

1 Introduction and Historical Background

The global polio eradication campaign has eliminated this devastating enteroviral disease from Europe, the Americas, and most of Africa and Asia. However, in the past 15 years, a related virus, human enterovirus 71 (EV71), has emerged across Asia, where it threatens to become the “new polio” [120]. EV71 first appeared in California in the 1960s and subsequently caused sporadic cases or small outbreaks of hand-foot-and-mouth disease (HFMD) [120], neurological disease, or both. In 1997, the virus caused an unexpectedly large and severe outbreak with many fatalities in Sarawak, Malaysia. Since then, countries of the Asia-Pacific region have been hit by regular epidemics of EV71, including an epidemic in Taiwan in 1998, in which millions of people were thought to have been infected, and an outbreak of HFMD in China in 2009, during which nearly 500,000 cases were reported [13, 16, 33, 55, 56, 76, 98, 112, 146, 171, 177]. Although the virus circulates worldwide, the largest outbreaks of disease have so far been largely confined to the Asia-Pacific region, for reasons that are incompletely understood [12, 13, 16, 17, 33, 56, 70, 76, 98, 136, 146, 176, 178]. The neurological manifestations range from aseptic meningitis to acute flaccid paralysis and brainstem encephalitis. They are often accompanied by systemic complications such as severe pulmonary edema and shock, which are thought to be neurogenic in origin [22, 63].

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2 Descriptive Epidemiology

2.1 Initial Epidemiological Observations

Although EV71 was first isolated from the stool of a 9-month-old infant with encephalitis in California in 1969, a retrospective analysis of samples from the Netherlands showed that EV71 was circulating as early as 1963 [128, 147]. Before long small outbreaks of neurological infection including encephalitis and aseptic meningitis attributed to the newly identified neurotrophic virus were reported in New York, Melbourne, and Sweden in the early 1970s [9, 43, 74]. The dermatropic properties of EV71 were first recognized during epidemics of HFMD in Japan in 1973 [54, 67]. In the 1970s, two large and severe EV71 epidemics occurred in Europe. The first was in Bulgaria in 1975, initially attributed to polioviruses because its epidemiological, clinical, and pathological characteristics mimicked those of poliomyelitis [40, 137]. In fact, at the height of the epidemic, nationwide administration of live-attenuated poliovirus vaccine was instituted. Isolation of EV71 later confirmed this virus as the etiological agent in 347 (77 %) of 451 children who presented with nonspecific febrile illness or neurological disease, of whom 44 died. The second major European epidemic occurred in Hungary in 1978, with 1,550 cases (826 aseptic meningitis and 724 encephalitis) and 47 deaths. Unlike the Bulgarian epidemic, the Hungarian one included a small number of patients with HFMD [103].

2.2 EV71 Activity in the Asia-Pacific Region

After the Australian and Japanese EV71 epidemics of the 1970s, further small epidemics and sporadic clusters occurred in Hong Kong (1985) and Australia (1986) [51, 126]. Then in 1997, a large outbreak in Sarawak, Malaysia, heralded the beginning of a series of large outbreaks across the Asia-Pacific region. These continuing epidemics have established EV71 as a major public health threat across the region (Table 12.1) [11, 12, 27, 39, 64, 66, 71, 79, 82, 97, 136, 146, 177, 178].

Table 12.1 EV71 genogroups circulating in Asia-Pacific region between 1973 and 2012 [11, 12, 27, 39, 64, 66, 71, 79, 82, 88, 97, 105, 121, 125, 136, 143, 146, 166, 177, 178]

Countries	1973	1980	1986	1988	1990	1993	1994	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	
Singapore	-	-	-	-	-	-	-	-	B3, B4	B3, B4	B3, B4	<u>B4</u>	B4	C1, B4	B4	-	-	B5	-	<u>B5</u> , C2	-	-	-	-	
Peninsular Malaysia	-	-	-	-	-	-	-	-	<u>B4</u> , C1 ^a , C2	<u>B4</u> , C1	B4, C1	<u>B4</u> , C1	-	-	-	-	<u>B5</u> , C1	-	-	-	-	-	-	-	
Sarawak, Malaysia	-	-	-	-	-	-	-	-	<u>B3</u>	C1	None	<u>B4</u> , C1	None	C1	<u>B5</u> , C1	None	B5	B5	B5	B5	B5	-	-	-	B5
Perth, Australia	-	-	-	-	-	-	-	-	-	-	<u>B3</u> , C2	C1	None	None	-	-	-	-	-	-	-	-	-	-	-
Australia	-	-	-	-	-	-	-	-	-	C2	<u>B3</u> , C2	B4, C1	B4, C1	C1	C1	C4	-	-	-	-	-	-	-	-	-
Japan	B1	-	-	-	-	B2, C1	C3	B3, B4, C2	B3, B4, C2	C2	C2	B4	B4, C2	B4, C2	C4, B5	C4	-	-	-	-	-	-	-	-	-
Taiwan	-	B1	B1	-	-	-	-	-	-	<u>C2</u> , B4 ^a	B4	<u>B4</u>	B4, C4 ^a	B4, C4 ^a	<u>B4</u> , B5 ^a	<u>C4</u> , C5 ^a	<u>C4</u> , C5 ^a	C5	B5, C5	<u>B5</u> , C2 ^a , C4 ^a , C5 ^a	B5	B5	C4	-	-
Korea	-	-	-	-	-	-	-	-	-	-	-	<u>C3</u>	None	None	C4	-	-	-	-	-	-	-	-	-	-
Brunei	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B5	-	-	-	-	-	-	-
Vietnam	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C4
Thailand	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	B5, C1, C2, C4	B5 ^a , C1 ^a , C2 ^a , C4	-	B5	-
China	-	-	-	-	-	-	-	-	-	-	-	C4	C4	C4	C4	C4	-	-	-	-	C4	C4	C4	C4	-
Cambodia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C4
Hong Kong	-	-	-	-	-	-	-	-	-	C2	C4	B3, C1, C4	B4, C4	C1, C2	C4	C4	C4	C4	C4	C2, C4	B5, C2, C4	-	-	-	-

Subgenogroups underlined are those which caused large outbreaks

^aSubgenogroup that were isolated in a smaller number

None: No HEV71 was detected despite active surveillance

- No data

Table 12.2 Clusters and outbreaks of EV71 infection occurring outside the Asia-Pacific region, 1988–2010

Country	Year	Ages	N	Clinical features and comments	Reference
Brazil	1988–1990	Children	90	IgM to EV71 in 20 blood samples of patients with flaccid paralysis	[42]
Finland	1994–2010	Children <11 years	928	Neutralizing antibodies in 1.6 % of serial serum samples	[58]
	1996–2008	Children <6 years	359	PCR positive in 0.3 % of serial stool samples	
Central African Republic	1997–2006	Children	93	EV71 in stool samples of patients with flaccid paralysis	[7]
Canada	1998	Children	20 EV71	Aseptic meningitis; respiratory symptoms	[100]
Brazil	1998–2001	Children ≤15 years	389	Neutralizing antibodies in 222 serum samples	[14]
Kenya	1999–2000	Children	–	–	[15]
France	2000–2009	Mostly children	81 EV71	Neurological disease, HFMD, fever rash, gastrointestinal symptoms, respiratory symptoms, and gingivostomatitis; 2 fatal: cardiorespiratory failure and encephalitis	[141]
Austria	2001–2004	Children	181 with aseptic meningitis	16 with EV71 in stool	[113]
United Kingdom	2001–2006	–	32 EV71	Most with neurological disease or HFMD	[8]
Norway	2002–2003	Children	19 EV71	Asymptomatic	[161]
Denver, USA	2003, 2005	Children: 4 weeks to 9 years	15 EV71	1 fatal	[116]
Denmark	2005–2008	Mostly infants	29 EV71	Aseptic meningitis, gastroenteritis, HFMD	[130]
Netherlands	2007	–	58 EV71	Fever, gastrointestinal, and neurological symptoms	[147]
Greece	2009–2010	Children	6 EV71	Fever, rash, or HFMD	[138]

During the Sarawak outbreak, between May and July 1997, a total of 2,618 HFMD cases and 34 deaths were recorded. EV71 also caused four deaths in peninsular Malaysia and a number of cases of severe neurological disease in Japan [13, 76, 92]. Another large epidemic occurred in Taiwan in 1998 when 405 children were hospitalized for serious neurological complications and 78 died. Epidemiological studies estimated that almost 1.5 million people were infected with the virus [56]. The largest epidemic to date occurred in China in 2008; approximately 490,000 children including 126 deaths were reported nationwide. At the epicenter in Anhui Province, more than 6,000 HFMD cases and 22 deaths were reported [178]. Surveillance improved following designation of HFMD as a notifiable disease in China in May 2008, and regular massive epidemics with alarming number of deaths were recorded. There were 1.1 million cases (353 deaths), 1.8 million cases (905 deaths), 1.6 million cases (509 deaths), and 2.2 million cases (567 deaths) reported in 2009, 2010, 2011, and 2012, respectively (http://www.chinacdc.cn/tjsj/fdcrbbg/index_1.html, accessed 15/7/2013). In addition to these very large outbreaks, many countries, including Sarawak, Taiwan, Singapore, Vietnam, and Japan, have experienced cyclical epidemics at 2- to 3-year intervals [59, 101, 119]. The most recent EV71 epidemic occurred in Cambodia in 2012. The outbreak, first labeled as a mysterious illness by local media, was associated with more than 50 sudden deaths within 24 h of hospitalization in children who were 3 years of age and below [129].

