REVIEW ARTICLE

Enterovirus 71 in Malaysia: A decade later

Yoke-Fun CHAN *PhD*, I-Ching SAM *MRCPath*, Kai-Li WEE *BSc*, Sazaly ABUBAKAR *PhD*

Tropical Infectious Diseases Research and Education Centre, Department of Medical Microbiology, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia

Abstract

In the last decade, Malaysia has experienced several hand, foot and mouth disease (HFMD) epidemics, complicated by fatalities due to severe neurological involvement. Enterovirus 71 (EV-71) has been implicated as the major causative agent for these epidemics. EV-71 infection is a global public health problem with pandemic potential. In many parts of Asia-Pacific, the virus has emerged as one of the most deadly virus infections amongst young children. The virus is highly transmissible through faecal-oral route and respiratory droplets. A recent rise in neurological complications and deaths suggests that the viruses currently circulating may be more virulent. The major risk factor associated with more severe EV-71 infection is young age and poor cellular immunity. Rapid laboratory diagnosis and molecular surveillance is important to closely monitor the emergence of new EV-71 subgenotypes. Since vaccine and anti-virals for EV-71 are not available, control and prevention strategies remain the only ways to combat the infection.

INTRODUCTION

Since its first isolation in California in 1969, enterovirus 71 (EV-71) has caused sporadic outbreaks of hand, foot and mouth disease (HFMD), as well as serious neurological diseases such as meningitis, encephalitis, and acute flaccid paralysis (AFP). In the last 15 years, these epidemics have increased in number and involved large numbers of children in Asia, including Malaysia.

In this review, we will discuss the epidemiology, clinical manifestations, risk factors, diagnosis, and management of EV-71 infection, with particular reference to work in Malaysia, and our diagnostic virology laboratory in University Malaya Medical Centre (UMMC), in Kuala Lumpur, Malaysia.

VIROLOGY OF EV-71

EV-71 belongs to the *Picornaviridae* family. Picornaviruses are small, about 28 nm in size, non-enveloped, and consist of icosahedral particles with a positive-stranded RNA genome. Based on nucleotide sequences, physico-chemical properties, serological relatedness and pathogenicity in man or mice, EV-71 is classified in the genus enterovirus along with polioviruses, coxsackievirus A (CV-A) and coxsackievirus B (CV-B), echoviruses, EV-70, and bovine enteroviruses.[1-2] EV-71 belongs to the species *Human enterovirus A* (HEV-A), which also includes other important human pathogens such as coxsackieviruses A2-A8, A10, A14, and A16.[3-4]

The EV-71 genome encodes a long polyprotein with a single open reading frame followed by a poly A tract. The single polyprotein is flanked by untranslated regions at both the 5' and 3' ends, and are cleaved into different structural (VP1-VP4) proteins, which form the physical structure of the virus, and non-structural proteins (2A-2C, 3A-3D), which include enzymes important for host interaction and replication (Figure 1).

GLOBAL EPIDEMIOLOGY OF ENTEROVIRUS 71

Global prevalence of EV-71

After the first outbreak in California in 1969, EV-71 was subsequently associated with several other outbreaks, in Bulgaria[5], Japan[6], Hungary[7], Australia[8-9], China[10], Malaysia[11], Taiwan[12-13], Hong Kong[14], Korea[15-16], Vietnam[17], Macao[18], Brunei[19], and, more recently, China[20] and Singapore in 2008. European countries such as Germany[21], Norway[22], United Kingdom[23], Austria[24], Netherlands[25], France[26] and Hungary[27] have also reported EV-71 cases. EV-71 infection is therefore a global health problem with pandemic potential.

Address correspondence to: Yoke-Fun Chan, Department of Medical Microbiology, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia. Tel: 60379676677, Fax: 60379675752, Email: chanyf@um.edu.my; chanyf@ummc.edu.my
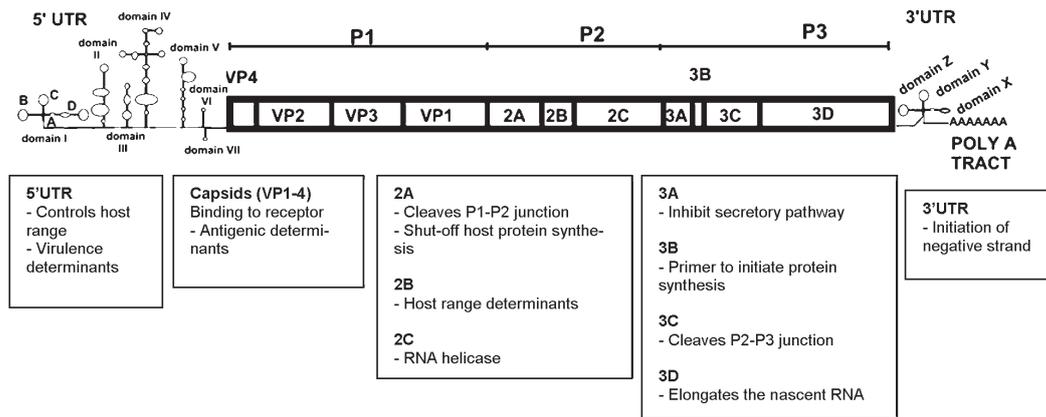
Figure 1. EV-71 genome organization. The genome organization and function of the EV-71 genes is similar to other enteroviruses.[4]

