

Forum on Hand Foot and Mouth Disease (HFMD) in Asia-Pacific Region

*Epidemiological, Laboratory, Clinical, and Public Health
Aspects*

York Hotel, Singapore
21 – 22 August 2008

FORUM REPORT

Sponsored by



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ABBREVIATIONS

ABG	Arterial Blood Gases
ABP	Arterial Blood Pressure
AFP	Acute Flaccid Paralysis
APNET	Asia Pacific Network for Enterovirus Surveillance
BP	Blood Pressure
CDC	Center for Disease Control and Prevention
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
CSL	Commonwealth Serum Laboratories
CVA	Coxsackievirus A
CVB	Coxsackievirus B
CVP	Central Venous Pressure
DSO	Defense Science Organization
EC	Echovirus
ECMO	Extra Corporeal Membrane Oxygenation
ELISA	Enzyme-linked Immunosorbent Assay
EV	Enterovirus
FETP	Field Epidemiology Training Program
GCS	Glasgow Coma Scale
HFMD	Hand, Foot and Mouth Disease
HIB	Haemophilus Influenzae Type B
HR	Heart Rate
ICU	Intensive Care Unit
IFA	Immuno Fluorescence Assay
IgG	Immunoglobulin G
IICP	Increased Intracranial Pressure
IVIG	Intravenous Immunoglobulin
JEV	Japanese Encephalitis Virus
KKH	Kandang Kerbau Women's and Children's Hospital
MOH	Ministry of Health
MOPH	Ministry of Public Health
MRI	Magnetic Resonance Imaging
MTA	Material Transfer Agreement
NUH	National University Hospital
NUS	National University of Singapore
NPHL	National Public Health Laboratory
NT	Neutralization Test
PCR	Polymerase Chain Reaction
PICU	Pediatric Intensive Care Unit
PVC	Polyvinyl Chloride
RD	Rhabdomyosarcoma
REDI	Regional Emerging Diseases Intervention
RNA	Ribo Nucleic Acid
RODS	Real Time Outbreak and Disease Surveillance
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAR	Special Administrative Region
SEARO	South East Asian Regional Office
SGH	Singapore General Hospital
SOP	Standard Operating Procedure
WBC	White Blood Cell
WHO	World Health Organization
VTM	Viral Transport Medium

EXECUTIVE SUMMARY

Introduction

Since the late 1990s the Asia Pacific region has experienced several large epidemics of hand, foot and mouth disease (HFMD) in children that have been accompanied by higher rates of severe neurologic and cardio-pulmonary manifestations than commonly seen. These severe cases of HFMD are associated with Enterovirus 71 (EV71) infection, which appears to be increasing in the region and its emergence has prompted concern that it will continue to spread in the region and globally.

To assess the HFMD situation in the Asia Pacific Region, the Regional Emerging Diseases Intervention (REDI) Center in partnership with the Ministry of Health (MOH) of Singapore and the Southeast Asia Regional Office of US CDC organized a forum on HFMD in Singapore on 21-22 August 2008. Eighty-eight participants from Australia, Brunei, China, Indonesia, Malaysia, Singapore, Taiwan, Thailand, USA and Vietnam attended the forum. The aim of the forum was to provide a regional platform for sharing knowledge and expertise on the clinical, epidemiological, laboratory, and public health aspects of HFMD and to identify opportunities to work on common solutions for preventing and treating the disease, especially the severe and potentially fatal consequences of EV71 infection.

At the forum international experts presented updates on the virology, pathogenesis, epidemiology, diagnosis and treatment of HFMD. Regional experts described their experiences of responding to HFMD outbreaks and managing the care of children affected by HFMD and its complications. Other speakers provided updates on the development of antiviral drugs, immune globulin preparations and monoclonal antibodies, and vaccines for prevention and treatment of EV71 infection. On the second day of the forum, participants formed working groups that were challenged to identify priorities for basic, clinical and operational research that is needed to improve surveillance, diagnosis and treatment and the public response to EV71 in the Region. The speakers and topics presented at the forum are listed in the attached list of presenters and presentation topics.

Hand Foot and Mouth Disease in Asia Pacific Region

HFMD is a common, self-limiting illness of children caused by a group of enteroviruses (Picornaviridae family) that includes Coxsackieviruses A and B, echoviruses and various other enteroviruses. The virus is excreted in feces and is also found in pharyngeal secretions. Transmission is associated with close contact among children and possible through environmental contamination. The disease is characterized by an acute onset of fever with a rash on the palms, soles, buttocks, and knees, and vesicles on buccal membranes that usually resolve in 7-10 days. Only a small proportion of children with HFMD develops severe forms of the disease. Severe HFMD, involving primarily the neurologic and cardiovascular systems manifesting as syndromes such as meningitis, encephalitis, acute flaccid paralysis, pulmonary

edema and cardiac failure appear to occur most frequently with EV71 infection. In the Asia-Pacific Region the most devastating neurological syndrome is brainstem encephalitis, which has a mortality of 40-80 percent. Children with severe HFMD may take months to recover, and in some cases the neurologic damage may be permanent. Currently, there is no specific antiviral treatment for HFMD and no vaccines to prevent EV71 infection.

EV71 was first isolated from a child who died of encephalitis in California in 1969 which was first reported in 1974¹. Although the virus has been detected worldwide since then, the recent regional epidemics of HFMD in Asia has raised concern that more pathogenic forms of EV71 may be emerging in the Region. Taiwan reported 129,106 HFMD cases in a 1998 epidemic with 405 having the severe form of the disease of which 78 were fatal². Singapore reported an epidemic of 9000 cases with 7 deaths during 2000-2001³, and since then has experienced recurrent epidemics every two to three years. During the first 8 months of 2008, Singapore reported 19,530 cases⁴ and one death⁵ due to HFMD. In Malaysia the first recognition of recent wave of outbreaks was in 1997 in Sarawak⁶. In 2006 Sarawak, Malaysia reported an epidemic of 14,875 HFMD cases⁷ with 13 deaths⁸. Thailand reported 3961 cases with 7 deaths in 2006. In 2007, Thailand reported an epidemic of 16,846 cases with 2 probable EV71 deaths without hand, foot or mouth lesions⁹. China reported 83,344 cases with 17 deaths in 2007, and in 2008 experienced an outbreak in Fuyang City in Anhui Province with 6049 HFMD cases and 22 deaths

¹ Schmidt NJ, EH Lennette, HH Ho (1974), An Apparently new enterovirus isolated from patients with disease of the central nervous system, J INFECT DIS. 129:304-309.

² Monto Ho, M.D., Eng-Rin Chen, Dr. Med.Sci., Kwo-Hsiung Hsu, M.S. *et al* (1999), An epidemic of Enterovirus 71 Infection in Taiwan NEJM. 341(13):929-935.

³ Committee on Epidemic Diseases. Surveillance of hand, foot and mouth disease in Singapore. Epidemiological News Bulletin 2001; 27:23-7

⁴ Ministry of Health, Singapore. Weekly Infectious Disease Bulletin. Epidemiological Week 35, 24-30 August 2008. Communicable Disease Surveillance Branch, Communicable Diseases Division, Ministry of Health, Singapore. Available at: <http://www.moh.gov.sg/mohcorp/statisticsweeklybulletins.aspx> (Last accessed on 3 September 2008).

⁵ Ministry of Health, Singapore. Press release, date of issue: 7 August 2008. Suspected Hand, Foot and Mouth Disease (HFMD) Death of 3-Year Old Boy in Singapore. Available at <http://www.moh.gov.sg/mohcorp/pressreleases.aspx> (Last accessed on 3 September 2008).

⁶ Cardoso M J, S Krishnan, P H Tio (1999) *et al* Isolation of subgenus B adenovirus during a fatal outbreak of enterovirus 71-associated hand, foot, and mouth disease in Sibul, Sarawak; THE LANCET 354:987-991.

⁷ Hand, Foot and Mouth Disease – Malaysia (Sarawak) Promed Archive Number: 20060313.0792

⁸ Hand, Foot and Mouth Disease – Malaysia (Sarawak) Promed Archive Number: 20060821.2355

⁹ Bureau of Epidemiology. Annual epidemiology surveillance report 2007, Express transportation organization of Thailand publishing. 2007, Thailand Ministry of Public Health Bangkok. p. 89.

among 353 cases with the severe forms of the disease¹⁰. These large outbreaks were widely covered by the press, which highlighted parental concerns about the health of their children and the social disruption from closing of schools and day care centers by public health departments in an attempt to break the chain of transmission.

Molecular Epidemiology of EV71

Molecular typing distinguishes four genogroups of EV71 designated A, B, and C. In the Asia-pacific region subgenogroups B and C have predominated. Subgenogroups B1 and B2 were first identified in US children during the 1970s and 1980s. The predominant subgenogroups of Genogroup B in the Asian HFMD epidemics predominantly in Malaysia and Singapore during the past two decades have been B3, B4 and B5. In the past decade Genogroup C has emerged in the Region with subgenogroups C1-C5¹¹ occurring in sporadic epidemics in Australia, Vietnam, Japan, Taiwan, and South Korea. In Malaysia there have been sporadic cases of subgenogroups C1 and C2 however no epidemics where genogroup C was predominant. Subgenogroup C4 was the cause of the serious cases in the recent HFMD epidemic in Anhui Province in China. Subgenogroup C5 was first identified in Vietnam 2005. In general, Genogroup C appears to be more prevalent in China and Vietnam while Genogroup B predominates in Malaysia and Singapore. Both B and C genogroups are represented in recent outbreaks in Taiwan. A new isolate of EV71 was recently obtained from a patient in India. The forum speakers concluded that they could find no association identified between virulence or severe disease and subgenogroup.

Natural History and Epidemiology

There are still large gaps in understanding the natural history and epidemiology of EV71 HFMD. Very little is known about the incidence, prevalence, and disease burden, seroprevalence during intra-epidemic periods, rates of asymptomatic and/or mild clinical disease and other indicators of the impact of infection and disease. More information is needed to fully understand the natural history of EV71 disease, especially the severe forms of the disease. There is also a need for a better understanding of the characteristics of natural immunity to EV71, the duration of acquired protection and the degree of cross-protection with other enterovirus infections.

The forum participants concluded that a collective effort was needed to gather disease incidence, mortality and cost data in order to assess the true impact of the disease and to identify areas for intervention, and evaluate current measures of prevention and control including examining the cost-effectiveness of measures taken. It is possible that many of these

¹⁰ Report on the Hand, Foot and Mouth Disease Outbreak in Fuyang City, Anhui Province and the Prevention and Control in China, China CDC/ WHO China May 2008

¹¹ Brown BA, MS Oberste, Jr JP Alexander, *et al.* (1999) Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. *J Virology*; 73(12): 9969 – 75.

questions can at least be partially addressed with a more comprehensive examination of existing data that have not been completely examined. An effort to systematize or harmonize regional surveillance data collection, including case definitions, case detection strategies (e.g. active or passive) and methods of diagnosis would strengthen documentation and analysis of data resulting from regional outbreaks. A more systematic approach would allow for regional comparison of epidemiological and clinical data, which could help identify risk or predictive factors for infection, progression to severe disease, and clinical outcome and to plan studies to address unanswered questions about the natural history of the disease.

The forum concluded that prospective coordinated clinical studies using standardized protocols are needed to identify the most effective strategies for early detection and treatment of severe HFMD. Given its rarity, a well-designed case control study would be best to identify risks for developing severe disease. Prospective studies need not necessarily be large however a lot could be gleaned from mining existing large databases. Early detection of a rare, severe manifestation of a common disease like HFMD is a challenge when there is no practical method to identify those with the greatest likelihood to progress to severe disease. Active case investigation of HFMD is resource intensive during an epidemic because it requires a clear understanding of the natural history of the disease, the clinical and laboratory correlates to test for, and the target population for surveillance. Some clinicians suggest that surveillance should be targeted to detection of severe disease in hospitals, although clinical and epidemiological data to support this approach remains to be established.

Prevention and Control of HFMD in the Asia Pacific Region

Several forum speakers described the extensive public health programs for controlling HFMD in the region. These programs include intensive campaigns to educate on good hygiene and basic sanitation, active and passive case finding, school and day care closures, public health information strategies and resource dissemination on good practices for affected and at-risk facilities and populations. In practice it has been difficult to document the attributable effectiveness of each intervention because of the natural dynamics of HFMD epidemics that confound the analysis. The epidemiological pattern of HFMD is likely influenced by multiple factors, including health-seeking behavior, socio-economic changes, school holidays, and media attention. Several speakers proposed more intense prevention programs that include enhanced surveillance and case detection, targeted case isolation or voluntary quarantine, and improved public health education and dissemination of information by practicing effective risk communication principles. However, it is important to have good documentation of outbreak investigations and outcomes from school closures, including publishing data. Specifically, when a school closes down, efforts should be made to document whether those children got HFMD anyway. Good descriptive data could provide some assessment of the value of school closure.

Laboratory Diagnosis for HFMD and EV71

The virologic diagnosis of HFMD takes on crucial importance because of the need to rapidly determine if the infection is caused by EV71, which is associated with more severe forms of the disease. Forum speakers stressed that the commonly used PCR tests for HFMD agents need to be standardized and quality control enhanced to improve virologic diagnosis. Most importantly the primers for the PCR test need to be constantly updated in response to the divergence of the HFMD causing viruses in the Region. In addition most participants agreed that a rapid point-of-care test using easy to access clinical specimens such as oral secretions is urgently needed to facilitate clinical management of HFMD. In addition, further work is needed to improve testing for markers of virulence or severity that could be studied using advanced techniques of proteomics and immunology.

From a molecular epidemiology perspective, there is a need to focus on partial sequencing the VP1 and VP4 domains of EV71 as well as increase the capacity to do full domain sequencing. Forum speakers acknowledged that molecular analysis of EV71 has been a challenge because of the lack of standardized procedures and nomenclature. Informal networks for molecular surveillance of EV71 such as the Asia Pacific Network for Enterovirus Surveillance (APNET) should be supported and similar networks could be formed to cover other regions. APNET provides laboratory training for sequence analysis to enable developing countries to participate in regional viral surveillance networks for molecular surveillance of EV71.

Drugs and Vaccines for EV71

Currently there are no vaccines or antiviral drugs effective against EV71 infections. Several experimental antiviral drugs including Pleconaril, Enviroxime, BPROZ-194 and Miltiorrhiza Type I interferons are in various stages of clinical evaluation. Some countries have used IVIG for treatment of the neurologic stages of EV71 infection. The forum participants encouraged further development of antiviral drugs and IVIG products and the conduct of the clinical trials to determine their clinical efficacy.

Vaccine companies based in Singapore, China and Taiwan are developing inactivated whole virion vaccines for EV71 that are currently undergoing preclinical toxicity and animal testing. Clinical testing of these products could begin in the next year or two. Early data from animal challenges studies indicate that the vaccines are immunogenic and protect against challenge infection in experimental animal models of EV71 infection.

Research Priorities

The forum strongly supported the development and promotion of multi-center collaborative studies across institutions and nations that could explore and evaluate the basic questions regarding the natural history and epidemiology of EV71 and its severe manifestations. Such national and international collaborations could enable studies of sufficient size and statistical

power needed to address important gaps in our current understanding of HFMD and EV71 infection. In addition, this network could be expanded to help answer research questions on the development of best practices for clinical management through the conduct of prospective cohort studies and randomized controlled trials that would provide an evidence base for improved care and treatment of EV71 and its severe manifestations. The priority research issues that need to be addressed include:

1. Establishing a better understanding of the natural history and epidemiology of EV71 disease, including risk factors for progression to severe disease
2. Determining the effectiveness of the commonly used prevention and control measures to contain outbreaks of HFMD
3. Understanding the reasons for the increasing magnitude of EV71 outbreaks, frequency of cases and frequency of severe cases and its sequelae in the Asia-Pacific region
4. Increasing knowledge of pathogen and host genomics that affect the outcome of EV71 infection.

Although recently published studies have provided new insights into the epidemiology, pathogenesis, diagnosis and clinical management of HFMD in the region, the forum participants also concluded that more research is needed to identify more effective ways of preventing and treating HFMD.