The clinico-epidemiological features of the EV71 epidemics in Asia in recent years differ considerably from earlier epidemics in that in addition to HFMD, aseptic meningitis, and flaccid paralysis, brainstem encephalitis associated with cardiopulmonary dysfunction also occurs, which has been the principal cause of death in most fatal cases in Asia [13, 63, 120, 146, 178]. The affected children have typically presented with a short febrile illness accompanied by subtle neurological signs, following which they develop signs of tachycardia, poor perfusion, and tachypnea, which rapidly develops into acute intractable cardiac impairment and fulminant, often fatal, pulmonary edema or hemorrhage [111]. Diagnostic imaging and autopsy examination indicate that this is associated with encephalitis in the brainstem, especially the medulla, and neurogenic pulmonary edema is thought to be the primary pathogenic process [22, 63, 91, 92]. Such rapidly fatal HFMD was not observed in epidemics caused by EV71 in the 1970s and 1980s, where aseptic meningitis was the predominant clinical manifestation [9, 74].

2.3 EV71 Circulation Outside the Asia-Pacific Region

Outside the Asia-Pacific region, EV71 has continued to circulate at low levels of activity in America, Europe, and Africa, producing sporadic cases or small outbreaks. The outbreaks involve mainly young children who develop aseptic meningitis, hand-foot-and-mouth disease, and other complications (Table 12.2).

Clearly, EV71 has spread across the world. Why the huge epidemics have been confined to the Asia-Pacific region, and whether they might be seen elsewhere in the future is not fully understood, though recent molecular epidemiological work on the evolution of the different genotypes of virus has offered some key insights into this important question.

3 Molecular Epidemiology

3.1 Virus Genogroup, Evolution, and Geographical Distribution

Before 1999, data about the molecular epidemiology of EV71 were sparse. However, systematic laboratory surveillance established in several Asian countries following the first epidemics in the late 1990s has provided invaluable information on the geographical distribution, spread, and evolution of the virus.

The first complete phylogenetic analysis of EV71 based on the structural VP1 gene identified three independent lineages of EV71 and designated them as genogroups A, B, and C [11]. A sequence diversity of at least 15 % in the VP1 gene was used to distinguish genogroups. Genogroup A consists of a single member, the prototype BrCr strain, which was first identified in California in 1970 and until very recently had never been reported outside the USA (see below). The genogroup B viruses, subdivided into subgenogroups B1 and B2 with divergence of 12 % at the nucleotide level, were the predominant circulating strains in the 1970s and 1980s. The genogroup C viruses, also similarly subdivided into C1 and C2, were only identified later in the mid-1980s (Fig. 12.1). A recent phylogenetic analysis suggests that EV71 may have emerged from the genetically closely related coxsackie virus (CV) A16 as recently as 1940 [144].

A number of new subgenogroups within genogroups B and C have emerged in the Asia-Pacific region in recent years. Subgenogroup B3 and B4 viruses are thought to have co-circulated in the region since 1997 [12, 97, 136]. Subgenogroup B5 was first isolated in Sarawak and Japan in 2003 and was responsible for epidemics in Sarawak, Taiwan, and Brunei in 2006 [1, 66, 101, 119]. Except for the major community outbreak in Sydney in 1986, subgenogroup C1 viruses have mostly been isolated from sporadic cases since the mid-1980s, suggesting a low level of endemic circulation across the globe [11, 127]. Subgenogroup C2 viruses were responsible for the large EV71 epidemic in Taiwan in 1998 and the Perth outbreak in 1999 [12, 82, 96, 98]. Subgenogroup C3 was isolated in Japan in 1994 and Korea in 2000 [12, 68, 70]. Since 2000, subgenogroup C4 has been the predominant circulating genogroup in mainland China, through the most recent epidemic in 2008, and has occurred in Japan, Vietnam, and Taiwan [82, 136, 146, 178]. Subgenogroup C5 has been reported from Southern Vietnam and Taiwan [66, 146].

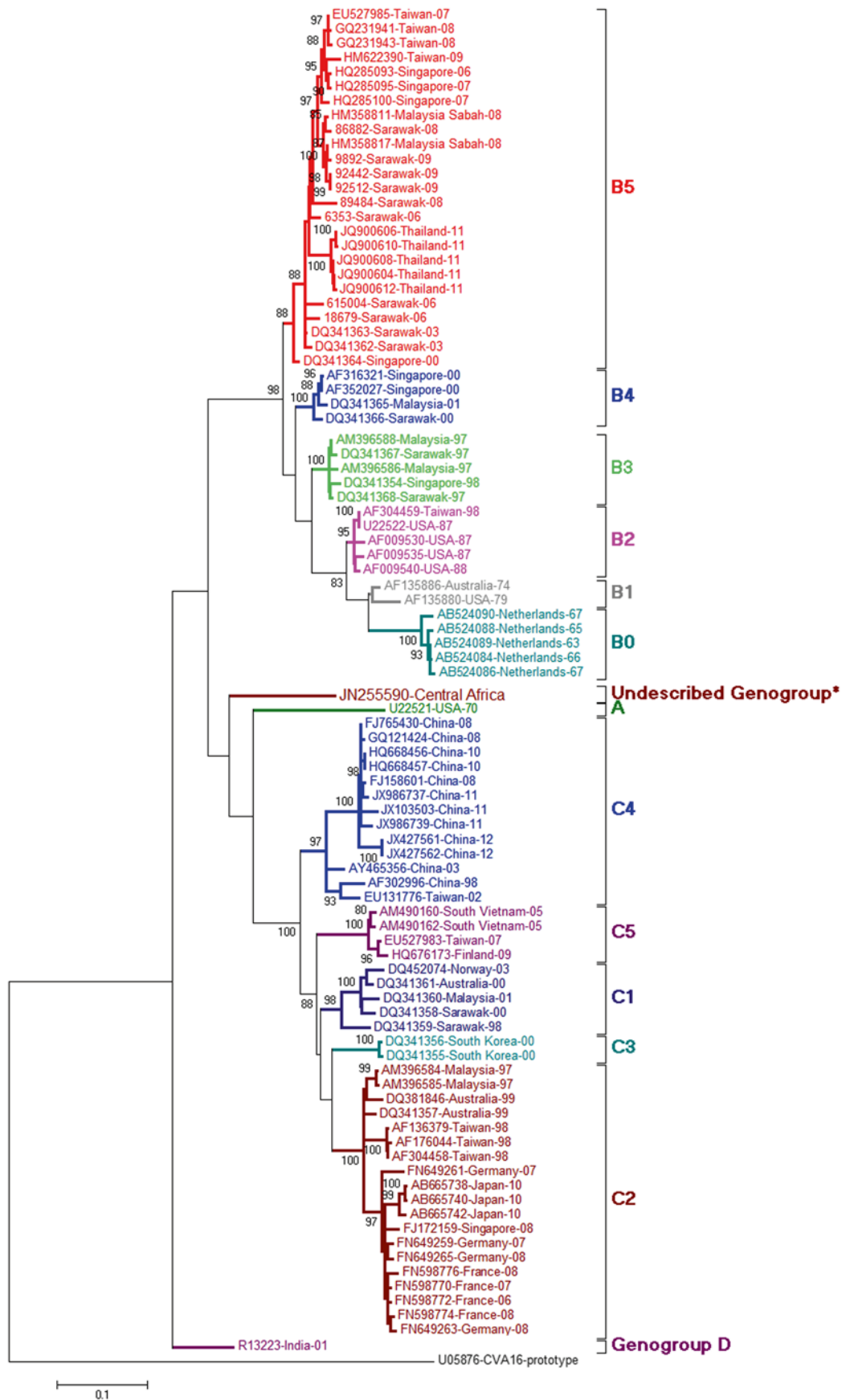
A genetically distinct EV71 strain (R13223, GenBank accession no. AY179600 to AY179602), with no genetic relationship to other strains recently isolated in the Asia-Pacific region, was isolated from a child with acute flaccid paralysis in India in 2001; it has been assigned as the only member of genogroup D [44]. In short, two major EV71 genogroups (B and C) have co-circulated and coevolved into subgenogroups in the Asia-Pacific region over the past 15 years. In Malaysia and Singapore, the genogroup B viruses have dominated, whereas in East Asia, particularly in mainland China and Vietnam, genogroup C viruses have dominated.

Interestingly, EV71 isolates of genogroup A were reported from 5 of 22 children presenting with HFMD in Anhui province of Central China during the 2008 outbreak that was the first ever reported detection of genogroup A viruses since the original prototype was isolated in America [174]. The nucleotide sequence of the complete VP1 gene of the isolates clusters very closely with that of the prototype genogroup A virus, with minimal divergence. This highly unexpected occurrence could indicate that the genogroup A virus has been circulating undetected in central China with very little evolutionary change for four decades or it could raise doubts about the source of the virus templates that were sequenced. Surveillance from the same outbreak by the Chinese Center for Disease Control does not appear to have identified any genogroup A viruses [178]. Hence, it is important to await confirmation by other laboratories before concluding that genogroup A viruses have reemerged. Another genetically distinct EV71 strain (NMA-03-008, GenBank accession no. JN255590) belonged to a previously unknown novel genogroup has been reported in Central African Republic; this indicates the existence of an additional African genogroup, which may have restricted geographical distribution so far [7]. Clearly, good surveillance programs are required in many different geographical regions in order to gather accurate and relevant information about EV71 transmission and evolution.

3.2 Transmission and Epidemic Potential of Genogroups

Following the initial outbreaks in Sarawak and Taiwan in the late 1990s, surveillance systems for EV71 were established in a number of countries in the Asia-Pacific region; while primarily aimed at monitoring transmission and spread, they have also provided invaluable insights into how the virus evolves in the community during outbreaks. For example, a virological surveillance system in Sarawak, Malaysia, has shown that increased EV71 circulation occurred every 3 years (1997, 2000, 2003, 2006, and 2008/09), closely paralleling increased reports of HFMD in the community [119]. The phenomenon of regular cyclical epidemics has also been observed in Fukushima Prefecture, Japan [59]. Such cyclical activity is presumed to relate to the availability of new birth cohorts of

Fig. 12.1 Phylogenetic analysis of EV71 VP1 gene sequences. A neighbor-joining tree constructed using the Kimura-2 parameter as a model for nucleotide substitution. The robustness of the tree was determined by bootstrapping using 1,000 pseudoreplicates. Sequences are labeled according to the following convention: “GenBank accession number” – “Country of origin” – “Year of isolation.” The scale bar represents nucleotide changes per site per year



susceptible children who have not been exposed to the virus during the earlier epidemics [23, 89]. Trying to make predictions about the epidemic potential of specific subgenogroups has proved difficult, beyond the observation that some subgenogroups, such as C1, appear to be less virulent and cause endemic disease rather than large epidemics, whereas other subgenogroups, particularly those that appear to have emerged in Asia in recent years, are associated with large epidemics.