Global increase of EV-71 activity

There has been increased EV-71 activity in the Asia Pacific region since 1997.[28] We compared surveillance data from USA, Japan, Singapore and Taiwan. In the USA, more than 50% of the 1,520 HEV-A reported were CV-A16 (40.5%) and EV-71 (17.8%).[29] In the 1970s and 1980s, CV-A16 was most frequently isolated, but was then replaced by EV-71 after the 1990s.[29] In Japan, enterovirus surveillance from 1982-2010 showed that of the 28,882 HEV-A isolates, 24.4% were CV-A16 and 17.5% were EV-71.[30] Of 4,181 HEV-A isolates reported in Taiwan between 1998-2005, the most common enteroviruses were EV-71 (40.9%) and CV-A16 (40.7%).[31] Surveillance from Singapore from 2001-2007 also revealed similar trends, with CV-A16 (40.8%) and EV-71 (31.4%) as the main enteroviruses.[32]

ENTEROVIRUS 71 IN MALAYSIA

Outbreaks of fatal hand, foot and mouth disease in Malaysia

In mid-1997, an outbreak of acute viral infection in Sarawak, Malaysia caused 31 deaths among young children aged between five months to six years.[11, 33] In most cases, the children died within hours of admission to hospitals due to acute congestive heart failure and cardiovascular collapse, which was suggestive of acute viral myocarditis. Occurrence of the fatal infections in the midst of simultaneous outbreaks of EV71-associated HFMD raised the possibility that EV71, or another enterovirus, was associated with the deaths. Based on the clinical presentation resembling myocarditis, group B coxsackieviruses

(CV-B) were initially thought to be the causative agent. However, CV-B was not detected in these fatal cases. Instead, a novel adenovirus was isolated from some of the fatal cases, in addition to EV-71.[34-35] A few months following the outbreak in Sarawak, fatal brainstem encephalomyelitis was reported in at least four young children in Peninsular Malaysia.[36] In all cases, EV-71 was isolated and identified from central nervous system tissue samples.[11]

In 2000, another fatal HFMD outbreak hit Malaysia. In addition to EV-71, echovirus 7 was also identified as a possible causative agent for three cases of acute encephalomyelitis.[37] Large outbreaks with fatalities continued to be seen in 3-yearly cycles in Sarawak in 2003, 2006[38] and 2008/2009.[39]

EV-71 in Malaysia before 1997

No data is available on EV-71 or HFMD prior to 1997. Laboratory records at UMMC showed that samples were received from patients with HFMD between 1982 to 1997, but no virus isolates are available. A seroprevalence study on archived sera will help to show if EV-71 was present in Malaysia before 1997.

EV-71 in Malaysia after 1997

After the 1997 EV-71 fatal outbreak in Malaysia, our laboratory began confirmation of EV-71. From 1997 to 2008, 1,098 samples from patients suspected with enterovirus infection were received in the virology laboratory in UMMC, and of these, 202 (18.6%) samples were culture-confirmed positive for EV-71, particularly during the outbreak years of 1997, 2000, and 2006. No

EV-71 was isolated in 2002 and 2004. From 2000 onwards, the laboratory also began confirmation of CV-A16 and since then, CV-A16 has been identified in 48 patients. Of these, 33.3% (16/48) and 37.5% (18/48) were reported in 2000 and 2002, respectively, while no CV-A16 was isolated in 2001 and 2003. The number of patients with confirmed CV-A16 infection was relatively small which suggests that it is uncommon for CV-A16 to cause more severe forms of HFMD that warrant hospitalization, at least in UMMC. In Sarawak, EV-71 was seen during the outbreak years, while CV-A16 was seen in both outbreak and inter-outbreak periods.[40]

The average incidence rate (per 100,000 population) for 2000-2008 was 25.0 (range, 1.5-60.6). Data from Ministry of Health Malaysia (MOH)[41] showed that the incidence rate of HFMD was as high as 56.1 and 60.6 in 2007 and 2008, respectively. This may be because HFMD was declared a notifiable disease in 2006. Nevertheless, it is highly likely that the disease remains underreported. The average mortality rate (per 100,000 population) for 2000-2009 was 0.01 (range 0-0.3). No deaths were reported in 2002, 2004, 2007 and 2009.

EV-71 genotype distribution

Generally, EV-71 is divided into four broad genotypes: A, B, C and D (previously designated as C4), based on analysis of complete genome sequences of many strains.[42] Genotyping and phylogenetic analysis provides useful information on the evolution and epidemiology of EV-71, such as confirming outbreaks and demonstrating geographical spread. Genotype A contains the prototype virus BrCr. Genotype B is further divided into B1-B4 (B4 was recently proposed to be merged with B5 based on its complete genome relatedness) while genotype C is further divided into C1-C3 and C5 (Figure 2).