List of Presenters and topics

Session 1. Overview and Update on Epidemiology and Status of EV71 Outbreaks in the Region

A. Overview of HFMD and historical perspective

- | | | |
|---|--------------------------------|---|
| 1 | Dr. Peter Mc Minn
Australia | Overview of current knowledge on regional distribution and disease burden of HFMD and EV71 and implications from clinical and public health perspective |
| 2 | Dr. Mark A. Pallansch
USA | Historical perspective and current status of HFMD and EV71 in the west |

B. Epidemiology and Surveillance Regional Panel

- | | | |
|----|----------------------------|---|
| 3 | Dr. Zhang Jing
China | The outbreak of HFMD in Fuyang city, Anhui province |
| 4. | Dr. Hui-Chen Lin
Taiwan | Surveillance of Enterovirus Infection, Taiwan |

5. Dr. Steven Ooi
Singapore HFMD – an overview
6. Dr. Andrew Kiyu
Malaysia Epidemiology of HFMD, Sarawak, Malaysia

C. Outbreak Response and Control

7. Dr. Steven Ooi
Singapore Outbreak Management – The Singapore Experience

D. Outbreak Response and Control Regional Panel

8. Dr. Huilai Ma
China Risk Factors for Transmission of Hand, Foot and Mouth Disease During Confirmed Enterovirus 71 Outbreaks in China, 2008
9. Dr. Kun-Bin Wu
Taiwan Outbreak Response and Control of Enterovirus in Taiwan, 2008
10. Dr. Wanna Hanshaworakul
Thailand Outbreak of HFMD and EV71 in Thailand

Session 2. Diagnostics and Molecular Epidemiology

11. Dr. Jane Cardosa
Malaysia Diagnostic methods and molecular epidemiology

A. Diagnostics and Molecular Epidemiology Regional Panel

12. Dr. Phan Van Tu
Vietnam Current Diagnostic Strategies
13. Dr. Wenbo Xu
China Identified an Outbreak of EV71 in Fuyang City of Anhui, 2008
14. Dr. Dustin C. Yang
Taiwan Laboratory Diagnosis of Enterovirus in CDC Taiwan
15. Dr. Raymond Lin
Singapore EV71 surveillance in Singapore
16. Dr. Ratigorn Guntapong
Thailand Laboratory Diagnosis and Molecular Epidemiology in Thailand: EV71

Session 3. Pathogenesis, Clinical Spectrum and Management of HFMD

17. Dr. Wong Kum Thong
Malaysia A Review of the current understanding of HFMD and EV71 pathogenesis and implications for clinical management
18. Dr. Tzou-Yien Lin
Taiwan Clinical aspects of enterovirus 71 infections: Taiwan experience

A. Pathogenesis, Clinical Spectrum and Management Regional Panel

19. Dr. Chong Chia Yin
Singapore HFMD in Singapore – The Clinical Aspects
20. Dr. M. H. Ooi
Malaysia Clinical Spectrum of HFMD/EV71 and case management approaches
21. Dr. Piyarat Suntarattiwong
Thailand Hand, Foot and Mouth Diseases from Enterovirus 71: Clinical Aspects

Session 4. Public Health Response

22. Dr. Lyn James
Singapore Surveillance of HFMD in Singapore - what we know and what we don't know
23. Ms. Melinda Frost
US CDC, China Societal impact – Emergency Risk Communication Strategies – The China Experience
24. Dr. Kamaliah Mohd Noh
Malaysia Prevention & Control of HFMD - The Sarawak Experience

Session 5. Drugs and Vaccines

25. Dr. Vincent Chow
Singapore Novel therapeutic strategies against Enterovirus 71 infections
26. Dr. Joe Santangelo
Singapore Update on progress in vaccine development against EV71- SingVax
27. Dr. Shanshan Dong
China Update on progress in vaccine development against EV71- Sinovac Biotech
28. Dr. Chiang Jen- Ron
Taiwan Update on progress in vaccine development against EV71-Taiwan

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1 SCOPE OF THE REPORT

Over the last decade, outbreaks of hand, foot and mouth disease (HFMD) in the Asia-Pacific region have been increasingly reported. The disease is caused by different serotypes of enterovirus, including enterovirus 71 (EV71), coxsackievirus A (CVA), and coxsackievirus B (CVB). Although HFMD is commonly a self-limiting illness, outbreaks associated with EV71 as the primary causal agent have been particularly alarming because of its association with serious and fatal disease resulting from neurological and cardiopulmonary complications.

The overlap in neurological presentation with that caused by the more well-known enterovirus, poliovirus has also led some to speculate and suggest that EV71 could become an important cause of acute neurological disease in the world with the eradication of poliovirus from the affected regions. This prospect should urge the regional stakeholders in public health, medical care and research to work together in order to improve the level of understanding and knowledge about this disease. Neither vaccines nor specific treatment is available for the disease, which means that rapid and accurate detection and recognition of EV71 disease and early intervention is critical to mitigating its potentially severe and fatal consequences. Sharing information and expertise and comparing experiences among the affected areas could improve the state of knowledge on the disease epidemiology, laboratory diagnosis, clinical management as well as control and prevention strategies, and identify priority issues around which collaborative activities and additional research are needed.

Hence, a regional forum on HFMD in the Asia-Pacific region, was convened jointly by the Singapore Ministry of Health and the REDI Center on 21-22 August 2008 in Singapore. Regional public health officials, medical practitioners, and research scientists from national and international public health organizations gathered and discussed clinical, epidemiological, laboratory diagnosis and public health aspects of HFMD and EV71 infection.

This report is the outcome of the consultation and is presented with the hope of providing important up-to-date and relevant knowledge for public health and medical personnel, as well as helping to inform future strategies on surveillance, prevention, control as well as management of the disease, and identifying programmatic and research priorities for the regional groups and interested parties to consider.

2 NATURAL HISTORY AND EPIDEMIOLOGY OF HFMD

2.1 Overview

Hand, Foot and Mouth Disease (HFMD) is a common illness of young children caused by a group of Enteroviruses (Picornaviridae family) that includes Coxsackievirus type A (CVA), B (CVB), echoviruses and various other enteroviruses). Serotypes EV71 and CVA16 are the most frequent causes of HFMD in the region. The natural history of the disease is not well known. HFMD is characterized by acute onset of fever with a rash (described typically as maculopapular or papulovesicular) on palms, soles, buttocks, knees and vesicle lesions on the buccal membranes. Although HFMD is distressing and temporarily debilitating for both child and caretaker, the illness is for the most part self-limiting. Complications of HFMD including neurological disease accompanied with pulmonary edema and cardiovascular collapse rarely occur.

Since the late 1990s the Asia Pacific region has experienced several large epidemics of hand, foot and mouth disease (HFMD) in children that have been accompanied by higher rates of severe neurologic and cardio-pulmonary manifestations than commonly seen. These severe cases of HFMD are associated with Enterovirus 71 (EV71) infection, which appears to be increasing in the region and its emergence has prompted concern that it will continue to spread in the region and globally.

The genus enterovirus, which includes poliovirus, coxsackie virus, echovirus and enterovirus 68-71 belong to the family of single-stranded RNA viruses known as Picornaviridae. Enteroviruses have been classified into groups A-D and to date over 100 types of enteroviruses have been identified. The enteroviruses most widely implicated in outbreaks of HFMD include CVA 16 and EV71, are group A enteroviruses. EV71 is a highly neurotropic virus that was first isolated in California, United States from a child who died of encephalitis¹ almost 40 years ago. Molecular typing divides EV71 into three genogroups, A, B and C. Genogroups B and C are further subdivided into B1-B5 and C1 to C5¹¹.

Figure 1 and Table 1 illustrate phylogenetic analysis of circulating EV-71 virus Genogroups and Subgenogroups since 1970-present.

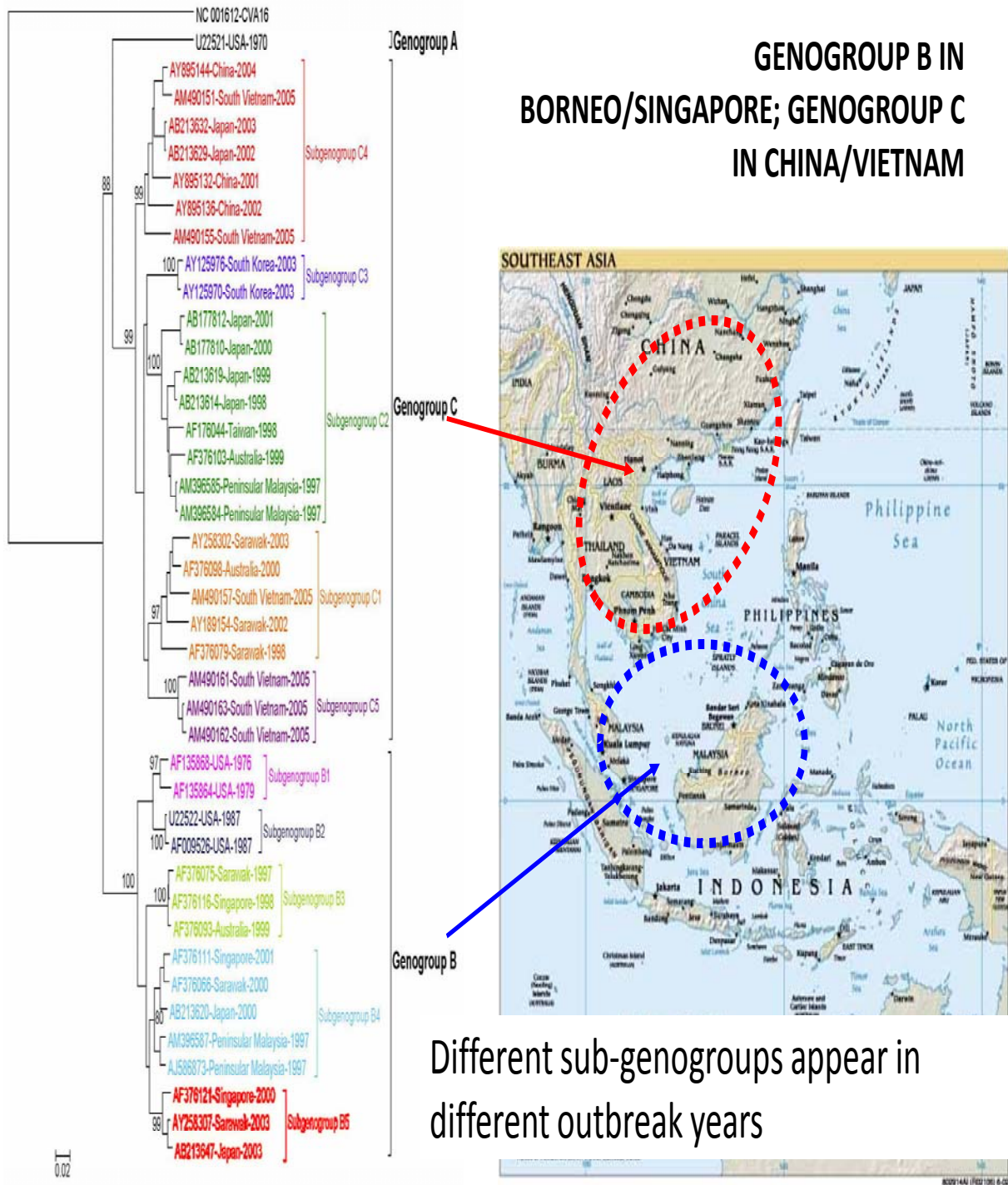


Figure 1 EV71 Dendrogram and location of global circulating genogroups, 1970-2005
 Courtesy of Dr. Jane Cardosa (Malaysia)

EV71 SUBGENOGROUPS 1980-2008												
COUNTRY	1986	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007 2008
SINGAPORE		B3 B4	B3 C1	B3	B4	B4	C1 B4	B4			B5	B5(2008)
MALAYSIA		B3 B4 C1 C2	C1		B4 C1		C1	C1 B5			B5	B5
THAILAND						B4 C1		C1	C1		B5 C1 C2 C4	B5 C1 C2 C4 C5
TAIWAN	B1		C2 B4 C4	B4	B4	B4	B4	B4	C4	C4	C5	C5 B5 C4
JAPAN		B3 B4 C2	C2	C2	B4 C2	C2	B4	C4 B5	C4			
CHINA		C3	C4		C4	C4	C4	C4	C4			C4
KOREA					C3							
AUSTRALIA				B3 C2	C1							
VIETNAM										C1 C4 C5		

Table 1 EV71 Subgenogroups: 1980 – 2008

References: Lin KH *et al.* J Med Virol. 78(2): 254-62, 2006
 Cardosa MJ *et al.* EID 9(4): 461, 2003
 McMinn P *et al.* JV 75(16): 7732-38, 2001
 Huang YP *et al.* Virus Res. 137(2):206-12, 2008

Genogroups A and B for the most part first appeared before 1990 with group C strains appearing later. Genogroup A was first identified in the United States in 1970. Genogroup B was identified from isolates in US during the 1970s and 1980s (B1, B2) but since 1997, strains identified from HFMD outbreaks in the Asia Pacific region have been classified as subgenogroups B3, B4 and B5. Subgenogroup B3 was identified from isolates in Sarawak (1997), Singapore (1998) and Australia (1999) whereas subgenogroup B4 was found in outbreaks in Peninsular Malaysia (1997), Sarawak (2000), Japan (2000) and Singapore (2001). Subgenogroup B5 has been found predominantly in Singapore (2000), Sarawak (2003) and Japan (2003).

Genogroup C has emerged relatively recently with C1 isolated in Sarawak (1998, 2002, 2003), Australia (2000) and South Vietnam (2005); C2 identified in Peninsular Malaysia (1997), Taiwan (1998), Japan (1998, 1999, 2000 and 2001), Australia (1999) and C3 in S Korea (2003). Subgenogroup C4 has been the focus of interest in particular in mainland China and Taiwan where it has been the predominant strain in recent outbreaks. Subgenogroup C5 was first identified in isolates from South Vietnam (2005) but since then has been identified in Thailand and Taiwan as well. At the meeting it was presented that a novel Genogroup single isolate originated in India in 2001 and its role as a major player in viral epidemiology remains unclear.

The HFMD outbreak years and predominant subgenogroups for each country or region have differed so far. In some countries for example Malaysia, China, Vietnam and Thailand, outbreaks have been mainly associated with EV71, while it was hard to conclude for other countries like Singapore as the dominant circulating strain in some 'outbreak years' was not EV71. In Singapore, higher number of HFMD cases were observed in 2002, 2005, 2006–2008 compared to 2000–2001. However in Malaysia the outbreak years and the corresponding predominant subgenogroups were 1997 (B3), 2000 (B4), 2003 (B5) and 2006 (B5). In China it was not clear whether HFMD was caused due to EV71 subgenogroups in 1997 because no data were collected that year. In the subsequent years – 1998, 2000, 2001, 2002, 2003, 2004 and 2008, the subgenogroup found was C4. In Vietnam, the outbreak years were 2005, 2007 and 2008. Phylogenetic analysis of 23 HEV71 isolates showed that during the first half of 2005 viruses belonging to 3 subgenogroups, C1, C4, and a previously undescribed subgenogroup C5 cocirculated in southern Vietnam. In the second half of the year 2005, subgenogroup C5 predominated during a period of higher EV71 activity¹².

¹² Tu PV, TTT Nguyen, D Perera, *et al.* (2007) Epidemiologic and Virologic Investigation of Hand, Foot and Mouth Disease, Southern Vietnam, 2005. *EID*; 13(11):1733-1741.

2.2 Epidemiology: Incidence, Prevalence and disease burden estimates

HFMD is an endemic disease with global distribution and it is estimated that more than 50% of children under the age of 5 years are at risk. In response to increasing outbreak of EV71 in the region, public health authorities have initiated national disease notification and sentinel surveillance systems. A comprehensive analysis of regional data has not been undertaken and therefore it is difficult to get a regional picture of disease incidence, prevalence and disease burden. The following description is based on published data and by presentations by Singapore and Taiwan at the forum.

A recent review of the epidemiology of the disease in Singapore from 2000-2007 showed that incidence of cases of HFMD varied between 125.5 per 100,000 in non-outbreak years to 435.9 per 100,000 in 2007¹³ outbreak year. There appears to be a rise in total number of cases since reporting first begun in 2000 when the number of cases totaled 3790, to 16,228 in 2002. In 2008, up till August, 18,000 cases have been reported. Incidence of severe disease is not available for Singapore but overall, the case fatality rate was 0.06 in 2001 (3/5210 cases)¹². Incidence was highest in those ages 0-4 with a higher predominance in males.

Taiwan has had the largest epidemic to date in 1998 with 129 106 cases reported. In a recent publication on the epidemiology of HFMD in Taiwan, the incidence of severe disease was 8 cases per 100,000 for epidemics or outbreak years 1998 and 2001¹⁴. Annual fatality rates during the epidemic years were calculated by dividing the number of deaths of HFMD/herpangina for children < 15 years by the number of severe cases the same year as reported between 1998 and 2005 and these were 20.6% (48/233) in 1998 and 15.3% (36/235) in 2001. Analysis of data showed that those under the age of 5 were at a higher risk of

Epidemiology and Disease Burden – key challenges

Need to better understand key elements of natural history of HFMD, such as natural immunity, duration of acquired protection, degree of cross-protection with other enteroviruses

Need synthesis of regional data to improve knowledge on disease burden including disease incidence, prevalence, cost data and impact and investigate potential epidemiological and clinical predictors of outbreaks and disease severity

Need studies to explore host factors that may influence disease outcomes, in particular severe disease and mortality

Need to initiate assessment of effectiveness and costs of current measures of prevention surveillance data

¹³ Ang LW, KWB Koh, KP Chan, *et al.* (2008), Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007, ANNALS ACAD OF MED., SINGAPORE, in press.