More than one subgenogroup may co-circulate, and a shift from one dominant genogroup to another was described during outbreaks in Sarawak and Vietnam [12, 119, 146]. In Japan and Taiwan, subgenogroup B and C viruses have caused epidemics at different times (Table 12.1) [66, 82, 149]. On the contrary in the Netherlands, a permanent shift appears to have occurred, from genogroup B viruses prior to 1986 to genogroup C viruses since 1987; cross-neutralization among the genogroup B viruses, but not with genogroup C viruses, is postulated as one explanation for this, although other experimental cross-neutralization data did not support it [77, 102, 147, 148]. While the older subgenogroups of virus have been circulating and causing low levels of disease for many years, some of the more recently evolved subgenogroups such as genogroup B5, which possess distinctive antigenicity from other viruses may have the potential to cause massive epidemics [64, 148]. Although these have been confined to the Asia-Pacific region so far, the fact that humans are the natural reservoir for the virus and that international air travel is increasing means that every region is at risk of EV71 epidemic.

4 Biological Characteristics

EV71 is a small, non-enveloped virus with a positive-stranded RNA genome about 7.4 kb in size [169]. The genome has a single open reading frame encoding a polyprotein that is cleaved into 11 proteins: the four capsid proteins (P1 – VP1, VP2, VP3, VP4) and seven nonstructural proteins (P2 – 2A, 2B, 2C; P3 – 3A, 3B, 3C, 3D) [99]. The VP1 open reading frame has considerable genetic variability, which confers antigenic variability and enables investigators to differentiate EV71 strains [53, 100]. VP1 is also important for attachment to cellular receptors and for viral virulence [169]. Other details regarding the biological characteristics of EV71 were provided earlier in the molecular epidemiology section; additional general information regarding the characteristics of enteroviruses can be found in Chap. 11.

5 Clinical Features

EV71 infection may present with a wide spectrum of clinical features, although central nervous system (CNS) infection and HFMD are the two most commonly recognized disease manifestations [114].



Fig. 12.2 Mucocutaneous lesions in hand-foot-and-mouth disease. This Malaysian child has ulcers that are seen inside the upper lip (*top*) and has vesicular and macular lesions on the wrists (*middle*) and soles (*bottom*) (Photos from T. Solomon) (Reproduced from Ooi et al. [111])

5.1 Mucocutaneous Manifestations

HFMD is a common childhood exanthema characterized by a short, usually mild, febrile illness with papulovesicular rashes over the palms and soles and multiple mouth ulcers (Fig. 12.2).

Table 12.3 Neurological syndromes associated with EV71 infection

<i>Purely neurological manifestations</i>	
Encephalitis, especially brainstem	Common
Acute flaccid paralysis (anterior myelitis)	Common
Encephalomyelitis	Common
Aseptic meningitis	Very common
Cerebellar ataxia	Uncommon
Transverse myelitis	Rare
<i>Neurological plus systemic manifestations</i>	
Brainstem encephalitis with cardiorespiratory failure	Common
<i>Manifestations indicative of immune-mediated (para-infectious) mechanisms</i>	
Guillain-Barré syndrome	Uncommon
Acute disseminated encephalomyelitis	Rare
Opsoclonus-myoclonus syndrome	Rare
Benign intracranial hypertension	Rare

Modified from McMinn [96]

Herpangina, a closely related childhood enanthema, is characterized by a febrile illness associated with multiple mouth ulcers at the anterior pharyngeal folds, uvula, tonsils, and soft palate. Although the classical HFMD picture is typically seen in older children with EV71, widespread and atypical rashes may occur in children aged 2 years and below. Besides EV71, another picornavirus, CVA16, is also a principal causative agent of HFMD in both sporadic and epidemic forms. The virus is not normally associated with neurological complication [114], but the rash it causes is indistinguishable from that caused by EV71. There may be other clues that HFMD is due to EV71 rather than CVA16; for example, studies from Sarawak and Taiwan show that children with EV71 are more likely to have a longer duration of fever (≥ 3 days), a higher peak temperature (≥ 38.5 °C), lethargy, and myoclonus [108, 152]. A household contact study in Taiwan during the 2001–2002 epidemic showed that, in addition to HFMD and herpangina, there is a broad range of mild clinical manifestations, including upper respiratory tract infection, gastroenteritis, and nonspecific rashes [24]. Other respiratory manifestations in young children include acute exacerbation of bronchial asthma, bronchiolitis, and pneumonia [100]. More than 20 % of adult contacts during one Taiwanese outbreak had symptoms of an upper respiratory tract infection, while asymptomatic infection occurred in more than 50 %, indicating that adults may be important source of infection for younger children [24].

5.2 Neurological, Respiratory, and Systemic Manifestations

Similar to other enteroviruses, EV71 can cause aseptic meningitis, acute flaccid paralysis, encephalitis, and other rarer manifestations (Table 12.3) [96].

Encephalitis typically affects the brainstem, and unlike most other enteroviruses, it is often accompanied by marked

cardiorespiratory dysfunction. This feature is also seen in poliomyelitis and has been attributed to neurogenic pulmonary edema [6], although the mechanism of disease remains controversial.

During a 7-year prospective clinical study of HFMD in Sarawak that covered several epidemics, 10–30 % of hospitalized children with HFMD due to EV71 had CNS complications [108, 112]. Brainstem encephalitis was the most common presentation, accounting for about 60 % of neurological syndromes, followed by aseptic meningitis (36 %) and brainstem encephalitis with cardiorespiratory dysfunction (4 %). Most children with CNS disease will also have features of HFMD; however, a small proportion may present with neurological disease only. Myoclonic jerks are seen more often with EV71 than with other enterovirus infections. This sign may be an early indicator of neurological involvement, particularly in the brainstem [90]. However, it is not pathognomonic for enterovirus infection; myoclonus has also been reported in other virus infections of the CNS including Japanese B encephalitis, subacute sclerosing panencephalitis, and Nipah virus, herpes simplex virus, varicella-zoster virus, and HIV infection. Myoclonic jerks are also often seen in otherwise healthy young infants, particularly when they are asleep and may occur spontaneously or be provoked by a loud noise.

Seizures, if they occur at all in EV71 infection, tend to be seen in younger children and are short-lived with rapid recovery of consciousness, suggesting that they are febrile convulsions, rather than due to CNS infection itself. Unlike those seen in other viral encephalitides, recurrent and prolonged seizures are very rare with EV71 infection, a distinction probably reflecting predominantly brainstem rather than cortical involvement.

Brainstem encephalitis with associated pulmonary edema has been the hallmark of EV71 CNS infection in Asia since the late 1990s. This distinctive clinical syndrome has a stereotypic clinical course characterized by a

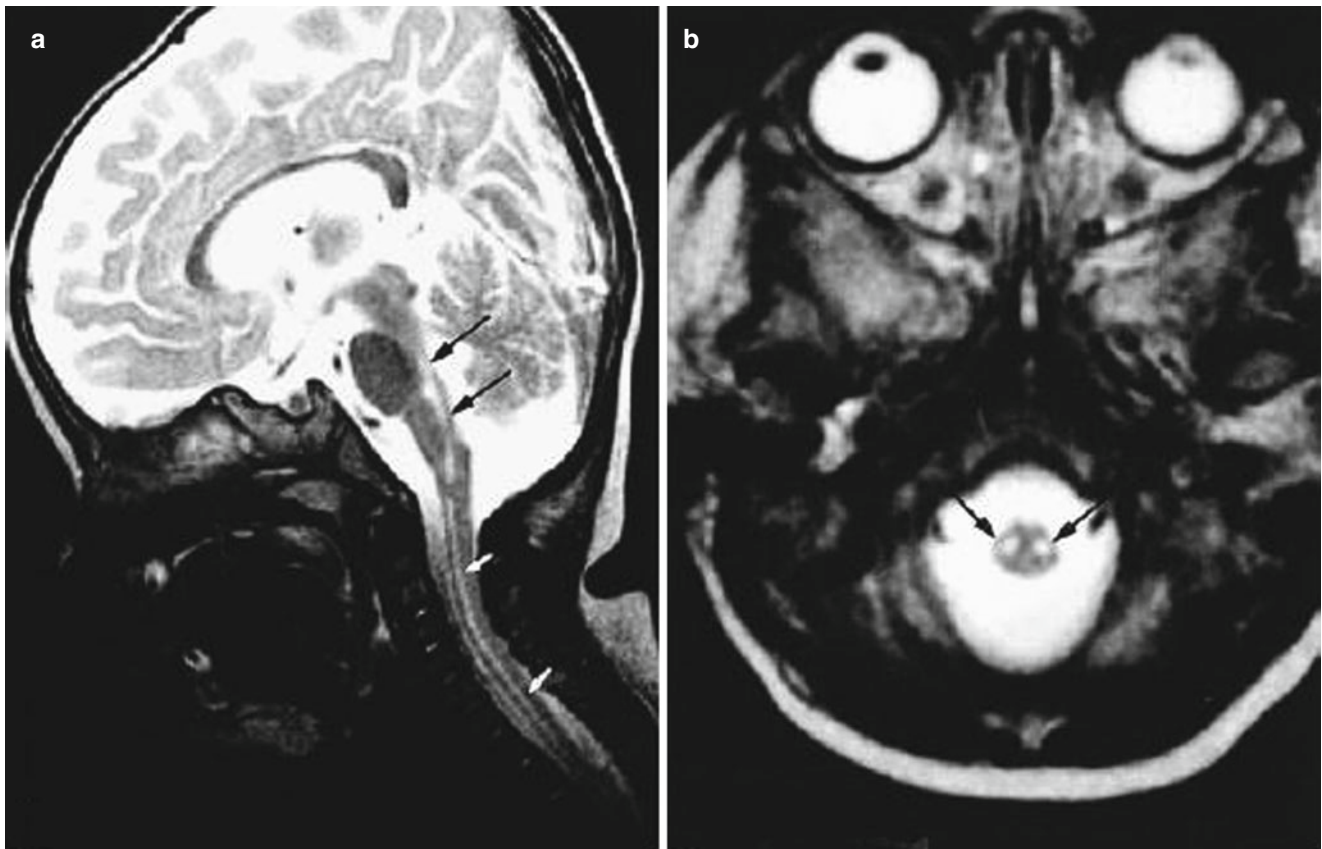


Fig. 12.3 MRI changes in EV71 encephalomyelitis (Modified from Shen et al. [131]). T2-weighted images of a 10-month-old female who presented 3 months earlier with somnolence tachycardia, tachypnea, and coma and who recovered consciousness but remained ventilator