Van Sanden et al. (2009)[25] reported the earliest EV-71 isolate, from 1963. Recent bioinformatics analysis showed that EV-71 probably evolved as early as 1942.[43] Based on molecular epidemiological studies performed using publicly available EV-71 VP1 or VP4 gene sequences, the distribution of different EV-71 in different parts of the world was mapped (Figure 3). Our review of the published literature shows that only several EV71 subgenotypes - B4 (previously B5), C1, C2, C5 and D are currently circulating in the world. While all these genotypes are found in Asia, only genotype D, subgenotype C1 and C2 are circulating in Europe (Figure 3). Subgenotype B1 was last seen in 1986, subgenotype B2 in 1994, while subgenotypes B3 and C3 only circulated briefly during 1997-1998 and 1997-2000, respectively. Of note, genotype A has been reported to have reemerged in China during the HFMD outbreaks in 2008 and 2009; however these isolates may not have evolved together with other subgenotypes because of its high similarity with the prototype BrCr.[44] In Malaysia, subgenotypes C1, C2, B3 and B4 have been reported, but recently, predominantly, subgenotype B4 (B5) is circulating in both Peninsular Malaysia and Sarawak.

Seasonal distribution and cyclical EV71 outbreaks?

Seasonal distribution has been observed for enteroviruses in temperate countries between June to October, the summer and early fall months.[4] HFMD surveillance in Singapore from 2002-2009 showed that annual peaks occurred between March to May.[45] Similarly, sentinel surveillance for enteroviruses from 1998-2005 in Sarawak also showed the occurrence of HFMD around March to June.[40] An 8-year epidemiologic study in Taiwan (1998-2005) showed that more severe HFMD occurred around April-June.[31] Limited surveillance data is available for Peninsular Malaysia. Based on the small number of 202 laboratory confirmed cases seen in UMMC in Kuala Lumpur, EV-71 was most frequently isolated between June to October. This hospital data reflects more severe cases and increased reporting due to public awareness in seeking treatment, in contrast to the active surveillance of HFMD cases from sentinel clinics in Sarawak and Singapore.

Some sites have reported cyclical patterns of EV-71 outbreaks, which we did not see with our limited hospital cases in Kuala Lumpur. In Sarawak, EV-71 HFMD outbreaks occurred in a cyclical pattern of approximately every two to three years.[40] The Infectious Disease Surveillance Center in Japan showed a similar cyclical trend, with predominantly EV-71 isolation in 1983, 1986, 1990, 1993, 1997, 2000, 2003, 2006 and 2010.[30]

TRANSMISSION OF EV-71

Humans are the only known reservoir for EV-71. The faecal-oral route is the commonest mode of transmission, although respiratory droplet spread may also play a role.[4] EV-71 shedding in the
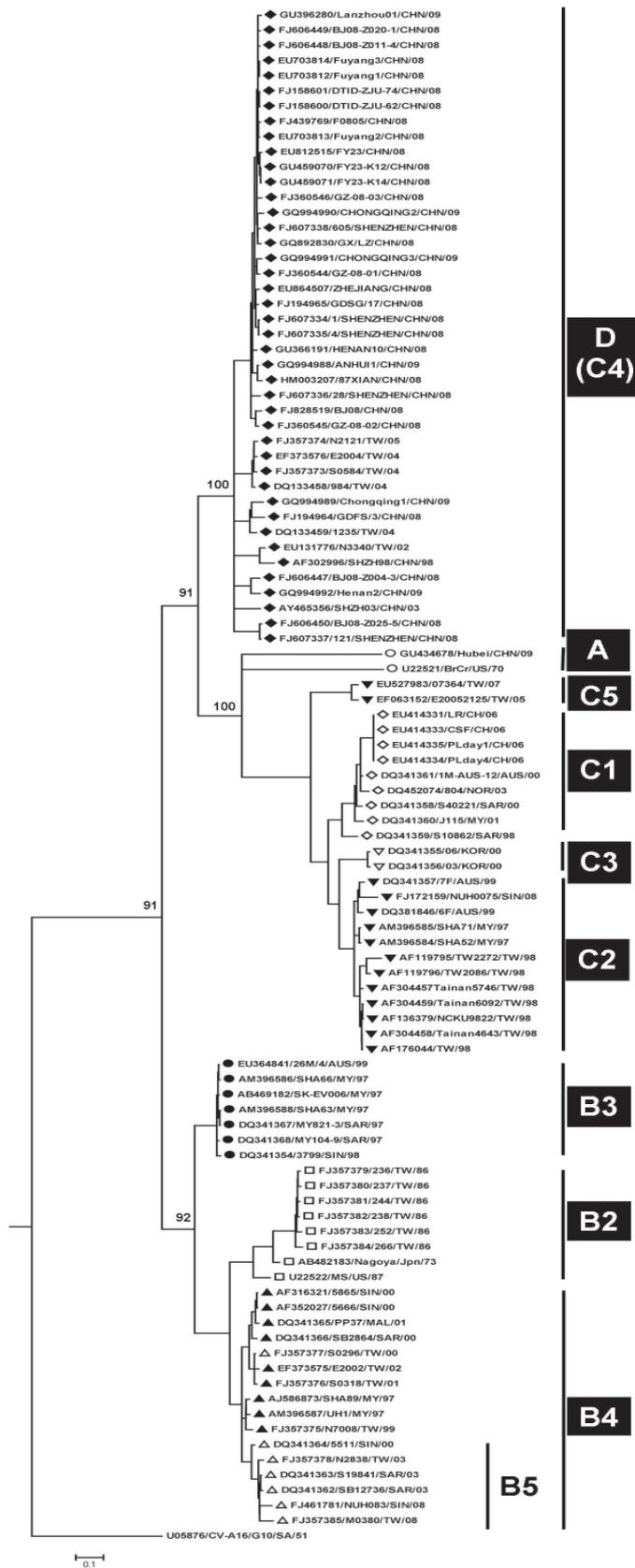
Figure 2. Phylogenetic tree based on EV-71 complete genomes. The maximum likelihood tree was constructed using PhyML and bootstrapped with 1000 samplings. The tree shows that EV-71 can be divided into four genotypes, A-D; genotypes B and C can be further subdivided into B1-B4 (with previously proposed B5 being incorporated as part of B4), and C1-C3 and C5 respectively. Subgenotype C4 has been placed into a new genotype, D.