¹⁴ Chen KT, HL Chang, ST Wang, *et al.* (2007), Epidemiologic Features of Hand-Foot-Mouth Disease and Herpangina caused by Enterovirus 71 in Taiwan, 1998-2005, PEDIATRICS, 120: p244-p252.

infection. Male children had higher rates than females. For both Singapore and Taiwan, the predominant serogroup reported is EV71 and CVA 16.

Not much is known regarding rates of asymptomatic or subclinical infections or seroprevalence of affected populations. Some data is available in the literature from Singapore and Taiwan. In a study in Singapore when comparing seropositivity with age, it was found that only 1 of the 124 (0.8%) samples from children ages 1–23 months had anti-EV71 antibodies compared to a seropositive rate increase of 12% per year in those ages 2-5 years suggesting that most infections occur in preschool-aged children¹⁵. In a 1996/1997 study carried out among 856 children aged 0-12 years at the National University Hospital (NUH), the average yearly sero-conversion rate of EV71 was 12% in children aged 2-5 years. EV71 antibody prevalence reached 50% after 5 years of age.

Seroepidemiological studies in Taiwan showed an inverse relationship between pre-epidemic seroprevalence and severe disease mortality suggesting age-related disease susceptibility or suggesting a protective effect by specific antibody¹⁶.

Knowing the pre-epidemic seroprevalence of EV71 could help in determining the risk factors associated with infection, severe disease and mortality during epidemics. Efforts should be initiated in gathering and synthesizing disease burden data in the region as well as expanding the studies on seroprevalence data. Such information will be important not only in identifying intervention strategies but will also help in assessing current measures for prevention and control including examining the cost-effectiveness of potential interventions.

Such regional studies may allow more detailed comparisons between key features of different epidemics, which may provide clues about factors that influence the severity of the outcome. For example, recently in Taiwan, the HLA-A33 gene which is common in Asians was found to be associated with greater susceptibility to EV71¹⁷. As yet, there has been no distinct association observed between specific genogroups and more severe clinical outcomes. More work is needed to determine whether any host-genomic factors are involved in resistance or susceptibility to EV infection.

¹⁵ Ooi E-E, MC Phoon, B Ishak, *et al.* (2002) Seroepidemiology of human enterovirus 71, Singapore, EID, 8(9):8995-7.

¹⁶ Chang LY, CC King, KH Hsu, *et al.* (2002) Risk factors of enterovirus 71 infection and associated Hand, Foot, and Mouth Disease/ Herpangina in children during an epidemic in Taiwan, PEDIATRICS, 109:6, p88.

¹⁷ Chang LY, IS Chang, WJ Chen, *et al.* (2008) HLA-33 is associated with susceptibility of enterovirus 71 infection, PEDIATRICS, 122(6):1271-6.

2.3 Transmission dynamics

The primary site of enterovirus replication is within the gastrointestinal tract. Patients with enteroviral illness excrete viruses in throat, nasal secretions or faeces before onset of symptoms and continue to excrete the virus for several weeks thereafter. Transmission through vesicles and upper respiratory secretions is highly efficient. Since 50-80% of HFMD enteroviral infections are asymptomatic¹⁸ the source of infection could be an asymptomatic case who transmits virus as readily as one who is ill. Young children are in the most susceptible age group and likely act as the primary reservoir for enteroviruses. In a study that measured seroprevalence of neutralizing antibodies to EV71 before and after an epidemic in Taiwan, it was estimated that up to 71% of children with EV71 were asymptomatic and may have served as reservoirs for EV71¹⁶.

Propagation of HFMD disease by the oral fecal route is very efficient in situations where children congregate such as in childcare centers and schools as well as within households. In an attempt to study the duration of enterovirus shedding, a study in Taiwan observed 12 children between the ages 1m – 5 years that tested positive for EV71 during the September 1998 to June 1999 epidemic. Virus isolation was carried out on throat swabs collected every 1 or 2 weeks for 1 month and stool specimen collected every 1 or 2 weeks for at least 2 months¹⁹. Shedding of EV71 was detected for up to 2 weeks in the throat and up to 6-8 weeks in the stool.

All enteroviruses have the potential to survive in the environment and it is thought that highly humid conditions in the region could play a role in prolonging virus survival. In the preschool or child-care setting where there may be asymptomatic cases in addition to symptomatic cases not being detected and isolated, children below 5 years of age may not have acquired the same immunity as their older counterparts. In addition, other factors such as long hours in crowded care and school centers, poor standard of hygiene leading to contamination of toys and furniture, sick children being sent to school may all contribute to facilitate rapid spread of the virus. There is no serologic or virologic evidence that domestic animals are reservoirs of EV in the environment.

¹⁸ Colorado Communicable Diseases Manual (2004)

<http://www.cdph.state.co.us/dc/Epidemiology/manual/AsepticMeningitisCD.pdf>

¹⁹ Chung PW, YC Huang, LY Chang, et al. (2001) Duration of enterovirus shedding in stool, J MICROBIOL IMMUNOL INFECT, 34(3), p167-70.

3 REGIONAL EPIDEMICS AND SURVEILLANCE STRATEGIES

3.1 Overview of regional outbreaks

Epidemics have been detected throughout the Asia-Pacific regions since the late 1990s, including in Taiwan, eastern China, Australia, Singapore, Malaysia, Vietnam and Thailand. Over the last year, the region has experienced a widespread occurrence of HFMD due to EV71 with outbreaks reported in China, Hong Kong SAR, Macao SAR, Taiwan, Mongolia, Vietnam, Malaysia and Singapore.

In Singapore about 19,530 cases had been reported in the first 8 months of 2008. One HFMD-related death was reported in August 2008, the first fatal case in seven years. The annual incidence pattern since 2005 has a bimodal pattern, with the incidence of HFMD showing peaks from March to May and a lower peak in August to October. The 2 peaks in each of the past 3 epidemic years were associated with CVA 16 in 2005 and 2007, and EV71 in 2006 and 2008. Higher hospitalization rates were associated with EV71 infections compared to CVA 16 infections. EV71 was detected in 4 out of 7 deaths during Sep 2000 -Feb 2001 and 1 death in Aug 2008. Three of the 4 serious cases hospitalized for encephalitis were infected with EV71.

The State of Sarawak, Malaysia, has experienced epidemic outbreaks of EV71 HFMD once in every three years with sporadic C1 cases in 1998 and then in 2008. Outbreaks due to EV71 occurred in 1997, 2000, 2003 and 2006. Prospectively collected HFMD sentinel data from 1998-2008 showed that non-EV71 enteroviruses, particularly CVA 10 and CVA 16 cause smaller clusters of HFMD in the inter-outbreak periods. Epidemics in the past decade have consistently peaked in March-April. Remarkably, it was noted that there have been very few EV71 cases in Peninsular Malaysia (including Johor Baru) even during the peaks of outbreaks in Singapore and Sarawak. There is speculation that this low number of EV71 cases could be due to under-reporting, under-detection or a low index of suspicion.

A 2-3 year epidemic cycle is described for state of Sarawak, Malaysia²⁰. A longer time series would be needed to ascertain whether EV71 epidemic occurs on a 2-3 year cycle in Singapore.

Thailand added HFMD into the disease under notification in 2000, following severe outbreaks in neighboring Malaysia, Singapore and Taiwan from 1997-2000. The case definition included fever and multiple oral lesions with pain and vesicles on either hand, foot or buttocks. EV71 cases were detected in the Queen Sirikit National Institute of Child Health and the Siriraj hospital since the start of this notification period, but no severe cases or deaths were reported until 2003. In 2006, Thailand experienced an epidemic of HFMD. Thirteen deaths were reported from several regions; 6 of the deaths were from the north eastern region clustering within a 2 week period. One of the six deaths was confirmed with EV71 infection. Active search for cases

²⁰ Podin Y, EL Gias, F Ong, *et al.* (2006) Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: Lessons from the first 7 years, BMC PUBLIC HEALTH, 6:180.

in the community found 4 additional confirmed EV71 infections. Coxsackie B virus was also detected in the same cluster. There have been several outbreaks since 2006, but it remains challenging to fully define the extent of the outbreak as the diagnosis is primarily clinical and most of infections are asymptomatic.

The first reported case of HFMD in Mainland China was reported in Shanghai in 1981. Since then several major outbreaks have been reported. In 2007, a major HFMD outbreak occurred in Shandong province with a total of 39,606 cases including 14 deaths. The majority of the cases were EV71 along with small proportions of ECHO3 and Coxsackie A16. In 2008, wide spread epidemics were detected during March through August, involving many provinces¹⁰. The provinces most affected include Guangdong, Zhejiang, Hebei, Shandong, Hunan, Anhui, Sichuan, and Jilin. The epidemic appears to have spread from southern and central provinces (Guangdong, Anhui) toward the north (Jilin) between March and July 2008 involving more than 176,000 reported cases of HFMD and 36 deaths (23 in Fuyang City, Anhui).

The majority of the severe cases and deaths occurred in Fuyang city in Anhui province which is one of the most densely populated areas in China. From March 1 to May 9, 2008 nearly 6000 cases were hospitalized and 23 deaths were identified from March 1- May 20. The incidence rate by districts/counties in Fuyang showed the epidemic beginning to decrease from end of April, 2008. The incidence of HFMD was seen to be higher in males as compared to females. The clinical manifestations were acute fever with vesicular lesions on hand, feet and oral mucosa in some cases. Severe clinical manifestations included respiratory failure caused by neurogenic pulmonary edema. Analysis of samples from some of the fatal cases by RT-PCR and sequencing confirmed the presence of EV71 in 6 out of 13 fatal cases. In the wake of the Fuyang city outbreak, China has instituted an enhanced surveillance policy, active case finding at hospitals and health care centers and mandatory reporting by health care workers.

Taiwan has experienced severe HFMD epidemics with EV71 infection since 1998. There was a large outbreak in 2008 in Taiwan. The case fatality rate was reported to be 3%-25%. There were 346 severe cases and 10 deaths, out of which 312 were laboratory confirmed as EV71 infection. The HFMD outbreak reached its peak in the 25th week of 2008 with 93% cases due to EV71 infection. The risk factors of the outbreak included infection with serotype EV71, age < 5 years of age, male gender and secondary cases within the same family. A contributing cause for the severe outbreak is thought to be the high proportion of susceptible children < 5 years. A seroprevalence study conducted in 2002 found that 90% of children \leq 3 years had no neutralizing antibodies to EV71¹⁶.

Observations in the state of Sarawak, Malaysia, indicate that the three-yearly EV71 epidemic cycle in Sarawak is associated with the emergence of new B subgenogroups phylogenetically related to the dominant viruses of the previous outbreak. Each outbreak appears to begin with a higher diversity of EV71 viruses which is rapidly replaced by a dominant new genogroup. For example subgenogroup B3 which was the predominant strain in 1997 appeared to be replaced by subgenogroup B4 in 2000 and subgenogroup B5 in 2003. In Singapore, subgenogroup B3 and C1 were reported in 1998, whereas in 2000 subgenogroup B4 was predominant and

subgenogroup B5 predominated in 2006. In mainland China, the etiology of the fatal and severe cases and HFMD outbreak in Fuyang in 2008 was predominantly caused by EV71 circulating viruses with EV71 isolates clustered within the subgenogroup C4 in the VP1 domain. There is evidence that recombination occurred between Fuyang EV71 strain and CVA 16 strain, but when and which strain is unknown.

In Taiwan the most prominent EV71 subgenogroups were B1, B4, B5, C2 & C4 in 2007-2008. B5 was the predominant strain of EV71 since 2006, as it was again this year. In Thailand, both EV71 and CVA 16 have been isolated during outbreaks of HFMD. EV71 played a major role during 2003-2004 and 2006 and CVA 16 was dominant in 2005 and 2007-2008. From 2001-2003 6

Surveillance – key challenges

Need a better understanding of the clinical spectrum of disease, elements to measure and populations to survey in order to tailor surveillance priorities

Need to share current surveillance practices and protocols in order to identify areas of collaboration as well as opportunities to harmonize collection of surveillance data

Need to improve regional monitoring of viral circulation, transmission and infection patterns

subgenogroups B4, B5, C1, C2, C4 and C5 were detected with C1 being the predominant strain of EV71 circulating in Thailand. Molecular analysis in 2007-2008 indicate that genogroups C1, C2, C4 and C5 were the predominant strains.

3.2 Regional Surveillance Systems and Strategies

Surveillance strategies in the region are driven by the public health need but limited by the existing capacity for clinical detection and laboratory diagnosis, and the availability of resources. Public awareness regarding the disease and distress at the loss of young lives is also another factor that drives demand for better surveillance.

A challenge of current surveillance efforts is to determine the parameters of surveillance and how different scientific questions should be prioritized. For most of the public health officials and clinicians, the priority is to detect cases, in particular severe cases, early in order to minimize the public health impact of

the disease. However, hospital-based surveillance, particularly active surveillance for early detection of severe cases is time- and resource-intensive, even if focusing only on neurological disease. The clinical manifestations of HFMD may be characteristic but there are no clear markers for distinguishing cases caused by EV71 that are at a higher risk of severe disease and those caused by other enteroviruses. In addition, some presentations are atypical. Confirmatory diagnosis requires virological testing consuming more time and resources.

Another key priority is the ability to respond in a rational evidence-based manner to an outbreak of HFMD in the community, with the understanding that the majority of cases will be mild and self-limiting. It is uncertain that the outbreak response that is initiated during an

epidemic as a result of surveillance is playing a significant role in decreasing severity of disease. More studies are needed to evaluate the effectiveness of individual and community level interventions.

Singapore

In Singapore, HFMD surveillance comprises the following 2 components:-

- Case Surveillance – Mandatory reporting to MOH
 - All clinical cases of HFMD by medical practitioners within 24 hours of diagnosis.
 - All outbreaks in the childcare centres reported by the centres, pre-schools and primary schools. An outbreak is defined as 2 or more cases of HFMD with onset of illness occurring within the same incubation period of 2-5 days in the same institution.
- Virological Surveillance – Stool samples, throat and rectal swabs and swabs from vesicular fluid and oral ulcers collected from selected outpatient and inpatient cases of HFMD at KK Women's and Children's Hospital (KKH) and samples obtained from HFMD cases at childcare centres, pre-schools and primary schools with parental consent are sent to Virology Laboratory of the Department of Pathology, Singapore General Hospital, Virology Laboratory, KKH and the National Public Health Laboratory for testing.

Malaysia

In Malaysia there are several approaches to surveillance for HFMD, which was made notifiable in 2006. The state of Sarawak began surveillance in 1998 long before HFMD was made notifiable, because the first large EV71 outbreak in the region was in Sarawak. Since 1998, Sarawak has implemented a sentinel clinic programme in which a few primary care clinics were identified in 2 towns (Kuching and Sibul). These clinics report all HFMD cases presenting to them and submit throat swabs and/or vesicle swabs for virus identification. This system provides data on epidemiological trends, information on circulating virus serotypes and functions as an early warning system for EV71 circulation.

A second surveillance is a passive hospital based system where all severe HFMD cases and all encephalitis cases are reported to the health department and specimens taken for virology investigations. The appearance of EV71 viruses associated with hospitalized cases normally happens well after EV71 appears in the sentinel clinics, but this surveillance programme provides information on severe disease and ensures that if EV71 infection leads to disease that is not manifested as HFMD the situation can be assessed as well.

The surveillance programme in Sarawak is a close collaboration between the Sarawak Health Department, Universiti Malaysia Sarawak and the private and public health care facilities in the state.

In summary, the surveillance programme in Sarawak focuses on monitoring the appearance of EV71 so that relevant public health responses can be implemented in a timely manner. HFMD due to non-EV71 enteroviruses are not expected to cause severe disease and public health responses are not of the same intensity as would be carried out if EV71 is found to be the causative agent.

Thailand

In addition to case reporting, clusters of HFMD or severe HFMD are investigated. Most information on HFMD and EV71 is gathered through active case finding during the outbreak investigation. After investigation of the cluster of deaths suspected of EV71 in 2006, the public concern increased towards EV71. The definition of suspected EV71 included children of age <12 years who get acute fever and one of the following symptoms including pulmonary edema, or cardiovascular collapse, or signs of CNS infection such as vomiting or seizure, or physician suspected EV71. If the suspected case gets pulmonary edema, left ventricle dysfunction, leukocytosis (WBC>15000), hyperglycemia and excluding other causes for cardiovascular collapse, the case will be classified as a probable EV71 case. Control measures to stop transmission include case isolation, social distancing, cleaning up the environment and hand hygiene. EV71 is determined by viral isolation and neutralization tests. In 2007, 3199 cases were reported. There were 3261 specimens isolated for EV71, and 2744 specimens underwent neutralization tests, with 5.6 % cases testing positive for EV71.