dependent. (a) Sagittal section showing high signal intensity in the posterior portion of the pons and medulla (*black arrows*) and anterior cervical cord (*white arrows*). (b) Axial section showing the high signal intensity in the two anterior horns of the cervical cords (*black arrows*)

prodromal illness of HFMD followed by a sudden deterioration that typically occurs after 3–5 days of fever. Children then develop acute rapidly progressing cardiorespiratory failure presenting as shock and pulmonary edema or hemorrhages. Without critical care support, most of these children die within 24 h of hospital admission and in some even before arriving at the hospital. A system of clinical staging (stage 1 through stage 4) has been used to help monitor the progress of the affected children during the clinical course of EV71 infection and to guide management, from uncomplicated febrile illness to CNS involvement to cardiorespiratory failure and development of neurological sequelae [20, 85, 86]. Such staging systems have not been adopted widely, possibly because they are not always easy to remember and they imply sequential progression through stages that do not always occur. In 2010, a WHO Informal Consultation on Hand Foot and Mouth Disease proposed the use of a simple clinical description of disease manifestation to assess the disease severity. It included uncomplicated HFMD/herpangina, HFMD with CNS involvement,

HFMD with autonomic system dysregulation, and HFMD with cardiopulmonary failure [160].

Findings on magnetic resonance imaging (MRI) of children with brainstem encephalitis correlated well with those of autopsy examination; both procedures demonstrate frequent involvement of the medulla oblongata, reticular formation, pons, and midbrain in several studies (Fig. 12.3) [29, 131, 162].

Acute flaccid paralysis is the primary presenting feature of a number of neurological syndromes caused by EV71 including poliomyelitis-like paralysis, Guillain-Barré syndrome, and transverse myelitis. Poliomyelitis-like paralysis is probably the most common of these, though it may be less severe than that caused by polioviruses, with a higher recovery rate [96]. Other respiratory manifestations in young children include acute exacerbation of bronchial asthma, bronchiolitis, and pneumonia [100]. More than 20 % of adult contacts during one Taiwanese outbreak had symptoms of an upper respiratory tract infection, while asymptomatic infection occurred in more than 50 %, indicating that adults may be important source of infection for younger children [24].

6 Clinical Management

During outbreaks of EV71, tens of thousands of children develop symptoms, and while most of them have mild self-limiting illness, in a small proportion of apparently well children, the condition can rapidly deteriorate to severe and fatal neurological and systemic complications over days or even hours. Whereas in the past children with mild HFMD tended to be managed at home, with increasing parental awareness about the risk of fatal complications, many are now brought directly to hospital, and health services can easily become overwhelmed. The challenges faced by primary care clinicians are recognizing which patients are likely to deteriorate, knowing which investigations yield the best diagnostic information, and deciding which treatments might be appropriate, without the benefit of guidance from controlled clinical trials.

6.1 Laboratory Tests

In mild disease, the blood count is usually normal, but in severe disease, the white blood cell count is often high with a neutrophilia [25]. Blood urea and electrolytes are typically unaffected, but there may be hyperglycemia in severe disease [25]. Creatine kinase is sometimes elevated in patients with cardiac involvement [46] and elevated cardiac troponin I has been reported as a predictor of imminent cardiopulmonary failure in children with brainstem encephalitis [65]. Chest X-ray characteristically shows a normal heart size, even in the presence of marked pulmonary congestion, indicating that neither acute viral myocarditis nor congenital heart disease is causing the illness. There are often nonspecific ECG changes [46], and continuous monitoring may show abnormal beat-to-beat variability, which may predict imminent cardiovascular collapse [83]. Echocardiography shows generalized left ventricular hypokinesia, occasionally accompanied by mitral regurgitation, in children who are hemodynamically unstable with tachycardia, hypotension, or pulmonary edema [46]. Pericardial effusion is rare.

Lumbar puncture is essential in children who are unwell with suspected CNS involvement. In some patients, the clinical features, such as meningismus or myoclonic jerks, may clearly point to CNS involvement. However in other children particularly those younger than 2 years of age, there may just be high fever, vomiting or lethargy, but a lumbar puncture reveals CNS disease. There is typically a mild CSF lymphocytic pleocytosis of 10–100 cells per mm³, but not always [116]. The CSF-to-plasma glucose ratio is usually normal but it can be low.

6.2 Virological Diagnosis

Laboratory diagnosis of EV71 is established primarily through virus isolation or molecular detection of the virus nucleic acid in appropriate clinical specimens.

6.2.1 Choice of Sample

A wide range of samples may be available, depending on the disease manifestations; these include throat and rectal swabs, serum, urine, and, when taken, cerebrospinal fluid (CSF), as well as fluid from vesicles and swabs from ulcers, if they are present. The sensitivity, specificity, and usefulness vary according to the sample [109]. In particular, virus detection in sterile sites such as vesicular fluid, CSF, serum, urine, serum, or autopsy material more reliably indicates a causative organism than does detection from non-sterile sites such as throat or rectum, which may indicate coincidental carriage. However, many of the sterile sites only occasionally yield virus. For example, virus is isolated from only 0–5 % of the CSF of patients with neurological disease [25, 40, 51, 67, 74, 103, 108, 112], because, as for poliomyelitis, the viral load in the CSF is very low [48]. The yield for serum is similarly low [112, 159]. Vesicular fluid, when present, is more useful. Although throat and rectal swabs are more likely to have an enterovirus detected, one study from Malaysia found that this was not always the same enterovirus as that isolated from a sterile site: using the isolate from vesicle swabs as a reference, 10 % of positive throat swabs gave a different isolate, and for rectal swabs the figure was 20 % [109]. Presumably, the isolate from the non-sterile site represented coincidental carriage, whereas that from the vesicles was actually pathogenic.

Prolonged viral shedding from the gastrointestinal tract (throat, rectum, or stool) may occur after complete resolution of EV71 infection, as it does for other enteroviruses; a study in Taiwan showed that EV71 may be detected in the throat up to 2 weeks after recovery from HFMD or herpangina; in the stool, it can be detected up to 11 weeks later [41]. During an outbreak, so many samples could potentially be positive that laboratories can soon become overwhelmed. In one study, the most efficient approach was to examine throat swabs for all patients plus swabs either from at least two vesicles when present or from the rectum when vesicles are absent [109].

6.2.2 Virus Isolation, Serotyping, and Nucleic Acid Detection

The gold standard for diagnosis of enterovirus infection is virus isolation. Several human and nonhuman primate cell lines may be used: rhabdomyosarcoma (RD), which is most efficient; human lung fibroblast cells (MRC5); and African green monkey kidney cells (Vero) [114]. In RD cells, a characteristic cytopathic effect is typically observed 7–10 days

after inoculation. However, to improve the yield, blind passage may be necessary before cytopathic effects become apparent. Once a cytopathic effect is observed, the virus is identified by neutralization tests using intersecting pools of type-specific antisera, by EV71-specific antisera, or by an indirect immunofluorescence assay using EV71-specific monoclonal antibodies [114]. More recently, a molecular “serotyping” approach has been devised. It involves amplifying part of the VP1 gene of the cultured virus, using polymerase chain reaction (PCR) and pan-enterovirus EV71-specific primers, and then sequencing the product [104]. To this end, several sets of primers directed at different regions of the VP1 gene of human enterovirus have been developed [10, 104, 115].

EV71-specific primers are now also being used to perform PCR directly on clinical samples. The advantage of this approach over virus culture is that it can provide rapid diagnosis in the midst of explosive EV71 outbreaks where urgent public health intervention is needed. Several sets of real-time RT-PCR protocols directed to detect EV71 and CVA16 in primary clinical samples have been published recently; however, their disadvantage is that the technique detects only the suspected virus for which primers are available, but they will miss any agents that are unexpected or for which no primers have been generated [34, 115, 142, 167]. DNA microarray technology is a powerful, though expensive, new tool designed to detect multiple pathogen targets by hybridization of pathogen-specific probes. Two groups have recently reported using such an approach to distinguish EV71 and CVA16 infection in primary clinical specimens [34, 134].

6.3 Serology

Serological diagnosis of an acute virus infection is classically established by demonstrating a fourfold rise in specific neutralizing antibody between the acute and convalescent samples [114]. However, in the case of EV71, very high levels of neutralizing antibodies are often detectable within the first few days of illness, and thus a fourfold rise cannot be demonstrated [40, 103]. Furthermore, although homologous antibody is produced when young children encounter their first enterovirus infection, heterologous cross-reacting IgG and IgM antibodies are produced by older children and adults following repeated infection with different enterovirus serotypes; this reduces their diagnostic usefulness [87]. Several rapid IgM ELISA tests for EV71 have recently been developed to try to overcome some of these limitations [153]; however, cross-reactivity remains an issue [168], and the duration of detectable EV71-specific IgM following an infection is also uncertain.