Figure 3. Genotype distribution of EV-71. The figure was tabulated based on the review of published literature and deposited sequences in GenBank (as of 15 November 2010).

stool was longer than in the throat[46], and may be for up to 42 days post-infection.[47] During the EV-71 outbreak in Taiwan in 2001-2002, the principal transmission was between children in childcare facilities. However, the transmission rate to household contacts was as high as 52%, especially to siblings and cousins.[48] Adults are rarely infected, as most adults already have previous immunity. However, infected adults may excrete the virus without signs and symptoms, and transmit virus to susceptible children.

EV-71 has been isolated from worldwide HFMD outbreaks every year, suggesting the continuous circulation of the virus in the population. The persistence of enteroviruses has been well documented in the environment, such as in sewage and water systems[4], and in spring water and environmental water in Taiwan.[49-50] EV-71 may also survive in the environment for at least three days at tropical room temperature.[51] The persistence of EV-71 in the environment may provide a continuing source of potential exposure for susceptible populations. A very low rate of enterovirus infections amongst blood donors has been documented, suggesting that blood components are unlikely to be an important route of transmission.[52] Perinatal transmission of enterovirus infections are common, however in EV-71 only one case has been documented.[4,53]

CLINICAL MANIFESTATIONS OF EV-71 INFECTION

Clinical manifestations of EV-71 range from asymptomatic to involvement of upper respiratory tract, gastrointestinal tract, central nervous system and cardiovascular system. Up to 71% of EV-71 infections in children may be asymptomatic, and may serve as a reservoir for transmission.[54] EV-71 is associated with HFMD, meningoencephalitis, poliomyelitis-like paralysis and aseptic meningitis (non-paralytic poliomyelitis). Chang *et al.* (2004)[55] classified symptomatic EV-71 infection into four stages.

Hand, foot and mouth disease and herpangina

Uncomplicated disease, or stage 1, mainly manifests as HFMD or herpangina. The most common causes of HFMD are CV-A16 and EV-71. Other enteroviruses associated with HFMD include CV-A4, A5, A10, B2 and B5. The incubation period for HFMD ranges from 3 to 7 days. The infectious period starts from several days before the appearance of symptoms and peaks within one week of disease onset. The common signs and symptoms are fever, sore throat, and exanthema. Vesicular or maculopapular rash can appear on palms, feet and buttocks (Figure 4). Vesicles and ulcers can also be found in the oral cavity especially on the tongue and soft palate (Figure 4). In herpangina, only oropharyngeal lesions occur. Most cases are mild, self-limiting and do not warrant any hospitalization.