Taiwan

In addition to the passive national notification of both HFMD/herpangina uncomplicated and severe cases, over the last 10 years, Taiwan has increasingly strengthened its surveillance strategy for HFMD. Taiwan also has sentinel clinical surveillance involving physicians from all over the island, hospital based surveillance for severe HFMD/herpangina, as well as a Real Time Outbreak and Disease Surveillance (RODS) system collecting events likely related to HFMD severe disease (ED-SSS) that present at enrolled emergency departments in hospitals. Based on sentinel surveillance for 2008, the severity of EV infection rose in April and declined in June. In all the EV cases, age group 1-5 years accounted for above 70% of all cases. The major peaks of EV activities usually occurred in summer and other minor peak sometimes happened from September to November.

3.3 Improving epidemiological knowledge and surveillance strategies – outstanding issues and questions

When considering reported data across the affected Asia-Pacific region, there appears to be different epidemiological patterns or cycles and different sub genogroups involved in the HFMD outbreaks with perhaps some differences when comparing outbreaks and predominant strains between regions in northeast Asia with those closer to the equator. For example, it would appear that genogroup C is more prevalent in China and Vietnam compared to in Malaysia and Singapore where genogroup B is more prevalent. However both B and C groups are prevalent in recent outbreaks in Taiwan. The seasonality of HFMD with EV71 infections in Taiwan occurred in late spring to summer (April-July). In Malaysia, epidemics in the past decade have consistently peaked in March-April, whereas HFMD/EV71 surveillance in southern Vietnam demonstrated an annual cycle of activity with a peak between September and December (pre-monsoon season).

Although HFMD, EV71 and associated severe CNS disease occur globally, the rate of severe CNS disease seems to vary geographically and the area of greatest public health concern is in the Asia-Pacific region. Despite useful information from the molecular analysis on viral isolates to date, little is known about the molecular mechanisms of host response to EV71 infection²¹. So far, viral genetic characterization has yielded limited information in relation to clinical disease or pathogenesis in humans. No virological predictors of severity have been identified over the last 10 years of surveillance. Although some clinical predictors have been identified through outbreak experiences in Singapore and Taiwan to help promote early detection and intervention, no clear clinical or laboratory marker of severity has been identified.

Overall it does not appear that current molecular virological data on EV71 and its phylogenetic evolution supports a distinct pattern of evolution towards greater virulence or pathogenicity in the virus. However, any interpretation of molecular virological data should be cautioned with the caveat that there is a lack of consistent virological surveillance in the region and relatively few isolates of EV71 isolates have been sequenced.

In order to obtain a more accurate picture of the evolution and linkages between isolates across the region and globally, surveillance and reporting of EV71 needs to be strengthened. More research is needed regionally, including studies to describe baseline prevalence studies, rates of uncomplicated and complicated disease, and relevant clinical outcomes. This could allow more comprehensive analysis that may help predict the virulence and severity of clinical outcomes, which would appear particularly important given the neurotropic nature of EV71 and the potential for its emergence as a leading cause of CNS disease previously associated with poliomyelitis.

Studies are also needed to improve management of the disease and determine if specific strategies confer any treatment difference towards an improved survival rate. In addition, collection of data using standard definitions of cases will be necessary to accurately determine disease burden and characteristics. It would be easiest to conduct prospective cohort studies through multi-center collaborations that could require large sample sizes. At the moment evidence that any specific intervention method has been proven to lead to improved survival rate is lacking. Studies done during the recent outbreak in China, found that one of the main risk factors for transmission is the lack of basic hygiene in the affected areas. Determining efficacy of these interventions will ultimately require multi-center clinical trials. In addition, the risk of EV71 transmission included secondary transmission among children in same family and kindergarten.

²¹ Shih S-R, V Stollar, JY Lin *et al.* (2004), Identification of genes involved in the host response to EV71 infection, *J NEUROVIROLOGY*, 10:5, 293-304.

4 CLINICAL SPECTRUM AND MANAGEMENT OF DISEASE

4.1 Overview

Hand-foot-and-mouth disease (HFMD) is a common childhood illness presenting with fever, sore mouth or throat, and characteristic rash on the palms, soles and oral cavity. The rash is typically papulovesicular lesions but may also be maculopapular in appearance. Young toddlers may also present with a rash on their buttocks, elbows, knees and trunk. Oropharyngeal lesions consist of vesicles which are rapidly ulcerating located on the buccal mucosa, tongue, gums and palate. In some cases, inflammation of the conjunctiva has been noted. The majority of the cases are self-limiting and resolve spontaneously in 7-10 days.

However, rarely, severe complications can result, primarily involving the central nervous system (CNS). The most common CNS manifestations are aseptic meningitis, acute flaccid paralysis and brainstem encephalitis. Other less common manifestations include cerebellar ataxia, Guillain-Barre syndrome, transverse myelitis, opso-myoclonus syndrome and benign intracranial hypertension. Most children with EV71-associated aseptic meningitis recover fully. EV71 has also been known to cause acute flaccid paralysis through destruction of anterior horn cells causing a poliomyelitis-like paralysis and other suspected immune-mediated neuropathological conditions. However, the most devastating neurological syndrome has been brainstem encephalitis, which in serious epidemics in the Asia-Pacific region has had a case fatality rate ranging from 40-80%. It is thought that the neuropathology leads also to the second devastating component of severe complications, cardiopulmonary failure resulting from neurogenic pulmonary edema, hemorrhage, and circulatory collapse. Those who do recover may face long-term serious neurological disability.

Clinical management – key challenges

Need to exchange information on common clinical practice in management of HFMD in particular around early detection, clinical and lab predictors of poor outcomes, approach to hospital admission and management, management of severe complications of HFMD

Need to collaborate on collection of clinical data of HFMD cases in a standard data collection format to better assess predictors of severity of outcomes or mortality

Need to conduct further clinical research studies on interventions used to manage severe complications such as the stage-based management strategy or the use of IVIG across institutions

Country	Year	Number of Cases	Number of Deaths
Taiwan	1998	129,106	78
Singapore	2000 (Sept-Oct)	3790	4

Table 2 **Number of HFMD cases and deaths occurring in Singapore and Taiwan during the first HFMD outbreak**

References:

Chan KP, Goh KT, Chong CY, *et al* (2003) Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore, *Emerg Infect Dis*, 8: p78-85.

Ho M, Chen E, Hsu K, *et al* (1999) An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group *N Engl J Med*, 341(13): p929-35.

Table 2 illustrates the number of cases and deaths that occurred during the first outbreaks experienced in Taiwan and Singapore in 1997, 1998 and 2000.

In Taiwan, in 1998, during the first and largest outbreak experienced so far, the total number of HFMD and herpangina reported by sentinel physicians was 129,106. Severe disease appeared to peak in early June, around the same time as uncomplicated cases and there were 405 severe cases with 78 deaths with 86% of deaths and 69% of the most severe cases occurring in children below 3 years of age. The most severe clinical syndrome was encephalomyelitis that resulted in fulminant cardiorespiratory collapse.

In Malaysia, most children died within 24 hours of hospital admission with apparently all the deaths being due to cardiorespiratory failure, and all had subtle neurological signs with CNS involvement at presentation. The clinical presentation of the 3 males and 1 female in the age group of 15 – 48 months who died from HFMD included one or more of the following symptoms: lethargy, cough, sore throat, shortness of breath, cyanosis, tachypnoea, sweating, cold extremities, fever, vomiting, rashes on hands and feet, with chest x-ray findings of diffused pulmonary oedema.

In Thailand, at Queen Sirikit National Institute of Child Health, an apparent seasonal distribution of HFMD and Herpangina was observed with the highest number of cases for Herpangina recorded in July (173/1323) and September 2001 (115/603). In early 2006 in Thailand, a cluster of 8 children who died from acute fever and pulmonary edema of unidentified cause were investigated. Three cases were found to have signs of probable EV71 with only 1 of 3 cases having typical HFMD skin lesions.

4.2 Diagnostic Features and differential diagnosis of HFMD

Increasing physician awareness regarding clinical manifestations and management of HFMD and implications in terms of severity of disease should the etiological agent be EV71 is critically important to help ensure early diagnosis and early intervention.

The main diagnostic features for the clinical spectrum of HFMD are outlined in the table below:

Disease	Case Definition
HFMD	Febrile illness, maculopapular or papulovesicular rash on palms and soles, vesicles/ulcers in mouth
Herpangina	Multiple oral ulcers on the posterior parts of the oral cavity
Aseptic Meningitis	CSF pleocytosis
Encephalitis	Lethargy, drowsiness/coma, seizures or myoclonus
Acute flaccid paralysis	Areflexic limb weakness
Cardiorespiratory Failure	Tachycardia, Respiratory Distress, Pulmonary Oedema, Poor peripheral perfusion, pulmonary congestion, reduced cardiac contractility on echocardiography

Table 3 Diagnostic features of HFMD and associated complications

Courtesy of Dr Ooi MH (Malaysia)

Although herpangina and a febrile illness with typical rash distribution affecting the mouth, and extremities are characteristic of HFMD, these features do not distinguish EV71 from HFMD caused by other enteroviruses. Thus as outlined in Table 3, clinical signs and symptoms of EV71 vary widely and patients may present with a wide spectrum of clinical manifestations, ranging from nonspecific febrile illness with rash to an encephalitis. In considering differential diagnosis, these features are also similar to that of other enterovirus infections. Herpangina is also caused by enteroviruses namely CVA 8, CVA 10, CVA 16 and EV71 with the difference being the distribution of the oral lesions (posterior pharynx instead of oral cavity). Other viruses that cause AFP in the region include non-polio enterovirus (e.g. Coxsackie virus and Echovirus), Japanese Encephalitis Virus (JEV) and West Nile virus. Other differential diagnosis for HFMD include chickenpox (varicella), herpetic gingivostomatitis, and aphthous stomatitis.

4.3 Pathogenesis and Pathological features

Data from autopsies conducted in Malaysia, Singapore and Taiwan have been useful to improve our understanding of the pathogenesis of severe disease and potential underlying pathological insult leading to death. The inflammatory response seen in EV71 encephalomyelitis is typical of viral encephalitides, consisting of neuronophagia, perivascular cuffing, focal oedema and macrophage/microglia infiltration. Although mild inflammation can be seen in the cerebral cortex, it is absent in the anterior pontine nuclei and cerebellar hemisphere.

The areas of inflammation appears to be localized to the brain stem and spinal cord. The stereotypical distribution of inflammatory lesions have contributed to making magnetic resonance imaging (MRI) studies diagnostically useful. MRIs often show hyperdense lesions in the spinal cord and posterior brainstem with the most severe inflammation located in the spinal cord, brainstem, dentate nucleus and hypothalamus.

It has been proposed that the topographic distribution of inflammation and virus suggests that the retrograde viral spread into and within the CNS could be by neural pathways, possibly involving motor pathways i.e. peripheral motor nerves, something that some groups are investigating in murine models.

It has been demonstrated that viral antigens/RNA appear to be localised to neuronal body and processes, and macrophages are participating in the neuronophagia seen in inflamed areas which provides evidence that neurons are likely the main viral targets, and viral cytolysis an important mechanism for neuronal injury. The primary cause of death is thought to be due to medullary inflammation and destruction, where the final pathway of apoptosis involves the activation of an inflammatory mediator, caspase 3 which is found in the glial cells.

Lung findings at autopsy is consistent with interstitial pneumonitis, pulmonary edema and hemorrhage and myocardial congestion. The pathogenesis of death resulting from pulmonary edema alone or pulmonary edema and concurrent pulmonary hemorrhage during EV71 infection is not completely understood. Based on the findings above, it has been proposed that the destruction of the medullary centers and resulting effect on cardiopulmonary function leads to the cardiopulmonary collapse and death. Although these and other additional causes for death including central nervous system dysfunction and myocarditis have been proposed, the alteration of cardiopulmonary function and its direct relationship to mortality have never been clarified⁵.

4.3.1 Pre-clinical studies into pathological mechanism of EV71 encephalomyelitis

Mouse models have been developed in order to study the pathogenic mechanisms of EV71 infections and to allow for testing of promising compounds or antigens for vaccine development. In one mouse model of EV71 encephalomyelitis, the distribution of virus infiltration and inflammation is similar to what is seen in human encephalomyelitis²², which has allowed for the generation and exploration of hypotheses regarding EV71 viral replication and spread in the CNS.

This is achieved through intramuscular hind limb-inoculation, which leads to early retrograde spread to ipsilateral lumbar efferent motor axons and anterior horn cells, motor cranial nuclei and contralateral motor cortex before affecting other parts. This suggests that motor pathways could be extremely important for viral transmission into and within the CNS. Viral antigens were found in the skeletal, muscle and brown fat. In the murine model, skeletal myositis was also severe but myositis has not been reported in human EV71 infections.

Pathogenic and anti-viral studies in these and other models may be useful for use in drug and vaccine development.

²² Ong KC, M Badmanathan, S Devi, et al. (2008), Pathological characterization of a murine model for human Enterovirus 71 encephalomyelitis, *J NEUROPATHOL EXP NEUROL*, 67:532-542.

4.4 Clinical Management

4.4.1 Overview

The key management strategy entails early recognition and early intervention. Unfortunately, most of the time, the viral diagnosis of HFMD is known only after the outbreak of severe cases. Management strategies are influenced by the evidence based on clinical as well as anecdotal case experiences and issues related to ensuring rapid and immediate access to hospital based management of severe complications. In some cases, analysis of clinical experience has identified potential risk factors that are associated with poor outcomes that could be useful predictors to be used in management. Treatment of cases are based on supportive management principles. There are no effective anti-virals available for HFMD. Some groups advocate the use of a stage-based management strategy, dependent on clinical signs and symptoms that denote involvement of the central or autonomic nervous system or cardiopulmonary compromise. In addition some groups also have resorted to the early use of intravenous immunoglobulin (IVIG) infusions in cases suggestive of CNS involvement. However conclusive data on the benefit of IVIG is lacking as no clinical trial of this approach has been undertaken.

4.4.2 Possible risk factors for severe outcomes and death

Analysis of cases in Singapore during the 2000 outbreak found that vomiting, absence of mouth ulcers, leucocytosis and an atypical presentation was associated with a fatal outcome. Atypical HFMD is described as having skin rashes but an absence of mouth ulcers, with vomiting, fever, diarrhoea²³.

Similarly in Taiwan, analysis of cases occurring in the 1998 outbreak combined with experience so far have identified several risk factors associated with severe EV71 infection. In a comparison of cases caused by CVA 16 and EV71, it was found that the significant difference in clinical features was that fever was higher (greater than 39°C) and of a longer duration (more than 3 days) in EV71 infections²⁴.

Table 4 summarizes the factors that are used to determine clinical management (admission criteria into the hospital and ICU) because of their association with severe disease.

²³ Chong CY, KP Chan, VA Shah, *et al.* (2003) Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases, ACTA PEDIATR, 92(10):1163-9

²⁴ Chang LY, TY Lin, YC Huang, *et al.* (1999) Comparison of enterovirus 71 and coxsackievirus A16 clinical illnesses during the Taiwan enterovirus epidemic, 1998, THE PED INF DIS JOURNAL, 18(12):1092-1096.

Risk factors for severe EV71 infections		
Severe manifestations	Risk Factors	Recommend
Common Clinical characteristics among patients with CNS involvement	Fever >3 days Fever > 39 C Lethargy Limb weakness Vomiting Seizure Hyperglycemia	Hospital Admission
Potential risk factors for Cardiopulmonary failure	Tachycardia Leukocytosis Hypertension Hyperglycemia	ICU admission and management

Table 4 Risk factors and hospital admission recommendations for severe EV71 infections in Taiwan

Modified from: Chang LY, Lin TY, Hsu KH, *et al* (1999) Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease LANCET, Nov 13;354(9191):1682-6.

Long-term studies of children who recovered from severe EV71 disease have found that serious impairment of neurological development as well as cognitive function can occur in some children. In a case-control follow-up of children aged 4-16 years with CNS involvement post-recovery, attention deficit hyperactivity related symptoms were seen in 20% of EV71 infected children and 3% of the control group children²⁵.

4.4.3 Stage-Based Management

The stage-based management approach developed in Taiwan has helped in reducing the fatality rate according to the Taiwan experience. The clinical course of EV71 infections is divided into 4 stages with corresponding features and management recommendations for each stage.

Table 5 presents a modified version of this approach resulting from discussions by the clinical management working group at the forum.

²⁵ Gau SS, LY Chang, LM Huang, *et al*. (2008) Attention-Deficit/Hyperactivity-Related Symptoms Among Children With Enterovirus 71 Infection of the Central Nervous System, PEDIATRICS, 122: e452-e458.