6.4 Diagnostic Imaging

Computer tomography scans are almost always normal in EV71 encephalitis, where the pathology is mostly in the brainstem. Conventional MRI may be normal in the early phase of EV71 encephalitis. Conversely, it may show characteristic high signal intensities on T2-weighted and fluid-attenuation inversion recovery (FLAIR) images in the dorsal pons and medulla, most of the midbrain, and the dentate nuclei of the cerebellum. Similar high-signal lesions may also be found in the anterior horn cells of cervical spinal cord (Fig. 12.3) [63, 131, 175]. Gadolinium-enhanced MRI examination improves the results [175]. Diffuse-weighted imaging (DWI), a sensitive tool for detecting of early changes in brain cellular function, seems to be better at detecting EV71 encephalitis than conventional MRI [81]. However, the value of MRI screening has yet to be demonstrated. In children with acute flaccid paralysis, MRI typically shows unilateral high-signal lesions in the anterior horn cells of spinal cord on T2-weighted images and contrast-enhancing ventral root on T1-weighted images [28, 63, 132].

6.5 Predictors of Severe Disease

Several clinical features and laboratory abnormalities have been associated with neurological and fatal EV71 disease, but few have been prospectively validated as prognostic indicators [25, 60, 65]. Younger age is associated with increased risk of severe disease [21]. A prospective clinical study of nearly 1,500 children presenting to one hospital in three EV71 outbreaks in Sarawak over 7 years suggested that peak temperature of 38.5 °C or higher, duration of fever for 3 or more days, and a history of lethargy were useful clinical predictors for neurological involvement. The presence of at least two of those three factors was strongly associated with the subsequent development of neurological disease [108]. The study corroborated the findings from early retrospective studies. However, it did not confirm other findings from earlier studies, such as the association between the absence of mouth ulcers and development of complicated or fatal disease [38, 112]. Hyperglycemia and leukocytosis have also been associated with fatal EV71 disease in a retrospective evaluation [25], and although these findings were confirmed in the prospective study, because they were late changes occurring about the same time as the fulminant disease, they were not helpful clinically in identifying children at high risk of complications and death [108].

Not all children with CNS involvement in EV71 infection will progress to cardiorespiratory collapse. A recent study in Southern Vietnam revealed that, in general, about 6 % of children with CNS involvement would develop autonomic

system dysregulation. Children with frequent myoclonus observed by doctors and healthcare workers, with or without other focal neurological signs, were at higher risk of disease progression compared with those children with no history of myoclonus (22.2 % vs 4.4 %). A small study involving 46 patients showed that children developed abnormal heart rate variability, an index of autonomic nervous system disease, about 7 h before the clinical onset of cardiorespiratory instability [83]. The authors proposed that screening children with CNS involvement for abnormalities in heart rate variability may provide early warning of imminent cardiorespiratory failure and allow timely institution of appropriate interventions. Cardiac troponin I is a cardiac-specific biomarker for myocardial damage, used for early diagnosis of acute coronary syndrome in adults. Elevated cardiac troponin I has been observed in children with brainstem encephalitis and cardiopulmonary failure [65]; in some cases, it was elevated prior to the development of cardiopulmonary failure, suggesting that serial measurement of troponin may be helpful in identifying children at risk of left ventricular failure. However, neither the evaluation of heart rate variability nor the measurement of troponin I level has become routine clinical practice in the management of EV71 infection, probably because the former requires relatively sophisticated equipment not widely available and the latter is relatively expensive in developing countries. Overall simple clinical parameters such as length of illness, height of fever, and lethargy are probably more useful indicators of potentially severe disease.

6.6 Outcome

Follow-up for as long as 7 years after infection shows that children who present with aseptic meningitis generally have a favorable outcome, although a recent report documented the incidence of attention-deficit/hyperactivity disorder-related symptoms, as reported by parents and teachers, is higher (20 %) in children who had recovered from CNS infection compared with the matched controls (3 %) [49]. Approximately one-fifth of those with more severe neurological disease, including encephalitis, poliomyelitis-like paralysis, and encephalomyelitis, have sequelae, particularly focal limb weakness and atrophy [21, 110, 145]. Cerebellar dysfunction is observed in about 10 % of patients who had moderately severe brainstem encephalitis, including cranial neuropathies, myoclonus, tremor, and ataxia. However, only a quarter of those with more severe brainstem encephalitis associated with fulminant cardiorespiratory failure make a full neurological recovery. Common sequelae include focal limb weakness and atrophy, swallowing difficulties requiring nasogastric feeding, central hypoventilation, facial nerve palsies, seizures, and psychomotor retardation.

7 Pathogenesis

7.1 Viral Determinants of Virulence

The factors that determine whether EV71 infection will be asymptomatic or result in HFMD or severe neurological disease remain unknown. For poliovirus, the 5'UTR and VP1 genes are known to contain virulence determinants [72]. Several studies have therefore examined the nucleotide sequence of these genes, or the whole genome, comparing EV71 isolates from fatal and nonfatal cases, but for the most part the isolates have been nearly or entirely identical, and significant changes have not been found [135, 139]. The incidence of CNS disease and other severe complications of EV71 infection seems to have varied among the recent outbreaks in Asia, leading to the postulation that differences in the virulence of the various genotypes may have a role. However, comparisons between outbreaks have been hampered by the retrospective nature of many of the studies as well as differences in inclusion criteria, definitions of disease severity, and viral diagnostic approaches and capabilities. Perhaps the strongest data supporting the hypothesis that strain virulence determinants play an important role in the pathogenesis of severe neurological disease come from studies in Perth and Sarawak. During the Perth epidemic in 1999, two subgenogroups, B3 and C2, co-circulated, thus providing a unique opportunity to examine the role of virulence determinants in a single epidemic setting [96, 97]. In this outbreak, subgenogroup C2 viruses linked to the Taiwan epidemic of 1998 were almost exclusively isolated from children with severe neurological disease, and only a single isolate came from a case of uncomplicated HFMD. Conversely, subgenogroup B3 viruses, similar to those from the Sarawak 1997 epidemic, were isolated mainly from children with uncomplicated HFMD, aseptic meningitis, or post-infectious neurological disease, none of whom died [98]. A detailed prospective clinical study of EV71 disease in Sarawak, which included 277 children with EV71-associated HFMD, provided further clinical and epidemiological evidence for different biological behavior of EV71 subgenogroups, with regard to risk of CNS disease and transmissibility within families [112]. Two discrete EV71 epidemics, caused predominantly by subgenogroup B3 and B4, respectively, but with small numbers of cases caused by subgenogroup C1, occurred [112]. Children infected with subgenogroup B4 viruses were less likely to present with CNS infection than those infected with C1 or B5 viruses, and they were also less likely to be part of a family cluster. On the other hand, children infected with B5 were more likely to be part of a family cluster, and there was a trend toward a greater incidence of CNS disease in these patients. These results suggest that subgenogroups do indeed vary in their propensity to cause neurological disease.

7.2 Dual Infection

During the first of the Asian EV71 epidemics in Sarawak in 1997, which was due to subgenogroup B3, an adenovirus type 21 was also implicated in the fatal cases as well as in some cases with acute flaccid paralysis [13, 110]. The adenovirus was isolated from sterile sites such as CSF, brain, and heart in fatal cases and indeed was more frequently detected than EV71 itself; this led to the suggestion that the fatalities were due to dual infection, rather than EV71 alone [13]. However, subsequent detailed studies, including longitudinal studies from Sarawak, have not found evidence of adenovirus 21 infection in other HFMD or neurological cases, though dual infection of EV71 with other viruses, including dengue and Japanese B encephalitis, has been found [112]. Furthermore, adenovirus 21 has never been isolated in Sarawak since 1997.

7.3 Host Susceptibility

A range of host factors may affect pathogenesis, particularly partial cross-protective immunity from prior outbreaks. Lack of cross protection may partially explain why young age is a risk factor for severe disease [23, 24, 89]. The potential for host genetic variants to explain differential susceptibility, clinical severity, and outcome of EV71 has also been studied. One study in Taiwan reported that HLA-A33 is associated with increased susceptibility to EV71, although the role of the major histocompatibility complex in the virus infection is still unknown [19]. HLA-A33 is more frequently found in the populations of Asian ancestry than in those of European ancestry, and a causal association of this allele could help explain the higher frequency of EV71 epidemics in Asia. In the same study HLA-A2 was linked to the risk of cardiopulmonary failure often observed in fatal cases [19]. HLA-G, an important immunotolerant molecule, is involved in the suppression of T lymphocytes, NK cells, and antigen-presenting cells and in the induction of regulatory T cells and tolerant dendritic cells. Increased levels of cell surface bound HLA-G and plasma sHLA-G were found in EV71-infected children and in children with pulmonary edema [179]. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an important regulator of T cell cytotoxicity and is involved in the regulation of immune response. Studies of polymorphisms in the gene encoding CTLA-4 in children with meningoencephalitis have yielded conflicting results [19, 173]. Th17 cells are effector cells in human immune response, and its related cytokines, IL-17 and IL-23, are important mediators in pro-inflammatory response. Chinese children with the IL-17 F 7488C allele, which has been associated with a blunted pro-inflammatory response, were more likely to have milder EV71 infection, and patients who were homozygous for the T allele had significantly higher level of C-reactive protein,

leukocytosis, and neutrophil counts when compared with patients with CC+CT genotypes [93]. Another Chinese study showed increased frequency of Th17 cells, as well as elevated serum IL-17 and IL-23, in peripheral blood of children infected with EV71 when compared to the healthy controls [32]. Interferon gamma, a Th1 antiviral cytokine, and IL-10, a potent anti-inflammatory cytokine that suppresses innate host defense, including interferon gamma production, have been implicated in the pathogenesis of severe EV71 infection. Susceptibility to EV71 encephalitis has been reported in children with the IFN-gamma+874 T/A genotype, previously associated with reduced IFN-gamma production, and in children with the IL-10-1082G/A genotype, previously associated with reduced IL-10 production [172]. All of these associations require replication.