Central nervous system involvement

The enteroviruses associated with central nervous system (CNS) infection are echoviruses, polioviruses and EV-71. In stage 2, several days after initial HFMD or herpangina, patients may develop CNS involvement, most commonly aseptic meningitis, encephalitis or acute flaccid paralysis (AFP). Aseptic meningitis is usually self-limiting. Encephalitis commonly involves the brainstem, cerebellum and spinal cord, and presents with myoclonic jerks, reduced consciousness, ataxia, and cranial nerve palsies. In a study of 1,548 severe cases, encephalitis was the most common manifestation of CNS disease.[31] AFP is the least common of the three main CNS complications. Children with AFP develop a poliomyelitis-like syndrome with acute onset of limb weakness with reduced reflexes.
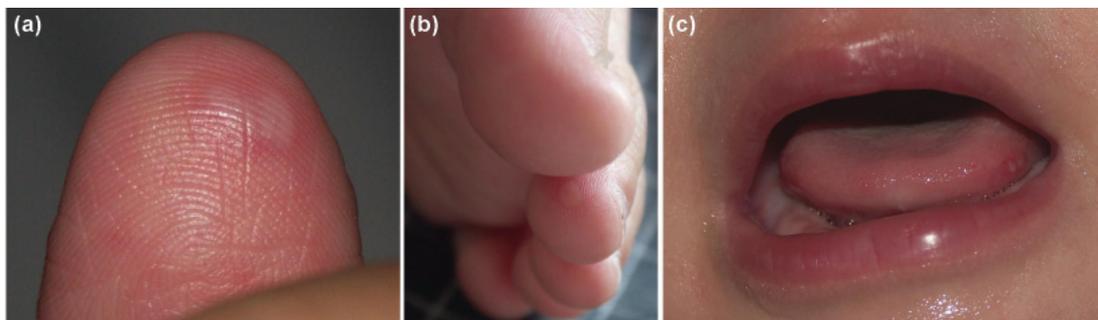

Figure 4. Cutaneous vesicles (a, b) and ulcers on the tongue (c) in hand, foot and mouth disease.

Cardiopulmonary involvement

Between several hours to two days following CNS involvement, stage 3 may occur, involving dysregulation of autonomic nervous system, and reduced myocardial function with progressive hypotension or pulmonary edema.[55] The onset of this stage may lead to death within hours. Extensive inflammation is always seen in the brainstem and spinal cord, but only occasionally in the lungs or myocardium. It is hypothesized that the cardiopulmonary failure is neurogenic in origin, due to EV-71 damage of the vasomotor centers in the medulla.[36]

Long-term morbidity

Stage 4, the convalescence stage, is characterized by the long-term neurological sequelae of CNS involvement, which includes limb weakness, poorer cognitive ability, motor deficits and neurodevelopmental delay.[13,56] Children recovered from EV-71 neurological illness may also have attention deficit or hyperactivity problems in schools.[57]

RISK FACTORS FOR SEVERE EV-71

Genetic variation of EV-71

One of the possible reasons that EV-71 continues to emerge as a more virulent virus is the presence of many subgenotypes within the virus. Ooi *et al*. (2007)[58] showed that subgenotype C1 and B5 were more likely to cause CNS infection compared to subgenotype B4. In Taiwan, outbreaks with CNS involvement due to subgenotypes C2, B4 and B5 were often preceded by shifts in the predominant circulating subgenotype of EV-71.[59-60]

Recombination in enteroviruses involves the breaking of viral nucleic acid before it is joined to another segment from a related virus. The resulting genetic variation may be beneficial as it may allow immune escape, or it may alter viral virulence. The possible emergence of these different subgenotypes through inter-typic recombination with other HEV-A has been shown.[61] It is now known that at least genotype D and subgenotype B3 are recombinants of EV-71 with mosaic sequences of CV-A16.[61-62] Interestingly, experiments performed in suckling mice showed that the non-recombinant subgenotype B4 and recombinant subgenotypes B3 had different neurovirulence.[63]

Sequencing studies have shown that different clinical outcomes of EV-71 infection are associated with mutations at 5'UTR[64-65], VP1[66], 2B[64], 2C[64], 3A[67], 3C[64,68], 3D[65], and 3'UTR[68] regions. However, there have not yet been any consistent findings of specific mutations that increase virulence that may be used in a clinically relevant manner.

Co-infection

The possible role of co-infection of other infectious agents in increasing virulence cannot be excluded. During the 1997 HFMD outbreak in Malaysia, adenovirus was also isolated[11,34], while in 2000, echovirus 7 was isolated in severe cases.[37] Nonetheless, it has also been recently reported that dual infection with other viruses (such as other enteroviruses or adenovirus) in Sarawak did not increase the risk of neurological complications.[58] It is likely that co-infection of EV-71 with other HEV-A provides opportunity for recombination.

Host factors

Age

Based on our laboratory records of confirmed HFMD cases from 1997-2007, the ages of children infected with EV-71 and CV-A16 were tabulated. Of the 145 confirmed EV-71 cases from 1997-2007, 9% were aged < 1, 65.5% were aged 1-3, 18.6% were aged 4-6 and 5.5% were aged 7-12. Of the 32 confirmed CV-A16 cases, 9.4% were aged <1, 71.9% were aged 1-3 and 18.8% were aged 4-6.