Stage	Clinical features	Management
Stage I (Non-complicated HFMD)	<ul style="list-style-type: none"> • HFMD • Herpangina • Fever 	<ul style="list-style-type: none"> • Symptomatic Treatment • Consider admission only if patients have high fever or prolonged fever
Stage II	<ul style="list-style-type: none"> • Aseptic Meningitis • Encephalitis • Encephalomyelitis, polio-like syndrome <p>Headache Vomiting Lethargy Limb weakness Upward gaze Seizure Myoclonic jerk</p>	<ul style="list-style-type: none"> • Monitor BP, HR, sugar, ABG, electrolytes e⁻, coma scale • Early Intubation and provide mechanical ventilator for GCS<9 or significant IICP (seldom noticed) or any deterioration signs. • IVIG: needs further evaluation 1gm/kg, i.v. 12 hours only for encephalitis, and polio-like syndrome • Fluid restriction:1/2-2/3 maintenance • Invasive monitoring: CVP, ABP • Consult cardiologist as needed and arrange echocardiography
Stage IIIa	<ul style="list-style-type: none"> • Autonomic Dysfunction <p>Tachycardia Hypertension Profuse sweating Paralytic ileus Leucocytosis Hyperglycemia Tachypnea Hypozeemia Pulmonary edema/hemorhage</p>	<ul style="list-style-type: none"> • Restrict preload: Fluid restriction, diuretics • Reduce after load cautiously: BP ↑,with normal cardiac contractility : vasodilator or β-blocker: <ol style="list-style-type: none"> 1. Nitroprusside 0.5-4 mcg/kg/min 2. Esmolol 50-300 mcg/kg/min 3. Milrinone 0.25-0.75 mcg/kg/min 4. Sedatives? midazolam, morphine or propofol* • Augment myocardium contractility <ol style="list-style-type: none"> 1. Milrinone 0.25-0.75 mcg/kg/min 2. Dobutamine 5-20 mcg/kg/ min <p>*not recommended in every hospital</p>

		and should be used very cautiously
Stage IIIb	<ul style="list-style-type: none"> • Circulatory Failure Hypotension Coma Loss of brainstem reflexes 	<p>Monitor HR, BP and Blood Sugar, Use of positive pressure ventilator, Inotropic agents and ECMO ventricular assist device</p> <ul style="list-style-type: none"> • Maintain adequate cerebral and vital organ perfusion during hypotension, optimize preload, after load and myocardium contractility • Inotropes - A very high infusion rate of inotropes may be needed to keep adequate BP <ol style="list-style-type: none"> 1. Dopamine 20mcg/kg/min 2. Epinephrine 0.05-0.4(?)mcg/kg/min
Stage IV	<ul style="list-style-type: none"> • Recovery/ Convalescence 	<ul style="list-style-type: none"> • Long Term care • Nutrition • Tracheostomy • Rehabilitation • Chest physical therapy-prevent pneumonia

Table 5 Proposed Stage-based management formulated by clinical care discussion group, at the forum

An assessment comparing outcomes of EV 71 infection with and without stage based management found that stage-based management reduced case-fatality rate in those with cardiopulmonary failure by 50% (from 80% to 30%). However, it was also found that among those who survived, there was a significantly higher occurrence of neurological impairment²⁶. Long-term follow-up of a group of children who were diagnosed with EV71 with CNS disease also found that long term care and support were needed for a number of these children, particularly those who had cardiopulmonary failure with up to 75% having a range of neurological sequelae such as ventilator dependency and assisted feeding (tube) and up to 20% with developmental delays²⁷.

²⁶ Chang LY, LM Hsia, CT Wu, *et al.* (2004) Outcome of enterovirus 71 (EV71) with or without stage-based management:1998-2002, PED INF DIS J, 23:327-32.

²⁷ Chang LY, LM Huang, SS Gau, *et al.* (2007) Neurodevelopment and Cognition in Children after Enterovirus 71 Infection, N ENGL J MED, 356:1226-34.

The use of IVIG remains controversial and is driven by several factors including anecdotal experience as well as pragmatic considerations. Some ways in which it is being used is described below:-

- a) In Singapore, IVIG was considered for a young patient < 4 years with the following symptoms: raised WBC, vomiting and the disease lasted < 5 days. Singapore did not specifically find a statistic correlation between severe disease and EV71 infection but most of the other sites did find a correlation between the severe disease and EV71.
- b) In Malaysia, early intervention using IVIG has been done using IVIG (Intragram P, CSL) 1-2 kg/kg over 10-12 hours infusion.

The need for testing this treatment approach in a clinical trial setting is something that needs to be considered.

5 Laboratory Diagnostic Strategies

5.1 Overview

The virological diagnosis behind a case of HFMD takes on a great significance mainly because of the need to rapidly and accurately determine if the virus is one associated with severe complications, in the case of EV71. The clinical hallmarks of HFMD do not distinguish between EV71 and other viruses and confirmation of diagnosis is by laboratory tests. In addition, there is a subset of infected patients who do not present with the typical characteristics and present a diagnostic challenge, particularly in the face of the risk of severe disease. Confirmatory diagnosis is based on tissue culture and virus isolation and detection which are both laborious and time-consuming. These methods therefore are not practical for clinical decision-making, particularly in deciding between interventions and treatment in patients where the clinical hallmarks are ambiguous or even absent. Antibody based tests and PCR are more widely available now and allow for more rapid diagnosis but are not yet widely used due to cost as well as concerns about the lack of sensitivity and specificity. Ideally, what would be most useful is a sensitive and specific method for the direct detection of EV71 antigen or RNA in a point-of care setting.

Presence of virus can be determined in primary specimens and detailed information can be obtained from the APNET(Asia Pacific Network for Enterovirus Surveillance) website: www.apnet.med.usyd.edu.au which acts as a resource for the region by

Diagnostic strategies – key gaps

Need to optimize the conduct of current diagnostic tests including the need to strengthen and harmonize assay parameters of quality assurance of assay performance

Need to support training in virological diagnostics to improve on the capacity to conduct improved regional surveillance (i.e. through networks such as APNET)

Need to optimize serological based diagnostic methods

Need to develop a rapid diagnostic, point of care test for EV71

making SOPs for clinical case definitions, virology story and epidemiology story available and also sending PCR primers and antibodies.

Current molecular techniques allow for the genomic analysis of the causal agents and allows for tracking of viruses such as EV71 as well as relationships of the virus strains in the region. In most cases, primary specimens are received from multiple sources depending on existing surveillance structures in place. As has been described in previous sections, many areas that have had to deal with outbreaks of HFMD in the region have some form of surveillance including active surveillance for cases and case investigation and have standard case definitions and protocols with algorithms to identify and find cases.

For some countries, particularly when the burden of testing is high at the peak of an epidemic, severe cases will be prioritized for rapid diagnosis through Real time RT-PCR for an initial, presumptive diagnosis. Positive cases will be prioritized for virus isolation in order to make the most of limited resources.

5.2 Laboratory Detection Methods

There is no widely available, sensitive rapid diagnostic test for EV 71. Laboratory diagnosis of EV71 infection depends on traditional virus isolation in cell culture followed by identification of virus serotype by neutralization with monoclonal antibodies, indirect immunofluorescence assay (IFA), or Real Time Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Primary specimens that can be sampled for culture include throat swab, vesicle fluid, stool samples, cerebrospinal fluid (CSF) and sera. The yield for both CSF and serum is very low. EV71 may be isolated in a variety of human and primate cell lines including African green monkey kidney (Vero) cells, human lung fibroblast (MRC5) cells and human rhabdomyosarcoma (RD) cells.

Serology can also be used in EV71 diagnosis but given that this requires acute and convalescent serum samples, this retrospective diagnosis may not be helpful in a clinical management or public health context.

5.2.1 Rapid presumptive diagnosis on primary specimens

The types of testing and diagnostic strategies favored are dependent on resources and capacity. However, with the need to both screen as well as provide more rapid diagnosis of the primary specimen acquired from the suspect case, most labs currently depend on direct RT-PCR as the method of choice allowing for the earliest, presumptive diagnosis of EV71. Sensitivity of detection between methods used on EV71 isolates and direct specimen RT-PCR are both high and comparable⁶ and so currently this is considered is the most rapid method for detecting EV and EV-71 in suspect samples before selecting positive samples for virus isolation and confirmatory sequencing, and typing into genogroups or subgenogroups.

Note on RT-PCR of primary specimens: A careful selection of primers is required. Pan Enterovirus primers are used in VP1 sequence to determine the serotype. As the virus evolves, primers need to be evaluated and reevaluated.

EV71 IgM-specific enzyme-linked immunosorbent assay (ELISA): Assays are being developed but still most have problem with specificity for EV71 resulting in many false-positive results and giving the tests low positive predictive value for EV71 infection.

5.2.2 Methods of identification of EV71 from virus culture

Several methods exist for the identification of EV71 from virus culture:

1. Confirmation of serotype achieved by neutralisation with a type-specific antiserum provides a specific and reliable serotype diagnosis of EV71. The current limitation is the availability of type specific anti-sera against EV71.
2. Indirect immunofluorescence assay (IFA) using anti-EV71 monoclonal antibodies can provide rapid, presumptive diagnosis. Although the IFA method is technically simple and rapid, the antibodies are relatively expensive to purchase. Supply of monoclonal antibodies is a limitation to wide access of this assay. Monoclonal antibodies are available commercially from Chemicon International and through APNET, which can provide a polyvalent antiserum against a recombinant EV71 VP1 antigen.
3. Reverse transcriptase – polymerase chain reaction (RT-PCR) amplification of viral RNA using various gene targets (5' untranslated region, VP1, VP4 genes).

5.3 Type of Specimen

One of the challenges of diagnosis is the type of specimen available for virus detection. The types of specimens that are collected and available for diagnosis vary depending on the clinical presentation as well as practice and may influence the test result. The sensitivity and specificity of the diagnostic methods vary between methods.

Specimens that may be collected are:-

1. Stool
2. Throat swab in Virus Transport Medium (VTM)
3. Vesicular fluid in VTM
4. Cerebrospinal fluid
5. Rectal swab (not recommended by WHO)

Serum is also collected and when available, tissue biopsy.

In Thailand, in a study of 29 hospital admissions with a diagnosis of HFMD or herpangina, testing of paired acute and convalescent sera found that, 45 out of 102 seroconverted for EV71. No EV71 infection was found in the Herpangina cases. The total of HFMD patients during this period was 60 with a mean age of 28 months. In all the viral agents were identified in 31/60

(52%) cases; 65% cases tested positive for EV71 (20/31) and 26% cases tested positive for non EV71 causes (8/31).

During the 2000 outbreak in Singapore, EV71 culture was tested positive in 44% stool samples, 43.6% vesicles, 25% mouth, 32% throat samples. In the non-HFMD patients, EV71 culture tested positive in 5.4% stool samples. EV71 was isolated from 73% of virus positive patients. Other serotypes isolated included - CVA 16, CVA 4, CVA 10, CVB 5, CVA 6, CVA 24, CVB 3, CVB 4, EV 18²⁸. During the 2008 outbreak, 28% EV71 tested positive from both throat and stool EV isolation for EV71 PCR. For the entire period in 2008, the EV71 positives contributed to 21% of the total number of tests for EV71 PCR.

In a comparison in Taiwan, in 1998, out of 117 throat and rectal swabs specimens, 105/117 (89.7%) throat swabs tested positive for EV71 and 80/117 (68.4%) rectal swabs tested negative.

²⁸Chan KP, KT Goh, CY Chong, *et al.* (2003) Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. *Emerg Infect Dis*; 8: 78-85.

The following table compares the advantages and disadvantages between the different specimens²⁹.

No.	Specimen Sample	Advantages	Disadvantages
1	Stool	<ol style="list-style-type: none"> 1. Easy to collect 2. VTM not required 3. Long duration of virus excretion 4. High rate of positivity 	<ol style="list-style-type: none"> 1. For in-patients only 2. Collection time depends on patient 3. Difficult for field investigation
2	Throat swab	<ol style="list-style-type: none"> 1. Easy to collect 2. For out-patients 3. For field investigation 	<ol style="list-style-type: none"> 1. Need VTM 2. Need well trained collector 3. Short duration of viral shedding 4. Low rate of positivity
3	Rectal swab	<ol style="list-style-type: none"> 1. Available for all patients 2. Does not require the presence of mucocutaneous stigmata 	<ol style="list-style-type: none"> 1. Not sterile site 2. Isolation of virus there might represent coincidental asymptomatic carriage rather than the causative agent
4	Vesicular Fluid	<ol style="list-style-type: none"> 1. Virus obtained from the fluid is a causative agent High rate of positivity 	<ol style="list-style-type: none"> 1. Difficult to collect vesicular fluid from very small vesicles 2. Need VTM
5	CSF	<ol style="list-style-type: none"> 1. Good to determine specific causative agent of viral meningitis 2. WBC count and biochemistry indicate CNS infection 	<ol style="list-style-type: none"> 1. Need experienced doctors to do lumbar puncture 2. Highly insensitive for isolating EV71

Table 6 Advantages and Disadvantages of various types of specimens
Courtesy of Dr Tu PV (Vietnam)

²⁹ Ooi MH, T Solomon, Y Podin *et al.* (2007) Evaluation of Different Clinical Sample Types in Diagnosis of Human Enterovirus 71-Associated Hand-Foot-and-Mouth Disease; *J Clin Microbiol* 45(6):1858-1866.

6 OUTBREAK AND PUBLIC HEALTH RESPONSE TO HFMD

6.1 Regional Prevention and Control measures

The extensive outbreaks of HFMD in the region has had a considerable impact on society and the public health, medical and diagnostic services. The rare but significant disability and death from EV71 causing HFMD has driven the prevention and control efforts in most of the countries in the region to respond to public and parental concerns. Overall, the strategies employed by areas and countries in the region are very similar in principle and they consist of :-

1. Educating on good hygiene and basic sanitation through intensive campaigns
2. Implementing and strengthening surveillance – Passive, Active Case finding, Case Investigation
3. Closing schools and daycare facilities
4. Applying public information strategies such as Risk Communication Principles
5. Disseminating resources on best practices

China

The prevention and control measures of HFMD in Fuyang city included closure of kindergartens, fever and rash inspection of pupils in morning, nosocomial infection control, clean environment campaign, health education including handwashing and disinfection of toys.

The risk factors of this disease included close contact with other children and exposure in crowded public areas. The personal hygiene habits enforced pre- and post-outbreaks include frequent hand-washing and avoiding thumb sucking and chewing nails. In several counties, secondary transmission between children in the same family was observed. Among the risk factors for transmission, closure of schools was found to be protective in urban Fuyang and risky in the more rural Qiaosi. The reason for difference in social distancing was not found to be clear. The data suggested that in rural provinces childcare centres may actually be cleaner than households and it may therefore be 'protective' to send children there than keep them at home. Basic hygiene practices such as frequent handwashing and avoiding sucking fingers and chewing nails can lower infection risk.

The following measures were taken to minimize the case fatalities –

1. Setting up of a patient triage system
2. Designating specific hospitals for treatment
3. Clinical Monitoring for the early detection and intervention of severe cases
4. Establishing a pediatric intensive care unit (PICU) on a 24 hour on-duty service
5. Reimbursing medical fare based on new rural cooperative medical care regulation

Singapore

Changes in social behavioural norms in Singapore such as a lack of an extended family structure with both the parents working has greatly increased the numbers of young children in childcare centres and pre-schools. Closure of affected childcare centres and preschools implemented from 22 April to 7 July 2008. Childcare centres and preschools were recommended to close when the transmission period was more than 15 days. Mandatory closure was required if there were more than thirteen cases or the attack rate was higher than 18%, with transmission period of more than 15 days. During the 2008 outbreak, as many as 28 public health officers are working on various aspects of the public health response to HFMD from outbreak investigation to managing press releases and media queries.

Control measures in Singapore are aimed at reducing the exposure of pre-school age children and interrupting transmission. The importance of maintaining high standards of personal and environmental hygiene to minimise risk of HFMD transmission was emphasised. The health screening measures instituted in childcare centres and preschools include early detection and prompt isolation, monitoring of children daily, and noting any unusual symptoms or behaviour or signs of illness. Parents were advised to consult a doctor early if their child has fever, mouth ulcers and rashes on the palms, soles or buttocks. They were also advised to keep the affected child at home until all the blisters have resolved. Parents were advised to practice proper hygiene at home to avoid transmission to other family members.

The Infectious Disease Act requires medical practitioners to notify all clinical cases of HFMD to MOH within 24 hours of diagnosis. Childcare centres, pre-schools and primary schools are also required to notify of any HFMD outbreak.

Sarawak, Malaysia

Surveillance such as sentinel site surveillance and high level of awareness regarding the disease among medical practitioners, teachers, child-minders and childcare centers is used to promote passive case reporting. Early detection and early intervention including early admission and intervention have been strengthened through guidelines that describe the case definition, admission criteria and clinical management of HFMD.