7.4 Viral Entry and Spread

EV71 is transmitted predominantly via the feco-oral route, with respiratory spread also implicated [114]. As for other enteroviruses, initial viral replication is presumed to occur in the lymphoid tissues of the oropharyngeal cavity (tonsils) and small bowel (Peyer's patches), with further multiplication in the regional lymph nodes (deep cervical nodes, mesenteric nodes), giving rise to a mild viremia. The majority of infections are controlled at this point and remain asymptomatic. However, *in vivo* studies show that if enteroviruses disseminate further, they reach target organs, particularly the reticuloendothelial system (liver, spleen, bone marrow, and lymph nodes), heart, lung, pancreas, skin, mucous membranes, and central nervous system, coinciding with the onset of clinical features.

The mechanism by which enteroviruses enter the CNS is not completely understood. A number of epidemiological and experimental animal studies on polioviruses indicate that the virus can invade the CNS system by permeation through a disrupted blood-brain barrier or by retrograde axonal spread along cranial or peripheral nerves. For EV71, this latter route has been implicated both in mouse models and by examining the distribution of virus and inflammation in fatal human cases [31, 107, 163].

7.5 Pathological Findings

The topographical distribution of CNS inflammation has been stereotypic and is observed predominantly in the neuronal areas of spinal cord, the entire medulla oblongata except the pyramidal areas, the tegmentum and floor of fourth ventricles in the pons but not the anterior pons, and the whole midbrain sparing the cerebral peduncles. In addition, the hypothalamus, subthalamic and dentate nuclei, and to a lesser degree motor cortex of the cerebrum and meninges are involved (Fig. 12.4) [62, 63, 91, 92, 131, 133, 163].

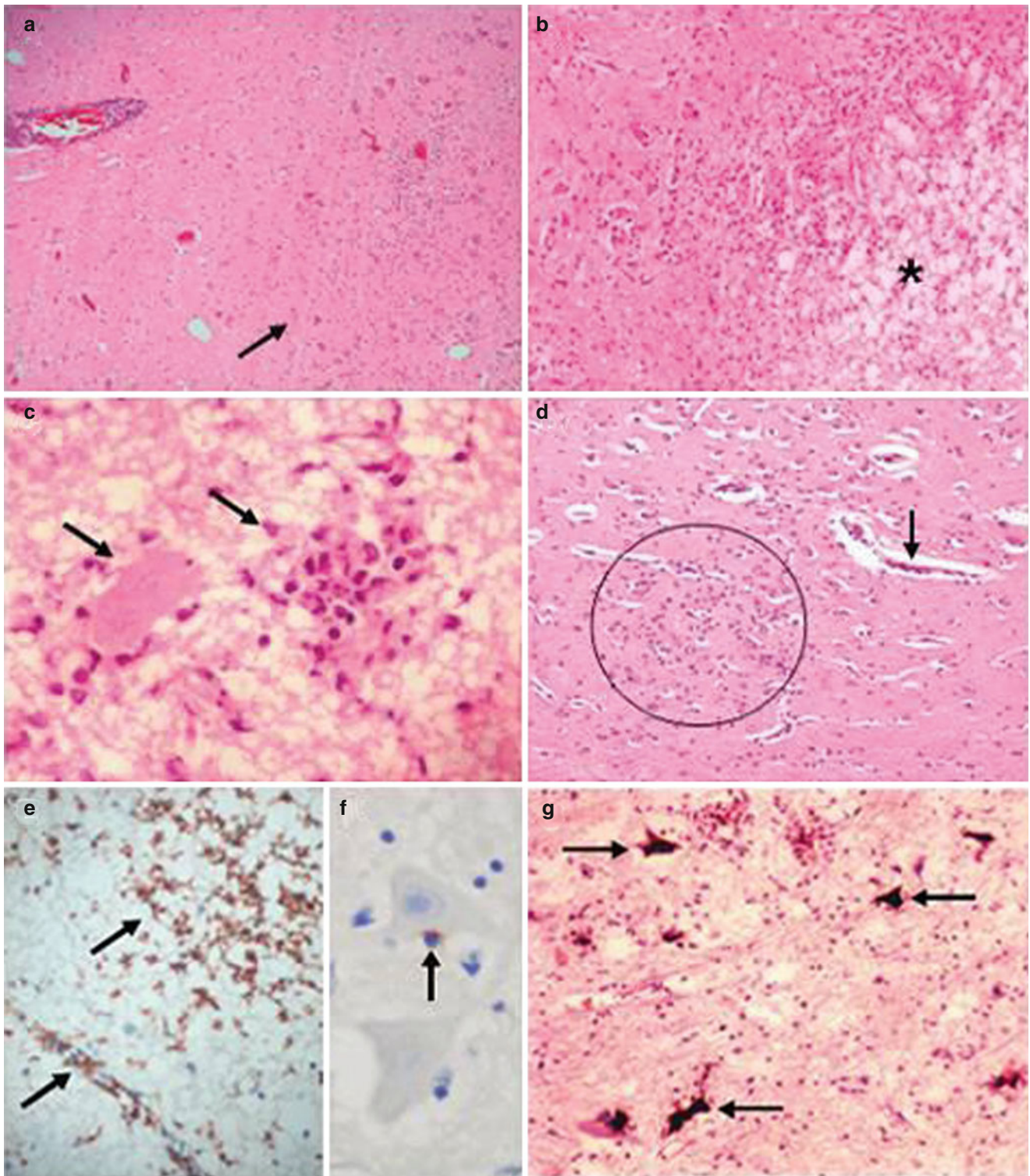


Fig. 12.4 Pathological findings in enterovirus 71 encephalitis (Modified from [163]). Parenchymal inflammation (*arrows*) and perivascular cuffing in the medulla (**a**); more severely inflamed areas (**b**), with edema (*) and neuronophagia (**c**, *arrows*). More subtle inflammation in the motor cortex with mild perivascular cuffing (*arrow*) and parenchymal inflammatory cells (*circle*) (**d**). Numerous CD68-positive

macrophages/microglial (**e**), CD8-positive lymphocyte adjacent to a neuron (**f**). Viral RNA in the anterior horn cells of the spinal cord (**g**). (**a–d**: hematoxylin and eosin stains; **e**, **f**: immunohistochemistry/ peroxidase/DAB; **g**: ISH/nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate stains. Original magnification: (**a**) 4 \times ; (**b**, **d**) 10 \times ; (**c**, **f**) 40 \times ; (**g**) 20 \times

Inflammatory changes were absent in the cerebellar cortex, thalamus, basal ganglia, mammillary body, hippocampus, temporal lobe, peripheral nerve, and autonomic ganglia. The histopathological changes, characterized by perivascular cuffing of macrophages, lymphocytes, neutrophils and plasma cells, variable edema and necrosis, focal neuronophagia, and microglia nodules, are similar to those in encephalitis caused by other viruses [50]. Neither virus inclusion nor vasculitis has been observed, and viral antigens and RNA can only be seen in a small number of neuronal processes and phagocytic cells [163, 164].

7.6 Pathogenesis of Severe Pulmonary Edema and Heart Failure

While it is clear that fulminant pulmonary edema is closely associated with, and preceded by CNS involvement, there is no consensus on its cause, especially the relative contributions of neurogenic pulmonary edema, cardiac dysfunction, increased vascular permeability, and a cytokine storm (Fig. 12.5).

Neurogenic pulmonary edema is classically seen following a head injury, where the associated raised intracranial pressure is thought to be important. Although the pathogenesis is not completely understood, studies from animal models suggest that the hypothalamus, and vasomotor centers of the medulla, and nuclei in the cervical spinal cord are important; lesions to various nuclei in these regions can increase activity along the sympathetic trunk, resulting in profound systemic and pulmonary hypertension and consequent pulmonary edema [57]. Pulmonary edema was also seen in poliomyelitis, and because it was associated with damage to brainstem nuclei, it was thought to be neurogenic in origin [6]. Thus when severe pulmonary edema was first seen in EV71 encephalitis, and brainstem inflammatory changes were observed too, the development of edema was attributed to neurogenic origin. Autopsy examination and magnetic resonance imaging studies of children with EV71 brainstem encephalitis showed that there was extensive inflammation of gray matter of the spinal cord and the whole medulla oblongata, as described above [62, 63, 91, 92, 131, 163]. The observations of hyperglycemia and leukocytosis were also postulated to be due to increased sympathetic discharges [25].

However, detailed hemodynamic observations of children with EV71 and pulmonary edema have not always shown the profound systemic and pulmonary hypertension that would be expected [46, 86, 89, 165]. This may be because the changes in vascular pressures in neurogenic pulmonary edema are only transient, as was shown for one child with EV71 [165]. Others have argued that cardiac impairment is a major contributor to the pulmonary edema. Although there is no histological or virological evidence of a viral myocarditis,

increased cardiac-specific troponin I level suggests some degree of cardiac injury [22, 46, 65, 91, 92]. A detailed echocardiographic study of 11 children with EV71 brainstem encephalitis shows that their cardiac function was impaired, with significantly reduced left ventricular ejection fraction [46]. Two children whose cardiac output was supported with a left ventricular assist device survived, whereas all the others died [45].

Although there is no myocardial inflammation, histological examination of heart ventricular tissue from six fatal cases and one survivor, obtained through a biopsy, revealed significant coagulative myocytolysis, myofibrillar degeneration, and cardiomyocyte apoptosis which are characteristic of catecholamine cardiotoxicity [46, 47]. Thus it is argued by some that the massive release of catecholamine caused by brainstem encephalitis may have a direct effect on cardiac function, as well as causing pulmonary edema through raised pulmonary pressures.