Many studies have showed that children below four years are the most susceptible to infection, and are summarized in Table 1. In Singapore, <1% of children of 6-23 months of age have neutralizing antibodies against EV-71, but by the age of five, the rate was >50%.[73] In Taiwan, seroprevalence of EV-71 in children aged 0.5-2 years was only 4%, but in children above six years and adults, seroprevalence was 57-67%.[54] In Brazil, about 85% of patients aged 0-3 years had no neutralizing antibodies against EV-71, but by the age of 12-15, >70% had neutralizing antibodies.[72] Another recent Taiwan study has shown that maternal neutralizing antibodies are present in about 50% of neonates, but are undetectable by 6 months of age.[71] Taking together all these findings, children between 6 months and 3 years are at highest risk of acquiring EV-71 infection due to lack of protective immunity. In addition, Chang *et al*. (2002)[54] also showed that more severe and fatal EV-71 cases occurred in this age group.

Table 1: Seroprevalence studies for EV-71 from different countries

Study population	Country	Prevalence of neutralizing antibodies		References
		Age group	Percentage (%)	
696 individuals aged 1- > 60	Germany	1-4	12	69
		5-9	49	
436 healthy individuals aged 10 months-75	Germany	0-3	27.3	70
		3-6	45.6	
		6-10	56.4	
		10-15	67.2	
		> 20	75	
459 pregnant women and their neonates	Taiwan	Adults (pregnant mother)	63	71
		< 6 month	51	
539 people (before the 1998 epidemic)	Taiwan	< 0.6	36	54
		0.5-0.9	4	
		1-1.9	4	
		2-2.9	22	
		3-5.9	36	
		6-11.9	63	
		12-19	66	
238 patients aged <15 years with symptoms of fever and exanthema	Brazil	0-3	14.8	72
		12-15	69.2	
856 children <12 years old	Singapore	1-23 months	0.8	73
		2-5	12	
		> 5	50	
4619 donors	Taiwan	0.5-3	4-26	74
		3-12	26-50	
		Adults	>50	
900 healthy children $\leq$ 5 years old	China	$\leq$ 5	32	75

Cross-protection

Cross-protection, or cross-neutralization, occurs when the host immunity induced by a virus also confers protective immunity against a different strain or closely-related virus. Generally, all enteroviruses are distinct serotypes but some enteroviruses such as CV-A3 and CV-A8, CV-A11 and CV-A15 and CV-A13 and CV-A18 have been shown to cross-neutralize.[4] However, with regards to the common HFMD viruses, previous CV-A16 infection will not provide cross-protection to subsequent EV-71 infection and vice-versa. The issue of whether cross-protection occurs amongst the different subgenotypes of EV-71 despite its high genetic variation is important, as it has implications for developing an effective vaccine. Kung *et al*. (2007)[76] demonstrated a high degree of cross-neutralization among EV-71 isolates of different subgenotypes of B and C. However Van Sanden *et al*. (2010)[77] has shown that antisera derived from genotype B2 poorly neutralized virus strains from genotype C. Further study is needed to resolve this question.

Antigenic variation in subgenotype B5 viruses did not fully protect the Taiwan population that had prior exposure to genotypes C and B4 and resulted in the Taiwan 2008 outbreak, the largest outbreak since 1998.[60]

Genetic factors

Genetic susceptibility to EV-71 infection has not been studied in detail. HLA-33 was strongly associated with EV-71 infection, while HLA-A2 was found more frequently in EV-71 cardiopulmonary failure patients.[78] As HLA-33 is an HLA type commonly seen in 17-35% of Asian populations, including Taiwan and Malaysia, and seen in <1% of white populations, it was suggested that this may explain why EV-71 outbreaks occur more frequently in Asian countries.[78] Glucose 6-phosphate deficiency has also been shown to enhance EV-71 infection *in vitro*, but remains to be determined in human subjects.[79]

CLINICAL AND LABORATORY BIOMARKERS

Reported clinical and laboratory markers of severe EV-71 infection are shown in Table 2. Increase of leukocyte counts in blood and CSF were noted in patients with pulmonary edema and encephalitis (Table 2). Generally, a poor cellular immunity response has been correlated with complicated EV-71 infection. Some studies have also reported the increase or decrease of cellular markers such as interleukins, however, most involved small number of patients with preliminary data and no conclusive findings were noted. Cardiac troponin 1 has been shown to be an early indicator of fatal EV-71 infection.[87,89] Understanding these risk factors will aid in the management of HFMD patients by allowing identification of those at risk of progression to severe neurological disease.

LABORATORY DIAGNOSIS

During a HFMD outbreak, rapid diagnosis is important to identify the causative agent and implement proper control measures. The method of choice must be able to differentiate between EV-71 and CV-A16, because the latter usually causes uncomplicated HFMD. In addition, it is also imperative to closely monitor the emergence of new subgenotypes which may predispose to neurological complications and deaths.

