: enteroviruses or other respiratory viruses, including adenovirus, influenza virus, parainfluenza virus, and others

**References**

1. Podin Y, et al. Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: lessons from the first 7 years. BMC Public Health, 2006, 6: 180.

2. Ang LW, et al. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. Annals of the Academy of Medicine Singapore, 2009, 38(2):106–112.

3. Chen KT, et al. Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. Pediatrics, 2007, 120(2): e244–252.

4. Chang LY. Enterovirus 71 in Taiwan. Pediatric Neonatology, 2008, 49(4):103–112.

5. Tseng FC, et al. Epidemiological survey of enterovirus infections occurring in Taiwan between 2000 and 2005: analysis of sentinel physician surveillance data. Journal of Medical Virology, 2007, 79(12): 1850–1860.

| | Age | Sex | Incidence Rate / Attack rates | No. of deaths / Case fatality rates | Wave | Laboratory findings EV71 involvement |
|---|---|---|---|---|---|---|
| | | | | 1997: 3 related to HFMD or EV71 | | |
| | | | | | | |



6. Taniguchi K, et al. Overview of Infectious Disease Surveillance System in Japan. Journal of Epidemiology, 2007, 17 Supple: S3–13.

7. National Institute of Infectious Diseases, Infectious Disease Surveillance Center. Infectious diseases weekly report: surveillance data table (page 2: Sentinel-reporting diseases). Available at: http://idsc.nih.go.jp/idwr/ydata/report-Eb.html (Last accessed on 5 March 2010)

8. Educational Committee of Infectious Agents Surveillance Report, National Institute of Infectious Diseases. Annual report on findings of infectious agents in Japan, 2003. Japanese Journal of Infectious Disease, 2004, 57 Suppl: 1–143.

9. National Institute of Infectious Diseases, Infectious Disease Surveillance Center. Infectious agents surveillance report: isolation and detection of viruses [Graphs 2 Updates]. Available at: http://idsc.nih.go.jp/iasr/prompt/s2graph-ke.html (Last accessed on 5 March 2010)

10. National Institute of Infectious Diseases, Infectious Disease Surveillance Center. Infectious agents surveillance report: isolation and detection of viruses [Graphs 2 Archives]. Available at: http://idsc.nih.go.jp/iasr/prompt/s2graph-pke.html (Last accessed on 5 March 2010)

# Appendix 2 : Benefits and limitations of specific prevention and control measures

| | Benefits | Limitations |
|---|---|---|
| Establishing and strengthening surveillance | Essential for risk assessments | Lack of standardized surveillance systems for HFMD<br>To provide timely data and information for risk assessment<br>Requires better understanding of the clinical spectrum of disease for surveillance priority<br>Limited existing capacity for clinical detection and laboratory diagnosis and standard assays, and resource constraints<br>Need to ensure sustainability |
| Conducting information and education campaigns on good hygiene and basic sanitation to reduce spread of disease | Protects individuals from infection<br>Cost-effective if high rate of compliance<br>Uncontroversial<br>Limited social and economic consequences compared with other societal level measures<br>Beneficial in reducing infections from other pathogens | Low compliance in some social and cultural contexts<br>Requires hand washing facilities and supplies in kindergartens and schools and in public places<br>Need to be culturally sensitive and ensure sustainability |

| | Benefits | Limitations |
|---|---|---|
| Providing assistance to kindergartens, daycares facilities and schools during outbreaks to protect children, reduce transmission or delay spread of disease to the community | Feasible approach, especially for mild cases<br>Targets high-risk groups<br>Less stressful and has good psycho-social support from family members<br>Easier to ensure essential services (e.g. food and entertainment etc.)<br>Promotes social and community support network | May need legal authority to suspend classes<br>Needs strong support from other sectors (e.g education authorities)<br>May need to arrange alternative education programme<br>Parents who are essential workers may be diverted from responsibilities to take care of their children<br>May cause public anxiety and psychosocial stress in students |
| Strengthening infection control measures both in health care facilities and in the community | Essential for all infectious diseases<br>Easier to ensure essential interventions<br>Cost-effective (resource saving)<br>Early detection to facilitate prompt isolation, monitoring of children | Some health care facilities do not have enough resources to implement<br>Need to train caregivers on infection prevention and control for patient management,<br>Compliance with infection prevention and control measures may be low |
| Coordinated risk communication to increase awareness of the risk through providing accurate and consistent messages to the public | Proactive measure to individuals and public<br>Cost-effective if compliance high<br>Uncontroversial<br>Limited social and economic consequence compared with societal level measures | Low compliance in some social and cultural context<br>Need to be culturally sensitive and ensure sustainability |