The other potential contributor to pulmonary edema is increased vascular permeability, which is postulated to have occurred following the systemic inflammatory response. Early studies that examined a narrow range of cytokines and chemokines have shown that interleukin (IL)-6, IL-1B, IL-10, IL-13, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ are all significantly higher in EV71 patients with pulmonary edema than in those with uncomplicated encephalitis, and several of these, including IL-1, IL-6, IL-13, and IFN- γ , are known mediators of increased vascular permeability [84–86, 151]. In addition, increased plasma levels of several chemokines including interferon gamma-induced protein (IP-10), monocyte chemoattractant protein (MCP)-1, monokine induced by interferon gamma (MIG), and IL-8 have been found in children with pulmonary edema when compared to those with uncomplicated brainstem encephalitis [156]. A study of 30 cytokine and chemokine levels in EV71-infected Malaysian children confirmed that cardiorespiratory impairment was associated with a widespread elevation of both pro-inflammatory and anti-inflammatory mediators in the serum and CSF. In addition, the study also showed that serum IL-1Ra and G-CSF levels were significantly elevated in patients who died, with a serum G-CSF/IL-5 ratio of >100 being the most accurate prognostic marker [52]. Children with edema also had depleted lymphocyte population particularly in CD4, CD8, and natural killer cells [151, 173]. Thrombocytosis, neutrophilia, and hyperglycemia are all thought to be indicative of a systemic inflammatory response [84, 151]. Fewer studies have looked at cytokines in the CSF. Elevated CSF IL-1b was found in patients with encephalitis complicated by edema when compared to those with encephalitis alone [84]. It has previously been proposed that the CNS may be the source of the inflammatory cytokines detected in the serum of patients with EV71-associated cardiac dysfunction [156]. A study examining the relative abundance of inflammatory mediators in the serum and CSF samples from

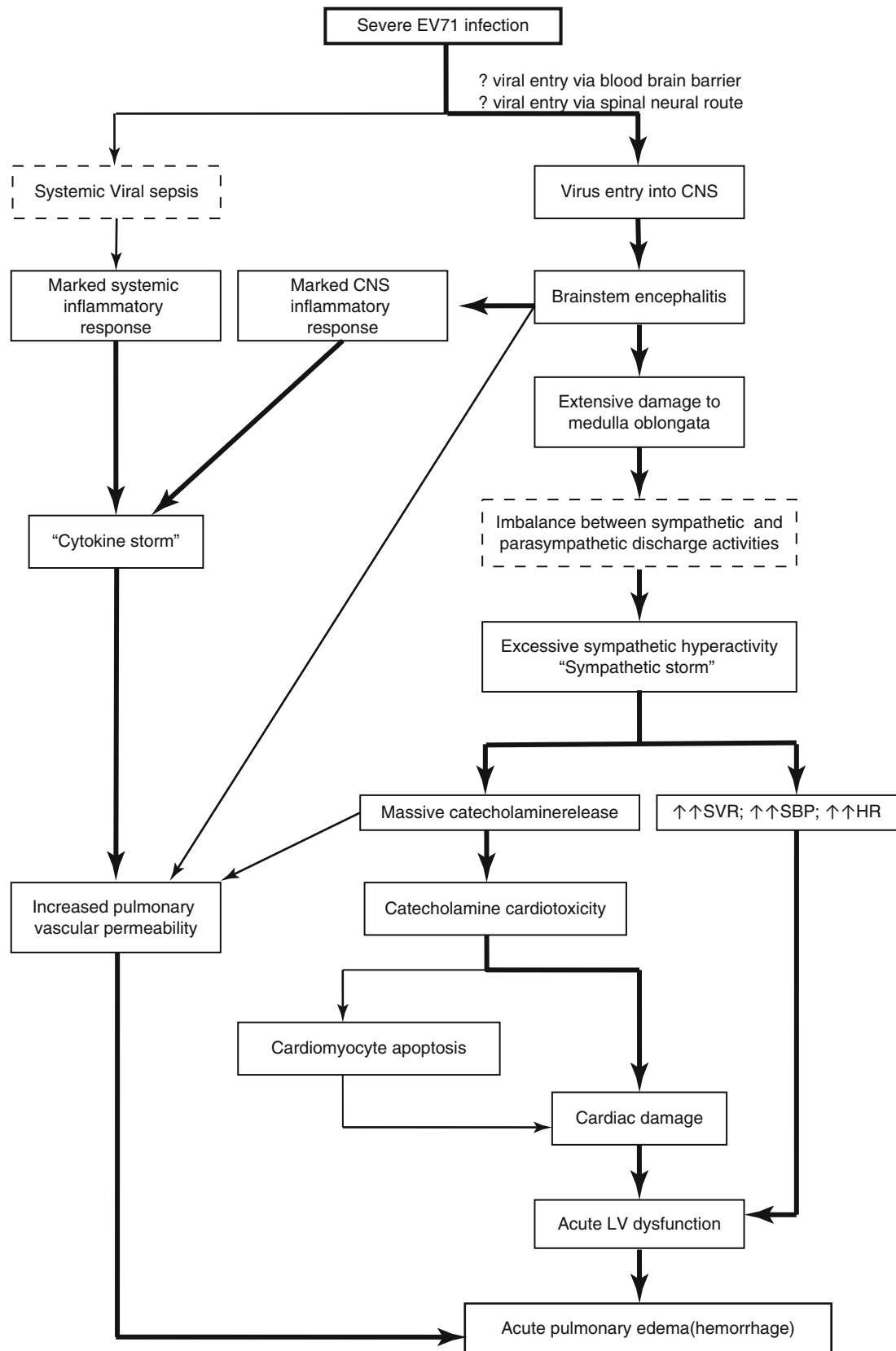


Fig. 12.5 The postulated pathogenesis of enterovirus 71-associated acute pulmonary edema Major postulated pathogenic pathways are shown with thick lines; lesser contributory pathways are shown with thinner lines. The solid boxes indicate strong supporting evidence from human clinical or pathological studies, while the dotted boxes indicate

hypothetical but unproven steps or evidence from animal models only. *EV71* human enterovirus 71, *BBB* blood-brain barrier, *CNS* central nervous system, ↑↑ markedly high, *SVR* systemic vascular resistance, *SBP* systemic blood pressure, *HR* heart rate, *LV* left ventricular (Reproduced from Solomon et al. [140])

88 Malaysian children showed two distinctive immune response patterns occurring independently in these two compartments [52]. Because the development of pulmonary edema in patients with EV71 encephalitis appears to be strongly associated with a dysregulated systemic and CNS inflammatory response, this observation has at least partly formed the basis for treatment with intravenous immunoglobulin, which appears to be effective [20, 85, 86, 108, 112, 154].

In summary, although still ill-defined, brainstem inflammation appears to be an important neurogenic mechanism for pulmonary edema in EV71 encephalitis. However, similar pathological changes are also observed in other encephalitides without pulmonary edema as a prominent feature. Cardiac compromise and the effects of the systemic inflammatory response on the vascular endothelium may also make an important contribution. In vivo mouse and nonhuman primate models have replicated some of the features of severe EV71 disease, such as neuroinvasiveness with marked inflammatory changes; however, no model has yet reproduced the severe systemic features such as pulmonary edema [5, 31, 36, 107, 158, 163].

8 Control and Prevention

8.1 Treatment

8.1.1 Antiviral Agents

There are no established antiviral treatments for EV71. Pleconaril is an antiviral drug that inhibits entry into cells for a number of enteroviruses by blocking viral attachment and uncoating. It has been used in clinical trials of aseptic meningitis [122–124]; however, it is not active against EV71 [35]. Several other newer capsid-function inhibitors have been investigated, and some have demonstrated promising anti-EV71 activities in preclinical studies [35]. Two human transmembrane proteins, P-selectin glycoprotein ligand-1 (PSGL-1) and scavenger receptor class B member 2 (SCARB2), have recently been identified to be functional receptors of EV71 [106, 170]. Analysis of the much-awaited crystal structure of EV71 revealed important structural differences from other enteroviruses. The mature virion of EV71 has a shallower canyon (believed to be the receptor binding site) on the viral surface and a relatively exposed “pocket factor” (which stabilizes the virus) compared to other enteroviruses [118, 157]. These discoveries are major steps forward and will guide rational design of antivirals against EV71.

8.1.2 Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) was used during the initial epidemics of EV71 in Sarawak and Taiwan in the late 1990s on the basis that its anti-EV71 neutralizing antibodies

and nonspecific anti-inflammatory properties might be therapeutic [112, 152]. Retrospective comparisons of patients who received IVIG with those who did not suggested possible benefit from IVIG if given early [20, 108]; for example, in Sarawak over 3 seasons, 204 (95 %) of 215 children who survived despite severe CNS complications had timely IVIG treatment, typically once severe disease occurred, whereas only one (11 %) of nine fatal cases received this treatment (OR 148.36, 95 % CI 16.34–6609.04, $p < 0.0001$) [108]. Proinflammatory cytokines measured before and after IVIG treatment were significantly lower in EV71 patients with autonomic dysfunction than patients with less severe disease [84–86, 151, 154]. IVIG has since become routine treatment for severe EV71 disease, and it has been recommended in the national treatment guidelines in Taiwan and Vietnam [20, 75, 85, 86, 108, 112, 150]. While remaining uncertainty over the efficacy of this treatment warrants randomized placebo-controlled trials, they would be logistically and ethically challenging to conduct in the face of the wide acceptance of IVIG as the current standard of care and the beliefs in their value strongly held by some. Carefully designed phase II trials would need surrogate end points for disease progression (e.g., failure of resolution of tachycardia).

8.1.3 Milrinone

Milrinone is a cyclic nucleotide phosphodiesterase (PDE) inhibitor currently used in the treatment of congestive heart failure. Inhibition of PDE subtype III by milrinone results in an increase in intracellular cyclic adenosine monophosphate (cAMP), which in turn leads to increased cardiac output and decreased peripheral vascular resistance. In a small nonrandomized retrospective comparison involving 24 children with EV71-induced pulmonary edema, those treated with milrinone had reduced tachycardia and lower mortality [150, 155]. Intriguingly, the peripheral white blood cell count, platelet count, and plasma IL-13 were also lower [155], suggesting an immunomodulatory as well as a cardiovascular effect of the drug. More recently a prospective, open-label, randomized controlled trial conducted by the same authors, involving 41 Vietnamese children 5 years of age and below, showed that milrinone significantly reduced the 1-week mortality from EV71-induced cardiovascular collapse without adverse effect. This encouraging finding has tentatively raised hope that earlier milrinone treatment might be useful in halting disease progression of cases with severe brainstem encephalitis [37].