Choice of specimens

For diagnosis of EV-71, clinical specimens can include throat swabs, ulcer swabs, vesicle swabs/fluid, cerebrospinal fluid, rectal swabs, stool and brain tissues. Blood is generally not useful, as enteroviruses may only be detected in blood at very early stages of infection.[90] Generally, enteroviruses can be recovered from these sites during the first few days of illness, and from rectal swabs and stool for many weeks afterwards.[4] In our laboratory, EV-71 and CV-A16 were most frequently isolated from vesicle swabs (47/147, 32%) followed by oral swabs (12/55, 21.8%), throat swabs (86/434, 19.8%), stool (34/180, 18.9%), rectal swabs (89/519, 17.2%), ulcer swabs (7/47, 14.9%) and cerebrospinal fluid (7/151, 4.7%). These findings were very similar to the evaluation performed in Sarawak and Taiwan.[91-92] As enterovirus infections are often asymptomatic, the isolation of enteroviruses from non-sterile sites such as throat and stool should be interpreted in the context of full clinical information, especially in the event of unusual or severe clinical disease. Isolation of enteroviruses from sterile sites such as CSF is always significant, but may be difficult to achieve due to lower virus loads.

Laboratory methods

The gold standard of EV-71 diagnosis is virus isolation using mammalian tissue cultures and subsequent confirmation with EV-71 specific monoclonal antibody. Mammalian cell cultures such as Vero (African green monkey kidney), human RD (rhabdomyosarcoma) cells, HeLa (cervical adenocarcinoma) cells and BGMK (buffalo green monkey kidney) support the growth of many enteroviruses.[4] Cytopathic effects can be observed as early as day 2. However, virus isolation is usually technically demanding, tedious, takes days to weeks, and has poor sensitivity. Detection of viral RNA by reverse-transcriptase PCR (RT-PCR) is increasingly becoming the method of choice, as it is rapid and sensitive. Careful interpretation of PCR results is necessary for non-sterile sites, as residual RNA and live viruses can be shed in throat and stool for weeks.[47] As genotyping is now considered an essential epidemiological tool, the right choice of genes for RT-PCR and genotyping is crucial since EV-71 frequently recombines and has high genetic variation. 5'UTR is frequently used for detection of enteroviruses while VP1 is used for genotyping. Recently, 3D has also been proposed for genotyping, as this gene is commonly involved in recombination in enteroviruses.[42,93]

Commercial IgM capture ELISA is available

Table 2: Clinical risk factors and cellular markers of severe EV-71 infection

Study population	Outcome	Risk factors or cellular markers for outcome	Reference
725 children with EV-71 (Sarawak, Malaysia)	CNS disease	Duration of fever ≥ 3 days Peak temperature ≥ 38.5°C Lethargy	38
78 children with EV71 infection (Taiwan)	Meningoencephalitis	Younger children, boys Increased total leukocyte count ↓ CD40-ligand expression in T cells G/G genotype at cytotoxic T lymphocyte antigen-4 (CTLA-4) position 49 exon 1	80
31 children with brainstem encephalitis (Taiwan)	Pulmonary edema	Plasma: ↑ Interferon-γ induced protein 10 (IP-10) ↑ Monocyte chemoattractant protein 1 (MCP-1) ↑ Monokine induced by IFN-γ (MIG) ↑ IL-8 CSF: ↑ Monokine induced by IFN-γ	81
57 children with brainstem encephalitis (Taiwan)	Pulmonary edema	CSF: ↑ IL-1 β ↑ IFN-γ	82
30 children with CNS disease (Taiwan)	Pulmonary edema	↓ interferon-γ ↓ IL-1β ↓ IL-6 ↓ tumor necrosis factor -α ↓ cellular macrophage inflammatory protein-1 α	83
73 EV-71 children with brainstem encephalitis (Taiwan)	Pulmonary edema	↑ leukocytosis ↑ thrombocytosis ↑ plasma IL-10, IL-13, IFN-γ ↓ circulating lymphocyte CD4$^+$ & CD8$^+$ T cells, natural killer cells	84
33 children with CNS infection (Taiwan)	Pulmonary edema	↑ blood IL-6 ↑ TNF-α ↑ IL-1β ↑ white blood cell count ↑ blood glucose	85
154 children with confirmed EV-71 infection	Pulmonary edema	Hyperglycaemia Leucocytosis Limb weakness Tachycardia, tachypnoea & cyanosis 1-3 days after onset	86
27 children with cardiopulmonary failure (Taiwan)	Death	↑ troponin 1 CSF white blood count ≥ 100/μl Initial diastolic pressure ≤ 100 mm Hg	87
138 children with HFMD (Singapore)	Death	↑ white blood cell count Absence of mouth ulcers Vomiting	88
21 children with CNS infection (Taiwan)	Death	↑ Cardiac troponin I	89

for diagnosis of EV-71.[94] This would be useful as most diagnostic laboratories have the capability to perform ELISA, but not virus isolation or PCR. Neutralization assays may be used to detect EV-71 antibodies, but are laborious and used only in research settings.