Legislation was also enacted to require mandatory notification of HFMD linked to notification of “myocarditis” and “life threatening infections”. From 12 October 2006, “myocarditis” and life threatening infections” were replaced by hand, foot and mouth disease. Surveillance also involves active case investigation per protocol by involving mobile teams. During epidemics, the number of cases reported may exceed the human resources to actively investigate each case. In this case, the MoH mobile field investigating teams prioritize the more serious cases for investigation.

In response to outbreaks the MoH also carries out school closures, conducts intensive education campaigns to promote handwashing and hygienic practices. During epidemics,

careful attention is paid to communication with the public especially the media, families, schools and other stakeholders.

Taiwan

In 2008, Taiwan implemented EV71 Control Policies in order to improve public health control and upgrade the medical service response to EV71. Control measures include public education about the disease and the importance of personal hygiene, cooperation with multiple ministries and announcement of mandatory school closure.

The medical service response was upgraded by constructing “Medical Care Nets for Severe EV Cases”, revising “Guideline for Treatment of Severe EV Cases” and adding this guideline to the “digital infectious disease learning net” to give physicians continuing education credits, disseminating the message pertaining to “Symptoms of EV71 and Timing of Referral for Severe Cases to Hospital”.

6.2 Risk Management and communication

One of the challenges of the HFMD situation has been the demand for greater and improved public health communication, primarily during the outbreak situation. There is growing awareness regarding the importance of accurate and consistent messages to the general public as well as importantly to the media. Information resulting from the 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 critical stakeholders such as public officials and the health provider community also need timely information. A concept that was discussed at the meeting is that of ‘risk communication’.

The main objectives of emergency risk communication are –

- 1) Providing accurate, timely and consistent information as well as essential coordination during an emergency
- 2) Informing the public of potential risks and steps being taken during an emergency
- 3) Aiding individuals, stakeholders or communities to accept the imperfect nature of choices and to make best possible decisions during an emergency

The principles of emergency risk communication are –

- 1) Announcing early and often
- 2) Being open and honest
- 3) Keeping messages simple and concise

- 4) Listening to and involve the public
- 5) Giving the public responsibility and actions
- 6) Displaying public empathy in public announcements when required
- 7) Stating the plan of action during emergency
- 8) Telling people where they can get additional information
- 9) Always planning ahead of time

For the recent outbreak in mainland China, it was found that health officials across China who received risk communication training used better risk communication strategies in post-training messages as compared to their pre-training messages. It did appear that this type of training and the resulting messages resonated well with the Chinese population and could be easily reproduced and adapted to other similar situations.

7 DRUGS AND VACCINES

7.1 Current therapeutic strategies

Currently there are no antivirals or vaccines effective against EV71 infection. Treatment options for EV71 infection under preclinical and clinical evaluation include anti-viral drugs: Pleconaril, Enviroxime, 3C protease inhibitors, BPROZ-194; and therapies such as Intravenous Immunoglobulin (IVIG), Salva Miltiorrhiza (Danshen), and Type I Interferon.

Anti-viral drugs

Pleconaril (WIN 63843) is an experimental antiviral that is used in phase 3 trials in the USA; preclinical and early trial data suggest that it is effective for most enteroviruses except for EV71. The antiviral drug salva miltiorrhiza is able to neutralize EV71 (in vitro) and hence reduce viral infectivity. Other potential therapeutic avenues being pursued include RNA inhibitors. Studies of RNA interference (RNAi) using chemically synthesized 19-mer siRNAs and 29-mer siRNAs show efficacy *in vitro* but not *in vivo*. Passive immunization by anti-EV71 immune sera has been effective in mice.

Immuno therapies

IVIG has shown to be a useful therapy with varying success rates for treatment of neurological cases of HFMD in Taiwan and Western Australia. The potential use of human monoclonal antibodies as passive immunotherapy is another promising candidate. Type I interferons have shown to enhance specific T-cell mediated immune responses but have shown limited effectiveness for EV71.

7.2 Novel Therapeutic Strategies

RNA interference

RNAi therapeutic agents are being developed for EV71 infection.

In vitro studies have been done using chemically synthesized 29-mer shRNAs and psiSTRIKE siRNA expression system. Both the chemically synthesized 29-mer shRNA and the plasmid siRNA expression systems inhibited EV71 replication and had activity against EV71 multiple genogroups. In the *in vitro* model siRNA targeting the 3D^{pol} region was the most effective.

The *in vivo* studies of RNAi in the murine model showed that the suckling mice recovered from EV71 infection after treatment with 19-mer siRNAs or plasmid-mediated siRNAs. The *in vivo* studies were effective against EV71 strains from other genogroups. However the 29-mer shRNAs failed to protect suckling mice from EV71 infection despite being more efficacious *in vitro*. The cause of death of mice in EV71 drug screening model is usually encephalitis. SRNAs are able to penetrate into the CSF.

RNAi may be a solution to treat EV71 infection but the problems of efficient delivery and sustained effect remain to be resolved.

7.3 Vaccine Development in Different Regions

The progress of vaccine development was discussed by three vaccine companies - SingVax (Singapore), Sinovac (China), and the Taiwan (CDC). All these use whole, inactivated virus because better safety was observed while using inactivated whole virion-vaccine as compared to the attenuated live vaccines.

However, they all faced similar challenges including:-

1. Difficulty in identifying an appropriate animal model and the crossreactivity of these vaccines against the different subtypes is unknown.
2. Lack of critical pieces of knowledge that will be required in order to design clinical studies and evaluate potential impact including cost-effectiveness of these vaccines. This includes better knowledge of virus transmission, disease burden, clinical stages of the disease as well as practical information of health care burden and current costs of providing public health response and care and treatment for HFMD.
3. Lack of funding.

Singapore

A number of studies have been carried out to demonstrate that neutralising titres can be achieved using purified inactivated EV71 WVP with adjuvant Alum, Proprietary adjuvant, used FCA/ICA as a "positive" control adjuvant. Proprietary adjuvant may be a suitable alternative to Alum for use with EV71 whole virus particle antigen. The neutralising titre levels generated in mice are very encouraging.

There is interest in Singapore to look seriously into the effectiveness of the vaccine and evaluate its efficacy in animal models and eventually in humans.

China

China CDC and Sinovac Biotech Co. Ltd. are collaborating on development of EV71 vaccine using different approaches such as a live attenuated vaccine, inactivated whole-virion vaccine, subunit vaccine and DNA vaccine.

Studies have been carried out to evaluate so far - propagation stability, exogenous factor examination, immunogenicity and development of virus seed bank for vaccine production. The manufacturing process has been carried out using culturing cell (Vero cell, human diploid cell), inactivation is done using formaldehyde, purification and formulation using adjuvant or nonadjuvant, different antigen dosages and BA 1B/c mice.

Taiwan

Taiwan CDC is developing an inactivated whole virion vaccine for EV71. In addition, the virus-like particle (VLP) technology is also being pursued. In 2000, a local isolate of EV71, E59 was found to be capable of eliciting antibodies that have broad spectrum of virus neutralization activities *in vitro*, and was selected as an EV71 vaccine strain candidate. In 2004, another local isolate, E36 (subgenogroup C4), was similarly found to have similar *in vitro* activity and was also selected as another potential EV71 vaccine strain candidate.

Sera from mouse raised against a vaccine based on the E59 strain of EV71 can neutralize most of EV71 genogroups isolated from different years (1998 to 2003) and different geographic locations. Sera from rabbits immunized with the E59 strain vaccine, can neutralize most (80.56%) of EV71 strains isolated from different years (1998 to 2005) and different geographic locations.

These locally selected strains have been adapted to the Vero cell. Promising products have undergone process development and are entering the next phase of pre-clinical and clinical development.

8 CONCLUSIONS AND RECOMMENDATIONS

The forum working groups identified challenges and research gaps and proposed approaches and regional activities to address these challenges.

- 1. Improve knowledge on disease burden and natural history:** There is a lack of information regarding the natural history of the disease, particularly the characteristics of natural immunity, duration of acquired protection and degree of cross-protection with other enterovirus infections. Not much information is available on disease burden (including incidence, prevalence, and cost data) to further document societal impact and anticipate the need for cost data in order to inform decisions about interventions such as vaccines should a vaccine become available. A few examples of information needed are seroprevalence of the infection at baseline and during an epidemic, rates of mild clinical disease and other indicators of impact of infection and disease. A collective effort to gather disease burden data should be undertaken as this may help not only to assess the true impact of the disease and the cost of responding to it, but also identify areas for intervention, and evaluate current measures of prevention and control including examining the cost-effectiveness of measures taken for vaccines or other preventive measures when they become available.
- 2. Synthesis of regional data:** More information is needed to fully comprehend disease epidemiology. Some of this information may be available but needs to be collected and analysed systematically throughout the region. An effort to synthesize regional surveillance data, including case definitions, case detection strategies (active or passive) and methods of diagnosis would clarify the picture of the disease in the region. Regional comparison of epidemiological and clinical data might identify risk or predictive factors for infection, disease, severe disease, clinical outcome. If the data are lacking, such an analysis would help identify the areas of study required in order to help answer those questions as well as promote increased harmonization of surveillance data in the future.
- 3. Determine and clarify the purpose of surveillance:** Tailoring surveillance to detect a common disease with rare consequences early is a challenge when there is no method to discriminate between those with the greatest likelihood to progress to the rare consequence quickly (i.e. those infected with EV71). Active case investigation is highly resource intensive and challenging in epidemic conditions. This implies having a clear idea around the clinical spectrum of disease including the clinical course and outcome of the disease, and the target population for surveillance.

Although some suggest that surveillance could be refined to focus only on detection of severe disease in hospitals, it is uncertain that current data supports this degree of refinement and focus of surveillance. Again this emphasizes the need to collect regional data in a way that could be compared and interpreted in a valid way.

- 4. Share best practices:** It was also proposed that there be an effort to collectively agree on some common thresholds for determining an outbreak status and the type and elements of response needed. This includes developing, sharing and comparing standardized protocols for case investigation.
- 5. Improve collective monitoring of trends and patterns in viral evolution and infection patterns:** There is still a lot unknown regarding the pattern of virus circulation and infection in the region and whether there are any significant changes compared to 10 or 20 years ago when this emerged as a public health concern. EV71 appears to circulate in a 2-3 year cycle in some areas but questions about this observation remains along with others related to the degree of susceptibles and non-susceptibles in the population. Unfortunately baseline seroprevalence data of either the assumed susceptibles or the general population is lacking and more studies are needed such as the one proposed by MOH, Singapore to conduct a national pediatric seroprevalence survey. The study involves collecting about 1200 samples among children aged 2-17 years from the two hospitals - KK Women's and Children's Hospital (KKH) and the National University Hospital (NUH). Increased vigilance should be maintained in view of the continued epidemics of HFMD in the Asia-Pacific region. A regional expert group could be established with expertise in the relevant areas in order to act as a resource for any advice on sudden outbreaks.
- 6. Collaborate on studies to inform on transmission and infection cycle:** There are no data on asymptomatic age-specific prevalence of EV infections. It is also unknown if prevalence for asymptomatic infections with viruses that cause HFMD differ among HFMD cases infected with different enteroviruses. One of the control strategies is interrupting the transmission cycle within schools, childcare facilities, families and communities. Identification of cases, isolation and breaking the chain of transmission should be effective. However, rates of asymptomatic infection in the different age groups is unknown and therefore it is impossible to determine if they are major contributors to transmission. Whether isolating siblings of affected children is necessary to prevent further spread of the disease within the family, schools and community, is unknown. There is some concern that there is a trend emerging where there is proportionally more cases in older children compared to younger children. Some have suggested that this may be an unintended consequence of the strict public health measures targeting the younger age groups and therefore leaving the older age groups more susceptible and vulnerable to the disease. It could also be related to differences in

age and activity level in different age groups. Studies to determine the asymptomatic infection rates in different age groups would require resources and collaboration across institutions to properly design and conduct a study of adequate power may need to be considered to help inform future policy and response.

- 7. Explore environmental surveillance:** There are also unanswered questions regarding transmission dynamics in particular the relationships with environmental and population factors such as temperature and humidity and crowded schools. Some have suggested that EV71 may survive longer in the environment and factors like this could be studied through environmental surveillance to provide information on actual virus survival time in the environment and potential reservoirs during inter-outbreak periods.
- 8. Optimize clinical management:** There is a need to share common clinical practice guidelines across the region. In addition clinical management particularly around use of IVIG needs to be evaluated in clinical research studies. Similarly, collection of clinical data in a standard data collection format across institutions in the region which have had to manage severe HFMD should be conducted in order to assess if the data would help with improving predictors of severity of outcomes or mortality, as well as improve management.
- 9. Support for improved lab capacity and diagnostics:** Diagnostic strategies should address clinical and epidemiological priorities. Early diagnosis of HFMD would help reduce morbidity and mortality but there is a lack of research and development on rapid methods of diagnosis. Clinical diagnosis for HFMD is nonspecific therefore diagnosis of EV71 is dependent on laboratory diagnostic tools for prompt detection of EV71. This gap leads to delays in implementing appropriate public health measures to contain EV71 transmission. Improving the situation involves weighing the availability of resources and capabilities with the current needs whether in the outbreak or inter-outbreak period. Support should be provided for improved diagnosis whether through clinical or laboratory diagnosis, so that public health preventive and control measures can be implemented to limit the further spread of the virus.

Some of the areas of improvement for lab and diagnostics include:

- a. Availability and type of samples:** Proving EV71 as a causative agent is dependent on the availability of the type of primary specimen collected. Although it is known that stool samples yield high positive rates, the collection of stool specimens is difficult in the outpatient setting, particularly with children. In Vietnam for example, stool specimens were obtained from hospitalized patients, as collection of stool samples from outpatients with mild illness of HFMD is very difficult. Similarly where viral detection from vesicular fluid or in CSF is

considered strong evidence of as a causative agent of HFMD or encephalitis, this is hampered by the difficulty of obtaining the specimen or insensitivity of detection methods or contamination. More needs to be done to optimize specimen collection and improve yield. Other more easily obtained specimens should be explored as primary specimens for diagnosis.

- b. Optimizing conduct of current diagnostic tests:** The diagnostics laboratories need new quality control methods, strains and updated primers in the face of the evolving virus. New primers need to be designed for circulating strains, including PCR and sequencing primers due to high Pan-EV IF positive rate versus low IF subtyping rate. Laboratory methods, materials, strains should be shared and exchanged.
- c. Strengthen and harmonize assay parameters of quality assurance:** Although it was felt that there was no need to standardize primers, the need to standardize the parameters for quality assurance was thought to be critical given the need to be able to relate the results with the clinical spectrum and the relevant virus sequences.
- d. Optimizing serological diagnostic methods :** There is a need to standardize neutralization tests for serum epidemiology and potential vaccine antigen development. An IgM-ELISA may be helpful for diagnosis but an improved assay with more specificity is not available and more work is needed in this area.
- e. Molecular epidemiology:** From a molecular epidemiology perspective, there is a need to focus on the VP1 domain partially and also on VP4 domain and as well as to increase the capacity to do full domain sequencing. Genome sequencing has been a challenge and more could be achieved through standardizing nomenclature and collaborating on regional studies to study virus evolution, particularly in areas where new viruses are likely to emerge i.e. India, Indonesia.
- f. Sharing information and specimens:** For most countries, limited funding affects the nation-wide laboratory surveillance although the sentinel surveillance approach seems a reasonable alternative. There is a need for the affected regions to further collaboration through informal networks that could share information regarding surveillance strategies, case definitions, and detection methods in order to improve virological information during epidemics as well as non-epidemic periods. Many agreed that there needed to be more informal collaboration and networks that would facilitate sharing of expertise, outbreak information and transfer of skills and capacity. Investigation of particular specimens or isolate will require sharing and shipment of specimens which raises

the issues around cost as well as requirements and authorizations around sharing and shipment of specimens, such as MTAs and managing publications. Informal networks should be able to develop standard procedures to approach these issues proactively so that they are not an obstacle to collaboration. Informal networks such as APNET which provides training in developing countries to support regional surveillance of EV71 should be supported and similar networks could be formed in other regions that would work together. It was agreed that APNET could possibly be a vehicle to drive HFMD surveillance and laboratory capabilities in the region.

10. Determine effectiveness of current measures of prevention and control: There is no evidence for effectiveness of prevention, control and treatment measures used in HFMD. The rapid evolution of HFMD epidemics makes it difficult to determine the effectiveness of control measures such as closure of childcare centers. The epidemiologic pattern of HFMD is influenced by many factors, including health-seeking behaviour, socio-economic changes, school holidays, and media attention. The importance of good outbreak investigations needs to be supported with well-conducted case-control or cohort studies and documenting as well as publishing findings. Several measures were proposed to address the situation including:

- a. Collaborating on comparative studies aimed at evaluating effectiveness of public health response measures across the region:** It is very important to evaluate the impact of interventions in order to understand its effectiveness. Large prospective studies are not the only way to obtain good data. There would be a lot of value in formal evaluations of school (preschool) closure policies. This would not have to be a large community-randomized trial although good documentation of the outcomes of the students in the schools (i.e., HFMD, EV71, healthy) and impact on families would be very beneficial if done for enough closed schools.