TREATMENT AND PREVENTION

Management

The majority of EV-71 cases are mild, do not require hospitalization, and can be treated symptomatically. However, the challenge, particularly during an outbreak situation with large numbers of infected children, is to identify the small numbers who are at risk of severe neurological disease. A prospective study in Sarawak identified and validated three risk factors for neurological involvement: fever for >3 days, peak temperature >38.5°C, and lethargy.[38] The stage management approach by Chang *et al*. (2004)[55] has improved the outcome of EV-71 infection in Taiwan. Management of EV-71 infection is mainly supportive, as there are still no effective anti-virals. Patients in stage 2 who demonstrate CNS disease should be hospitalized, and closely monitored. Intravenous immunoglobulin (IVIG) has been used at this stage to reduce acute mortality in Taiwan and Malaysia.[38,95] The mechanism of effect of IVIG is not known, but IVIG reduces plasma levels of cytokines which are raised in patients with EV-71-associated pulmonary edema.[95] Patients in stage 3, with cardiopulmonary failure, require the necessary intensive care support, and may also benefit from milrinone, which has inotropic and vasodilating properties to increase cardiac output.[96] For Stage 4 patients, rehabilitation is required for limb weakness/atrophy and dysphagia, while those with diaphragm dysfunction, apnea or central hypoventilation may require long-term respiratory support.

Developments in anti-virals and vaccines

EV-71 infection is often self-limiting and does not require any medication. However, in the case of neurological complications, anti-viral drugs would be beneficial to improve the outcomes. Many new developments in anti-viral therapy are on-going. Interferons, antibodies, capsid inhibitors, enviroxime-like compounds targeting the 3A and 3C protease inhibitors are potential anti-picornaviral compounds.[97] The capsid inhibitor pleconaril is one of the most promising enteroviral drugs, and is currently being tested in phase 2 clinical trials for viral meningitis. However, its use has not been demonstrated for EV-71.

No anti-viral drug is currently available for EV-71. Nevertheless, there are numerous studies showing *in vitro* effects of many compounds against EV-71. Ribavirin, a nucleoside analogue has been shown to reduce viral load in cell culture and in animals.[98] Lactoferrin has been shown to prevent entry of virus in the host cell by binding to EV-71 capsid VP1, protecting cells against EV-71 infection and mice against EV-71 lethal challenge.[99-100] Other compounds reported with anti-EV-71 activity include the Raf-1 inhibitor, GW5074[101], capsid-binding, pyridyl imidazolidinones[102-103] and the interferon inducer, aloe emodin.[104] In addition, the therapeutic use of small-interfering RNA targeting the capsid and non-structural genes of EV-71 showed promising viral inhibition activity.[105]

A vaccine is highly desirable for EV-71. The conventional formalin-inactivated EV-71[106] and attenuated EV-71 strain[107-108] are potential candidates for EV-71 vaccines. Other newer vaccines include oral vaccine with transgenic tomato[109] and milk of transgenic mice[100], virus-like particles[110], VP1 subunit vaccine[106,111-112] and synthetic peptides targeting VP1.[113]

Prevention and control strategies for EV-71

Hand-washing and environmental cleaning

The lack of proper hygiene and close contact between children facilitates faecal-oral spread. Simple handwashing with soap will remove the virus from hands. The virus is resistant to many disinfectants such as alcohol and phenolic disinfectants. The efficacy of Virkon S, a peroxide-based disinfectant has been tested and a minimum 1% Virkon is sufficient to kill the virus.[51] Diluted chlorine (bleach) has also been recommended for cleaning surfaces and toys in nurseries during outbreaks.

Public health control measures

Adequate surveillance systems should be in place to enable early detection and control of outbreaks. In Malaysia, the statutory notification of HFMD has been in enforcement since 12 October 2006, and HFMD guidelines were released by the Ministry of Health Malaysia in 2007.[114] As previously shown, most of the transmission of EV-71 occurs in childcare settings or between household members.[48,54,73] Under the guidelines,

when two or more cases occur in the same childcare facility, the premise is advised to close for ten days from the date of onset of the last case, and must be thoroughly disinfected before reopening. Infected children should always have minimal contact with other people, particularly other children in the kindergarten or household. Public education, particularly during outbreaks, will also help to alert the public to adopt preventive measures. These include washing hands before preparing food, after going to the toilet, and after handling stool-soiled clothes or diapers, and covering the nose and mouth when coughing or sneezing.

CONCLUSION

EV-71 is an emerging virus with pandemic potential to cause severe neurological disease. Although large outbreaks are mainly seen in Asia, EV-71 disease is also increasingly found in Europe. In the absence of effective anti-virals, a vaccine offers the best strategy for control. However, many questions have not been addressed in vaccine development such as the target vaccination group and possibility of an universal EV-71 vaccine for all subgenotypes. In Malaysia, seroprevalence studies are needed to understand the baseline population immunity, the role of cross-protective antibodies across the different subgenotypes, and reasons for the cyclical outbreaks every 2-3 years. Molecular surveillance at a national level will also help in understanding the viral genetic evolution that leads to recurring outbreaks, and contribute to development of vaccine and anti-virals. Meanwhile, improvement of surveillance and proper risk communication to the public will help keep the highly contagious and potentially life-threatening infection at bay.

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