For example careful descriptions of what happens after a school closure by following up the children to see how many end up getting HFMD anyway and evaluating the impact on families even without a control group can prove very valuable for understanding the utility and shortcomings of this intervention.
- b. Sharing and exchanging resources to inform on best practices:** Information sharing and exchange of resources on best practices with regards to prevention and control measures taken such as public education methods, regulation and management of school closures, criteria or rating systems for child care centers, communication, implementation and enforcement strategies across the region.

- c. Developing common principles or strategies for prevention and control:** These could be adaptable and applicable across different areas and countries. These principles could cover all aspects of detection, management and public health response. Sharing information on licensure and enforcement standards for schools and childcare centers was thought to be very helpful.
- d. Practising risk management:** A lot of important questions can be addressed in outbreak investigations by practising better public communication through risk communication strategies that accurately inform the public, including the politicians, public, media, parents and child care centres as was illustrated by the example of a project in China. As outbreaks will most likely happen, planning the investigations ahead of time would be highly desirable. Risk communication practices may need to be tailored toward gradually disseminating more information on HFMD in order to sensitize the public to evidence-based approaches toward public health response to HFMD.

11. Develop activities to address research priorities: Although recently published studies have provided new insights into the epidemiology, pathogenesis, diagnosis and clinical management of HFMD in the region, the forum participants concluded that more research is needed to identify more effective ways of preventing and treating HFMD. Forum participants strongly supported focused efforts to develop and promote collaborative studies across institutions and nations that could explore and evaluate some of the basic questions regarding the disease. Such national and international collaborations could enable studies of sufficient size and statistical power needed to address important gaps in our current understanding of HFMD and EV71 infection. In addition, this network could be expanded to help answer research questions on the development of best practices for clinical management through the conduct of prospective cohort studies and randomized controlled trials that would provide an evidence base for improved care and treatment of EV71 and its severe manifestations.

Among the priority research questions proposed are:-

- a.** What is the true picture of disease epidemiology including the clinical course, outcome and risk factors for severe disease?
- b.** What do the disease incidence, prevalence and cost data indicate about the disease burden of hand, foot and mouth disease?
- c.** What are the asymptomatic infection rates for EV infections in different age groups?
- d.** What is the effectiveness of commonly used prevention and control measures in an outbreak?
- e.** What are the reasons for the apparent increasing magnitude of HFMD epidemic, and the focus in the Asia-Pacific region?

- f. What are the relevant genetic associations of both pathogen and host that are related to infection, disease presentation and outcome?

Such collaborative efforts could provide the platform to answer questions regarding clinical management practices through randomized controlled trials and cohort studies to properly investigate interventions for HFMD such as IVIG treatment.

Most agreed that a rapid point-of-care test is critically needed using easy to access clinical specimens such as oral secretions and should be a research priority. In addition further work to improve testing for markers of virulence or severity or to detect viral shedding using advances in proteomics and immunology, should be undertaken. Seroepidemiological studies include virus evolution and environmental factors affecting it, host genetic susceptibility and ethnicity studies and the use of *in vitro* methods and animal models to study cross neutralization would also be helpful, particularly for the development of drugs or vaccines.

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Table A Investigation and Detection of HFMD and EV71 among selected areas in the region

No.	PARAMETER/COUNTRY	MALAYSIA	SINGAPORE	MAINLAND CHINA	TAIWAN	THAILAND
1	Outbreak Investigation	<p>Outbreak definition: 2 or more cases in the same locality within the incubation period.</p> <p>Outbreaks reported:</p> <p>1996</p> <p>1997: 34¹ deaths</p> <p>2000:3560² cases</p> <p>2003: 2113² cases</p> <p>2006: 14,875² cases with 13³ deaths(Sarawak and Borneo)</p> <p>EV71 has been shown to have a 3 yearly cycle as compared to non-EV71 (endemic and low-level) and may increase during the three year peak.</p>	<p>Outbreak definition: 2 or more cases of HFMD with onset of illness within 10 days in the same institution for investigation and management by MOH.</p> <p>Epidemics reported:</p> <p>2000: 3790 cases, 4 deaths⁴</p> <p>2001: 5210 cases, 3 deaths with a CFR of 0.06%⁵</p> <p>2002: 16228 cases⁵, 0 deaths⁷, incidence rate 388.6/100,000⁵</p> <p>2006: 15,282 cases⁵, 0 deaths⁸, incidence rate 347.2/100,000⁵</p> <p>2008: 19,530 cases⁵, 1 death⁹, 4 cases hospitalized for encephalitis who were epidemiologically linked to HFMD clusters, including the HFMD-related death.</p> <p>Age distribution: 2001-2008 >50% children, <5 years of age.</p> <p>Pattern of the epidemic curve: unimodal(2000-2004) to bimodal (2005-2008).</p> <p>Type of strains in Singapore causing HFMD: CA16 and EV71</p>	<p>Outbreaks reported:</p> <p>Mainland China¹⁰, (2007), Shandong Province (2007), Anhui province, Fuyang City (March 1 - May 9, 2008)</p> <p>2007 Mainland China - 83344 cases, 17 deaths</p> <p>Incidence: 6.34/100,000</p> <p>Case Fatality Rate(CFR): 0.02%.</p> <p>Half of the cases occurred in children in childcare centers</p> <p>2007 Shandong Province - 39,606 cases, 14 deaths</p> <p>2008: Anhui province, Fuyang City - 6049 cases, 22 deaths, 3023 cases hospitalized (50%), 353 severe cases (6%), 0.4% CFR</p> <p>Age Group: 78% cases <3 years</p> <p>Main etiologic agent: EV71.</p>	<p>Outbreaks reported:</p> <p>1998, 2008¹¹</p> <p>1998: 129,106¹²cases, 405¹²severe cases, 78¹² deaths, CFR 86% < 3y/o12</p> <p>2008: 346¹¹severe cases, 10¹¹ deaths, 3%-25%¹¹ age distribution: 90% of cases for age group < 5 years¹¹</p> <p>EV71 reached peak in 25th wk of 2008 with 93% cases due to EV infection¹¹</p> <p>312 cases laboratory confirmed as containing isolate EV71 as Genogroup B5¹¹.</p>	<p>Outbreak years:</p> <p>2001: 1547 cases, 0 deaths</p> <p>2002: 3533 cases, 2 deaths</p> <p>2003: 1218 cases, 2 deaths</p> <p>2004: 769 cases, 2 deaths</p> <p>2005: 4646 cases, 0 deaths</p> <p>2006: 3532 cases, 7 deaths</p> <p>6 deaths were from the north eastern region clustering within 2 wks of disease outbreak</p> <p>2007¹³: 16846 cases, 2 deaths probable of EV71 without hand, foot and mouth lesions.</p>

Table A Investigation and detection of HFMD and EV71 among selected areas in the region

No.	PARAMETER/COUNTRY	MALAYSIA	SINGAPORE	MAINLAND CHINA	TAIWAN	THAILAND
2	National surveillance (Physicians/Laboratories)	Notifiable under national notification of Infectious Diseases since Oct 2005; legislated in Oct 2006. Notification must be done within 24 hours via telephone and by notification form. Mandatory notification of death and hospital admissions of children under 10 of HFMD	Notifiable since October 2000 Medical practitioners are required to notify all clinical cases of HFMD to MOH within 24 hours of diagnosis. Can be done by fax or online Childcare centres, pre-schools and primary schools are required to notify MOH of any HFMD outbreaks (defined as 2 or more cases of HFMD with onset of illness within 10 days in the same institution for investigation and management by MOH).	Notifiable since May 2008 (See below for details)	Notifiable since 1999 (See below for details)	
3	Sentinel surveillance (Clinical cases and Laboratory)	In Sarawak, surveillance is enhanced through physician sentinel surveillance sites consisting of private and government clinics in the areas of Kuching and Sibiu. Reported cases as well as word of mouth cases is actively investigated by state mobile teams. Teachers & child minders play in detecting and informing on suspect cases. Laboratory Surveillance – Two sentinel labs per state are designated to conduct testing for enterovirus.	Hospital based reporting for serious and fatal cases from the two main hospitals seeing pediatric patients in Singapore - KK Hospital and NUH Laboratory Surveillance – Samples collected from inpatient and outpatient HFMD cases at KKH and obtained from childcare centers are sent to Virology Laboratory of the Department of Pathology, Singapore General Hospital, Virology Laboratory, KKH and the National Public Health Laboratory for testing.	Enhancement of surveillance by active screening through patient triage system Designation of specific hospitals for treatment Clinical Monitoring for the early detection and intervention of severe cases Encouraging those ill to seek medical care by reimbursing medical fare based on new rural cooperative medical care regulation Enlisting daycare centers and schools to conduct fever and rash inspection of pupils in morning	Sentinel Physicians Surveillance (established 1990; HFMD included in 1997) enlisting 850 representative sentinel physicians from all 22 cities and counties on the island to report suspect cases among the outpatient population. Emergency Department Syndromic Surveillance System (Taiwan ED-SSS) receives real-time data on patients seen at the ED to detect 11 syndromic illnesses that represent clinical presentations of enterovirus and influenza for example. Hospital based reporting of hospitalizations due to HFMD reported to the Taiwan CDC Laboratory Surveillance – Surveillance of Sentinel Viral Laboratories for enterovirus	

Table A Investigation and Detection of HFMD and EV71 among selected areas in the region**References**

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- 4 Committee on Epidemic Diseases. Surveillance of hand, foot and mouth disease in Singapore. *Epidemiol News Bull* 2001; 27: 23-7
- 5 Ang LW, KWB Koh, KP Chan, LT Chua, L James, KT Goh (2009), Epidemiology and control of hand, foot and mouth disease in Singapore, 2001-2007. *Ann Acad Med Singapore* 2009 (in press).
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- 12 Monto Ho, M.D., Eng-Rin Chen, Dr. Med.Sci., Kwo-Hsiung Hsu, M.S. *et al* The New England Journal of Medicine 1999
- 13 Bureau of Epidemiology. Annual epidemiology surveillance report 2007, Express transportation organization of Thailand publishing. 2007, Thailand Ministry of Public Health Bangkok. p. 89.

Table B Laboratory Detection Methods used by different countries and regions to detect the presence of EV71

No.	PARAMETER/ PLACE	CHINA	MALAYSIA	SINGAPORE	TAIWAN	THAILAND	VIETNAM
1	¹ PREDOMINANT SEROTYPE/GENOGROUP/SUBGENOGROUP	1997 EV71: C3 1998, 2000, 2001, 2002 2003, 2004, 2007-2008 EV71: C4	1997 EV71: B3 2000 EV71: B4, 2003 EV71:, B5 2006 EV71: B5 C1 (sporadic) most years	1997 EV71: B3, B4 1998 EV71: B3, C1 1999 EV71: B3 2000 EV71: B4 2001 EV71: B4 2002 EV71: C1, B4 ² 2008 EV71: B5 ,CV:A16	1998 EV71: C2/B4/C4 1999-2003 EV71: B4 2004-2005 EV71: C4 2006 EV71: C5 2007 EV71: C5/B5 2008 EV71: B5	2001 EV71: B4 2002 EV71: C1, B4 2003 EV71: C1, B5 2005, 2007-2008 CV: A16	2005 EV71: C1, C4, C5; CV: A16
2	CLINICAL SPECIMENS USED	Throat swab Stool CSF Serum Autopsy tissues	Throat Swab Stool/ Rectal Swab CSF Vesicle Swab Endotracheal Tube Aspirate Serum	Throat Stool Rectal Swab CSF	Throat swab Stool CSF Rectal swab Blood samples Organs	Throat Swab Stool CSF Nasopharyngeal Swab or Secretion Paired Serum	Throat Swab in VTM Stool CSF Vesicular fluid in VTM
3	LABORATORY DETECTION METHODS REPORTED	Virus Isolation RT- PCR NT IgM and IgG by ELISA RT-PCR, Genetic Sequencing	Virus Isolation using RD, Vero Cells RT-PCR of primary specimens and/or isolates followed by sequencing; RT PCR using pan EV primers in 5'UTR and in VP4 or VP1 as well as EV71 specific primers.	Virus Isolation using HEL, RD, Hep-2C and MRC-5 cell lines RT-PCR, Genetic Sequencing	Cell culture (IFA), NT Serological Testing (IgM capture ELISA) RT-PCR/CODEHOP	Virus Isolation using RD, Vero or GL-37 Cells Serological diagnosis using IgG RT PCR, Nucleotide Sequencing	Virus Isolation PCR IFA NT RT-PCR

References

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2 Communicable Diseases Division, Ministry of Health, Singapore

Abbreviations used: CODEHOP: Consensus Degenerate Hybrid Oligonucleotide Primers
RD: Rhabdomyosarcoma PCR: Polymerase Chain Reaction NT: Neutralization Test

Table C Clinical Spectrum of HFMD observed in the region

No.	PARAMETER/ PLACE	MALAYSIA	SINGAPORE	TAIWAN	THAILAND
1	DIAGNOSTIC FEATURES	<p>HFMD</p> <ul style="list-style-type: none"> - febrile illness - maculopapular or papulovesicular rash on the palms and/or soles - vesicles or ulcers in the mouth <p>Herpangina</p> <ul style="list-style-type: none"> - multiple oral ulcers predominantly affecting the posterior parts of the oral cavity 	<p>Typical HFMD:</p> <p>Skin rashes: vesiculo-papular rashes over hands, feet, buttocks AND Mouth ulcers</p> <p>Atypical HFMD:</p> <p>Skin rashes: vesiculo-papular rashes over hands, feet, buttocks</p> <p>Without mouth ulcers</p> <p>± Fever</p> <p>± Diarrhoea, vomiting</p> <p>± Contact with known HFMD</p> <p>If no skin rashes and only mouth ulcers, diagnosed as Herpangina</p>	<p>HFMD</p> <ul style="list-style-type: none"> - febrile illness - maculopapular or papulovesicular rash on the palms and/or soles - vesicles or ulcers in the mouth <p>Herpangina</p> <ul style="list-style-type: none"> - multiple oral ulcers predominantly affecting the posterior parts of the oral cavity 	<p>Fever, rash involving distal extremities, buttocks, extensor surfaces of knees and oropharyngeal ulcers; history of contact; age group</p>
2	SEVERE DISEASE MANIFESTATIONS	<p>Aseptic meningitis (CSF pleocytosis, fully conscious, had headache, meningism, and no focal neurological signs)</p> <p>Encephalitis (presence of impaired consciousness including lethargy, drowsiness or coma, seizures or myoclonus)</p> <p>Acute flaccid paralysis (acute onset of areflexic limb weakness)</p> <p>Cardiorespiratory failure (presence of tachycardia, respiratory distress, pulmonary oedema, poor peripheral perfusion requiring inotropes; pulmonary congestion on CXR; reduced cardiac contractility on echocardiography)</p>	<p>Aseptic meningitis</p> <p>Encephalitis</p> <p>Cardiopulmonary manifestations - myocarditis, interstitial pneumonitis;</p> <p>Secondary infections i.e. RSV bronchiolitis HIB meningitis, pneumonia</p>	<p>CNS and/or cardiopulmonary manifestations:-CNS involvement- aseptic meningitis; encephalitis; poliomyelitis-like syndrome; encephalomyelitis (headache, vomiting, lethargy, upward gaze, seizure, limb weakness, myoclonic jerk)</p> <p>Cardiopulmonary manifestations- autonomic dysfunction (tachycardia, hypertension, profuse sweating, paralytic ileus, leucocytosis, hyperglycemia, tachypnea, hypoxemia, pulmonary edema, hemorrhage); circulatory failure (hypotension, coma, loss of brainstem reflexes)</p>	<p>Seizure</p> <p>Encephalitis</p> <p>Pulmonary edema and hemorrhage</p> <p>Cardiopulmonary collapse</p> <p>myocarditis</p> <p>pneumonia</p>

Table C Clinical Spectrum of HFMD observed in the region

No.	PARAMETER/ PLACE	MALAYSIA	SINGAPORE	TAIWAN	THAILAND
3	RISK FACTORS OF A COMPLICATED OUTCOME	CSF pleocytosis	<p>Vomiting Absence of mouth ulcers Atypical presentation Raised WBC</p> <p>EV 71 positivity did not show any statistically significant association with complicated disease. Fatal cases in 2000-2001 epidemic died from interstitial pneumonitis alone or with myocarditis or encephalitis</p>	<p>Risk factors associated with CNS involvement : Fever > 39°C Fever >3 days Seizure Vomiting Lethargy Limb weakness Hyperglycemia Risk factors associated with cardiopulmonary collapse: Tachycardia Hypertension Leucocytosis Hyperglycemia</p>	to be determined
4	SEQUELAE	not mentioned	not mentioned	<p>Long-term effect seen on neurodevelopmental aspects such as limb, weakness and atrophy, prolonged ventilatory and feeding support, cognitive impairment. Attention deficit/hyperactivity related disorders were observed raising the possibility that EV71 infected children may face problems in long term regulation of attention and emotion after recovery.</p>	not mentioned

Table C Clinical Spectrum of HFMD observed in the region

No.	PARAMETER/ PLACE	MALAYSIA	SINGAPORE	TAIWAN	THAILAND
5	PATHOLOGY FINDINGS	CNS inflammation found on autopsy typical of viral encephalitides - perivascular cuffing, neuronophagia; EV71 is neuronotropic and the distribution of inflammation in EV71 is stereotypic in the posterior brainstem and spinal cord areas. (MR scans show hyperintense lesions in the spinal cord, medulla, pons, cerebellum up to the midbrain in a stereotypic fashion that aid diagnosis)	Interstitial pneumonitis(alveolar congestion, inter-alveolar hemorrhage and interstitial lymphocytic infiltrate) alone or associated with myocarditis(lymphocytic infiltration, myocardial necrosis and interstitial edema) or encephalitis	Intense inflammation with perivascular cuffing and neuronophagia of the CNS in particular the brainstem and spinal cord; marked pulmonary edema with focal hemorrhage in the lung; EV71 isolated from CNS tissues	Diffuse brain edema; small numbers of lymphocytes and histiocytes focally present in the subarachnoid spaces; Scattered foci of necrosis present in thalamus, pons and medulla Diffuse congestion in the heart but No definite pericarditis, myocarditis or endocarditis No area of infarction Diffuse pulmonary edema and hemorrhage
6	MANAGEMENT	Screening for suspect cases (fever with mouth ulcers or rash on palms and soles) for admission with a low threshold for hospital admission for risk factors of complications or manifestations of severe disease; Risk factors include prolonged fever, tachycardia, tachypnea. Note for Sarawak: Early intervention with IVIG in those with marked CNS symptoms	Symptomatic supportive management The use of IVIG is being considered for patient < 4 years with the following symptoms: raised WBC, vomiting and the disease lasted < 5 days.	Management is based on clinical stages that denote disease severity and progression. This stage based management is illustrated further in Table x An evaluation has shown that the approach does achieve a reduction in fatality rate but conversely increases the long-term sequelae in survivors. Strict infection control practices	Symptomatic supportive management

LVAD: Left Ventricular Assist Device, ECMO: Extra Corporeal Membrane Oxygenation

Table D Outbreak and Public Health Response to prevent and control HFMD in the region

No.	STRATEGIES	CHINA	MALAYSIA	SINGAPORE	TAIWAN
1	Enhanced surveillance and case detection	Enhanced surveillance through early notification and case detection and case investigation	Emphasis on early detection and investigation of suspect cases according to the MOH 2007 Guidelines i.e. active case investigation by involving mobile teams; from 12 October 2006, “myocarditis” and “life threatening infections” were replaced by hand, foot and mouth disease	The Infectious Diseases Act requires medical practitioners to notify all clinical cases of HFMD to MOH within 24 hours of diagnosis, since Oct 2000; Enhanced surveillance also through community notification by childcare centres, pre-schools and primary schools of any HFMD outbreaks.	Widening surveillance network through sentinel physician surveillance and real-time outbreak disease reporting systems. Network for detection of severe disease consisting regional hospitals, commanders and labs called “Medical Care Nets for Severe EV Cases”
2	Optimized Case management of HFMD	Development of guidelines, diagnosis and treatment of HFMD; training of health workers from 16 cities in Anhui province on diagnosis, and ICU management; Designation of Fuyang City hospitals that are assisted in establishing pediatric ICUs; Designating specialized medical team to be available on round-the-clock service; Defining triage areas, procedures and protocols. Setting up reimbursement scheme for HFMD patients	Guidelines and protocols made available to enable early detection and admission, including hospital admission criteria. Guidelines also cover clinical management. Designation of hospitals experienced in the management of HFMD Special note on Sarawak: Analysis of past experience has identified CSF pleocytosis as associated with cardiorespiratory failure and death. This criterion is used as early detection of those at high risk for severe disease and requiring hospital admission. Early intervention using IVIG is also practised.	Guidelines for clinical management made available on the MOH Website to inform healthcare officials and general public of the disease	Guidelines on diagnosis and management including treatment for severe disease revised and updated; linked to physician continuous education system by offering credits online through “digital infectious disease learning net”. Dissemination of information on “Symptoms of EV71 and Timing of Referral for Severe Cases to Hospital”; establishing cooperation with relevant medical associations to improve care and treatment; strengthening infection control in the hospital setting

Table D Outbreak and Public Health Response to prevent and control HFMD in the region

No.	STRATEGIES	CHINA	MALAYSIA	SINGAPORE	TAIWAN
3	Case Isolation/ Voluntary Quarantine	Patient triage system established to promote case isolation. Promote social distancing	Designated hospitals have isolated areas and wards, increased awareness and encourage voluntary social distancing	Early detection by health screening to isolate suspect cases before entering the school	Case isolation is promoted but practice has been a challenge
4	Institutional Closure and accompanying measures of hygiene and sanitation	Closure of kindergartens and schools in highly affected cities Health screening (fever and rash inspection in the morning) and reporting to local CDC daily Health education including hand washing, avoiding thumb sucking and chewing nails Disinfection of toys. Secondary Transmission between siblings of the same family	Voluntary and mandatory closure of childcare centers, pre-schools and kindergartens. Health education (hand washing and personal hygiene) practices and standard of hygiene. Health inspection by checking the standard of hygiene (hand washing and personal hygiene) practices	Voluntary and mandatory closure of childcare centers, pre-schools and kindergartens. Health screening (fever and rash inspection in the morning) Implement hand washing, sanitary practices in diapering and handling of soiled items, good housekeeping and avoiding communal bathing. Washing and disinfection of toys, play items and other school equipment; mattresses should have waterproof PVC protection which can be cleaned. Domestic Bleach (undiluted) can be used as a good sanitizing agent	Voluntary and mandatory closure of childcare centers, pre-schools and kindergartens. The implementation of EV Control Policies in 2008 in Taiwan especially the announcement of mandatory school closure requires close cooperation with multiple ministries

Table D Outbreak and Public Health Response to prevent and control HFMD in the region

No.	STRATEGIES	CHINA	MALAYSIA	SINGAPORE	TAIWAN
5	Public health Education	Health education regarding the disease and promotion of hygienic practices; distribution of leaflets; other media to increase awareness.	Health education regarding the disease done by MOH by holding campaigns for example clean hands campaign and distributing leaflets and brochures to the childcare centers to promote good sanitary and hygiene practices.	The MOH provides number of notifications in MOH Weekly Infectious Diseases Bulletin, available online	Strengthening infection prevention practice in medical settings by ensuring temporary closure of playgrounds; educating medical workers about good hygiene practices for example hand washing
6	Dissemination of Information and Risk Communication	Applying a risk assessment and communication approach to dissemination of information and communication strategies: 1) Announce early and often and always plan ahead of time 2) Be open and honest 3) Keep messages simple and concise 4) Listen to and involve the public 5) Give the public responsibility and actions 6) Display public empathy in public announcements when required 7) State the plan of action during emergency 8) Tell people where they can get additional information	Risk Communication skills and interagency collaboration with licensing authority; surveillance	Public Health information is disseminated by distributing leaflets and brochures to the childcare centers which in turn inform the public. Childcare centres, pre-schools and primary schools send out notifications for any HFMD outbreaks.	Control Measures in Public Health included educating the public about the disease and the importance of personal hygiene, cooperation with multiple ministries

Appendix B List of Countries and Regions taking part in the HFMD Forum

No.	Countries and Regions	Number of Participants
1.	Australia	1
2.	Brunei	2
3.	China	7
4.	Indonesia	4
5.	Malaysia	6
6.	Singapore	46
7.	Taiwan	6
8.	Thailand	11
9.	USA	2
10.	Vietnam	3

Total number of Participants for the HFMD Forum
taking part from 10 different countries/Regions = 88

Appendix C List of Participants

No.	Name of Participants	Affiliation	Contacts
1	Alden Henderson	US CDC SEARO, Bangkok	akh0@th.cdc.gov
2	Alvin Lee	MOH, Singapore	alvin_lee@moh.gov.sg
3	Andrew Kiyu	Sarawak Health Department, Malaysia	kiyu.andrew@gmail.com
4	Ang Li Wei	MOH, Singapore	ang_li_wei@moh.gov.sg
5	Angela Huang	CDC Taiwan	huang.songen@gmail.com
6	Ariel Chua	MOH, Singapore	ariel_chua@moh.gov.sg
7	Bai Xin Lai	SGH, SINGAPORE	bai.xin.lai@sgh.com.sg
8	Bao-Ping Zhu	FETP, China	bpzhu@cfetp.org.cn
9	Chan Pei Pei	MOH, Singapore	chan_pei_pei@moh.gov.sg
10	Chiang Jen-Ron	CDC, Taiwan	jrc@cdc.gov.tw
11	Chong Chia Yin	KKH, Singapore	chong.chia.yin@kkh.com.sg
12	Cui Lin	NPHL, Singapore	cui_lin@ttsh.com.sg
13	Cynthia Kwok	MOH, Singapore	cynthia_kwok@moh.gov.sg
14	Darmawali Handoko	MOH, Indonesia	drwalisurv@yahoo.com
15	Duane J. Gubler	Duke-NUS Graduate Medical School, Singapore	duane.gubler@duke-nus.edu.sg
16	Dustin C. Yang	CDC, Taiwan	cxy1@cdc.gov.tw
17	Eileen Chen	SGH, Singapore	eileen.chen.x.q@sgh.com.sg
18	Elizabeth Crosson	Embassy of the United States of America, Singapore	CrossonEF@state.gov
19	Fu Xiuju	Institute of High Performance Computing, Singapore	fuxj@ihpc.a-star.edu.sg
20	Goh Kee Tai	MOH, Singapore	GOH_Kee_Tai@moh.gov.sg

21	Henry C. Baggett	US CDC SEARO Bangkok	Kipb@th.cdc.gov
22	Herna Harianja	NIHRD, Indonesia	herna@litbang.depkes.go.id
23	Hui-Chen Lin	CDC, Taiwan	lhuichen@cdc.gov.tw, lhuichen@gmail.com
24	Jane Cardosa	Institute of Health & Community Medicine Universiti Malaysia, Sarawak	jane.cardosa@gmail.com
25	Jeffery Cutter	MOH, Singapore	jeffery_cutter@moh.gov.sg
26	Jeremy Farrar	Hospital for Tropical Diseases, Ho Chi Minh, Vietnam	jfarrar@oucru.org
27	Joe Lew	MOH, Singapore	joe_lew@moh.gov.sg
28	Joseph Santangelo	SingVax, Singapore	J.santangelo@singvax.com
29	Justin Chu Jang Hann	NUS, Singapore	miccjh@nus.edu.sg
30	Kamaliah Binti Mohamad Noh	MOH, Malaysia	kamaliahmn@gmail.com
31	Kan Nyunt	MOH, Brunei	nyunt123@gmail.com
32	Kong Yeean Leng	REDI Center, Singapore	kong@redi.org.sg
33	Kun-Bin Wu	CDC, Taiwan	kunbin@cdc.gov.tw
34	Lai Wen Bin	SingVax, Singapore	wb.lai@singvax.com
35	Lalitha Kurupatham	MOH, Singapore	Lalitha_Kurupatham@moh.gov.sg
36	Linda Meng	NPHL, Singapore	meng_shu12@hotmail.com
37	Low Chew Ling	MOH, Singapore	low_chew_ling@moh.gov.sg
38	Lusiana Lim	NPHL, Singapore	lusiana_ls@yahoo.com.sg
39	Lyn James	MOH, Singapore	lyn_james@moh.gov.sg
40	Lynette Oon	SGH, Singapore	lynette.oon.l.e@sgh.com.sg
41	Ma Huilai	CDC, China	huilaima@cfetp.org.cn

42	Mark A Pallansch	US CDC Atlanta	map1@CDC.GOV
43	Melinda Frost	US CDC assigned to Beijing	MFrost@cdc.gov , mhf@CN.CDC.GOV
44	Meng Chee Phoon	NUS, Singapore	micpmc@nus.edu.sg
45	Michael Malison	US CDC SEARO, Bangkok	mmalison@th.cdc.gov
46	Ng Kah Ying	NPHL, Singapore	kypepper@gmail.com
47	Nguyen Thi Hien Thanh	National Institute of Hygiene and Epidemiology, Hanoi	thanh.enterovirus@nihe.org.vn, thanhpoliolab@yahoo.com
48	Ooi Eng Eong	DSO and Duke-NUS Singapore	oengeong@dso.org.sg
49	Ooi Mong How	Sibu Hospital, Malaysia	monghow@pd.jaring.my
50	Peter McMinn	University of Sydney, Australia	peterm@ichr.uwa.edu.au, pmcminn@med.usyd.edu.au
51	Phan Van Tu	Pasteur Institute, Vietnam	pvtu123@yahoo.com
52	Piyarat Suntarattiwong	MOPH, Thailand	drjunesunta@yahoo.com
53	Rahmah Hj Md Said	MOH, Brunei	rahmahms@gmail.com rahmahms@brunei.bn
54	Rassamee Tansirisithikul	MOPH, Thailand	artrassamee@yahoo.com
55	Ratana Tacharoenmuang	MOPH, Thailand	rt281039@dmsc.moph.go.th
56	Ratigorn Guntapong	MOPH, Thailand	ratigorn@dmsc.moph.go.th
57	Ray Chen	NIAID, NIH, USA	RChen@niaid.nih.gov
58	Raymond Lin Tzer Pin	NPHL, CDC, MOH, Singapore	Raymond_LIN@moh.gov.sg

59	Rismali SpA	Infectious Disease Hospital, Indonesia	uki.reivendra@gmail.com, info@infeksi.com
60	Rodney Hoff	REDI Center, Singapore	rhoff@redi.org.sg
61	Rohini Rao	REDI Center, Singapore	raorohini@redi.org.sg
62	Rome Buathong	MOPH, Thailand	romebua@hotmail.com, romebua@health2.moph.go.th
63	Shanshan Dong	Beijing Sinovac Co.	dongss@sinovac.com
64	Somsak Thamthitawat	US CDC SEARO, Bangkok	SomsakT@th.cdc.gov
65	Soo-Sim Lee	REDI Center, Singapore	Isoosim@redi.org.sg
66	Steven Ooi Peng Lim	CDC MOH, Singapore	OOI_Peng_Lim@moh.gov.sg
67	Susan Maloney	US CDC SEARO, Bangkok	szm7@th.cdc.gov
68	Sylvia Lim	MOH, Singapore	sylvia_lim@moh.gov.sg
69	Sylvie Alonso	NUS, Singapore	micas@nus.edu.sg
70	Tan Eng Lee	Singapore Polytechnic	englee@sp.edu.sg
71	Tang Yan Ling Nataline	NPHL, Singapore	nataline_yl_tang@ttsh.com.sg
72	Teo Junxiong	NPHL, Singapore	teo_junxiong@moh.gov.sg
73	Toh Hai Yin	MOH, Singapore	toh_hai_yin@moh.gov.sg
74	Tzou-Yien Lin	Chang Gung Children's Hospital, Taiwan	pidlin@adm.cgmh.org.tw, tzuen@adm.cgmh.org.tw
75	Vernon Lee	Ministry of Defense, Singapore	vernonljm@hotmail.com
76	Vincent Chow	NUS, Singapore	micctk@nus.edu.sg
77	Wai Jin Man	SingVax, Singapore	jm.wai@singvax.com

78	Wanna Hansaoworakul	Ministry of Public Health (MOPH), Thailand	wanna@health.moph.go.th
79	Wenbo Xu	CDC, China	wenbo_xu1@yahoo.com.cn
80	Widoretno	NIHRD, Indonesia	w_retno@litbang.depkes.go.id
81	Wong Kum Thong	University of Malaya, Malaysia	wongkt@um.edu.my, wongkt@ummc.edu.my
82	Wu Hong King	MOH, Singapore	wu_hong_king@moh.gov.sg
83	Wu Yan	NUS, Singapore	g0700115@nus.edu.sg
84	Xiaoqi Wang	CDC, China	wangxq@chinacdc.cn, doris_wang@126.com
85	Yew Yik Weng	MOH, Singapore	yew_yik_weng@moh.gov.sg
86	Za Hussain Reed	REDI Center, Singapore	zareed@redi.org.sg
87	Zainah Saat	Institute of Medical Research (IMR), Malaysia	zainah@imr.gov.my
88	Zhang Jing	CDC, China	jkccdc@vip.sina.com