8.1.4 Fluid Balance and Ionotrope Support

Hypovolemia and dehydration are the commonest causes of shock in children and are treated with rapid fluid resuscitation with good results. However, similar approaches used in the early EV71 epidemics in Asia precipitated massive pulmonary edema. Judicious use of intravenous fluid and early

institution of inotropic support are critical in children with severe EV71 infection. Where fluid management could be guided by central venous pressure monitoring, in Taiwan, management algorithms based on this approach appear to have improved outcome [20].

8.1.5 Novel Treatment Approaches

Although better recognition of early signs of CNS involvement and the disease progression has helped improve the clinical outcome, many children continue to succumb to severe EV71 infection because of late presentation or disease progression despite intervention. A left ventricular assist device with extracorporeal membrane oxygenation was associated with a higher survival rate in a small number of recent cases than in historical controls, but with significant neurological sequelae [69]. Hemofiltration has been employed to treat children with cardiovascular collapse in Vietnam [117].

8.2 Prospects for Prevention

8.2.1 Surveillance

The only measures available currently for disease control at the population level are public health approaches. Countries in Asia (Singapore, Taiwan, Japan, and Vietnam) have implemented heightened surveillance for EV71 [2, 3, 33, 101, 119]. HFMD has now become a notifiable disease in many places, including Malaysia, Singapore, Thailand, Taiwan, Vietnam, and China [3, 4]. However, HFMD may be caused by a number of enteroviruses including CVA8, 10, and 16, and concurrent virological surveillance is necessary. Virological surveillance also provides invaluable molecular epidemiological data about the circulating EV71 genotype and may thus help to track the spread of the virus across the region.

Because humans are the only known natural hosts of human enteroviruses, outbreak control measures are targeted primarily at interrupting person-to-person virus transmission via contact with throat and nose secretions, saliva, stool, and vesicular fluid but also at minimizing contact with contaminated surfaces, toys, or fomites. Because of the lack of a lipid envelope, EV71 has considerable stability in the environment. It can remain viable at room temperature for several days and has been recovered from surface and ground water and hot spas [30, 61]. Hence, health education focuses on personal hygiene and good sanitation including frequent hand washing, proper disposal of soiled diapers, and disinfection of soiled surfaces with sodium hypochlorite [73]. Like other enteroviruses, EV71 is resistant to alcohol. Consequently, use of the widely available alcohol-based (70 % ethanol or isopropanol) disinfectants alone for hand hygiene is ineffective in preventing EV71 transmission [26]. A recent study showed that EV71 can be destroyed by virucidal disinfectants such as Virkon [18].

Transmission of enteroviruses including EV71 is most efficient in overcrowded settings, and most countries in the region including Malaysia, Singapore, Taiwan, Hong Kong, and China have adopted “social distancing” measures during epidemics. These measures include closures of childcare facilities and schools and cancellation of public functions involving children [2, 3]. Although there has been little systematic research to examine the effectiveness of public health interventions, a few studies of school closure or other approaches from Singapore and Hong Kong appeared to show some benefit [2, 95]. It nevertheless remains uncertain whether social distancing measures are effective or what the optimal timing for instituting them may be—at the onset of an HFMD outbreak or at the time of laboratory confirmation of an EV71 etiology. Although this widely adopted empirical measure has considerable socioeconomic impact, the cost of the disease and its control measures have not yet been studied. If experience with other directly transmissible viruses applies to EV71, then such measures as school closure could decrease the peak incidence but prolong the outbreak, with no reduction in the overall number of cases (Fig. 12.6) [140].

Further epidemiological work to elucidate the transmission dynamics of the virus will guide the formulation of evidence-based interventions to control the spread of EV71. Critical pieces of information such as precise estimates of the incubation period, ratio of asymptomatic to symptomatic cases, and time and duration of infectiousness are needed. The reproduction number (R_0) for EV71 has been estimated to be higher than that of CVA16 (median 5.48, IQR 4.20–6.51 compared with 2.50 [1.96–3.67], respectively; $p=0.002$) [94].

8.2.2 Vaccine Development

The success story of the control of poliomyelitis indicates that vaccines would be the best approach for future disease control, and the target population should be younger children, especially those less than 3 years of age, because they are at highest risk of severe disease. In fact, an inactivated EV71 whole virus vaccine was developed in Russia in 1976 after the Bulgaria epidemic. But it received no further evaluation because no further outbreaks of EV71 occurred [78]. More recent candidates for an EV71 vaccine include inactivated whole virus, live-attenuated, recombinant viral protein, virus-like particle, and DNA vaccines. These are at different stages of the development in China, Taiwan, and Singapore [78, 176]. Among these, inactivated whole virus vaccine candidates, the development of which modeled that of inactivated polio vaccine, are at the final stages of the clinical evaluation. A phase 3 randomized double-blinded, placebo-controlled trial of inactivated alum-adjuvant EV71 vaccine (Beijing Vigoo Biological, EV71 strain FY7VP5/AH/CHN/2008, genogroup C4) involving 10,245 Chinese children between the

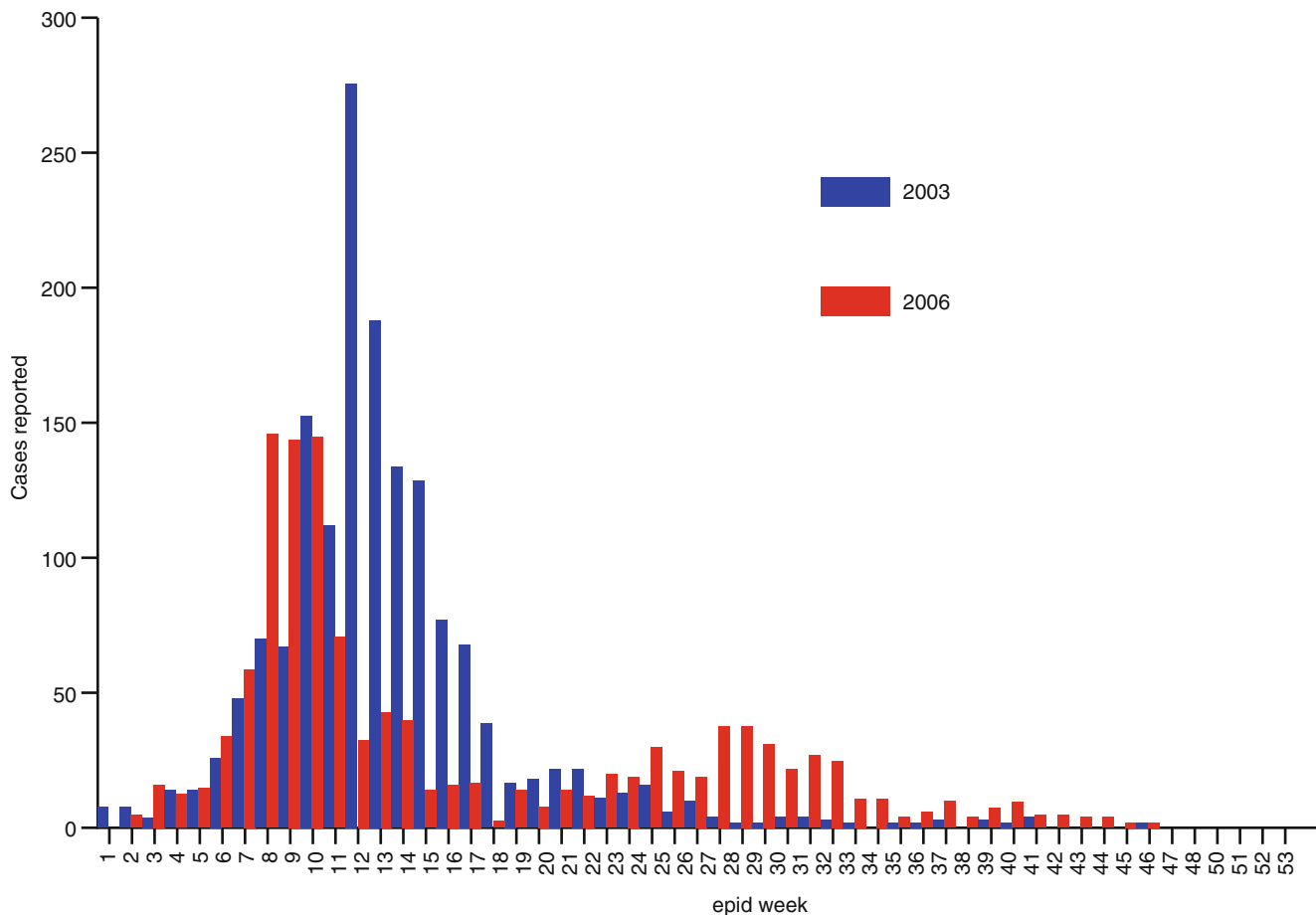


Fig. 12.6 The effect of public health interventions on hand-foot-and-mouth disease outbreaks, comparing sentinel surveillance data from the 2003 outbreak in Sarawak, Malaysia, when the public health

response was limited, and the 2006 outbreak, when more rigorous social distancing measures were encouraged. (Reproduced from Solomon et al. [140])

ages of 6 and 35 months has shown encouraging vaccine efficacy, immunogenicity, and safety [180]. Another alum-adjuvant inactivated whole virus candidate (EV71 strain H07, genogroup C4) produced by Sinovac Biotech Co., LTD) was reported to be well tolerated and highly immunogenic in a phase I trial in infant populations in China [80]. While an eagerly awaited vaccine will likely be available for routine use in the near future, it remains uncertain whether the vaccine developed from a specific genogroup would provide adequate cross protection against all genogroups. This concern is critical because genogroup B and C virus so far appear to have different geographical distributions in Asia, and the data to date on cross protection between genogroups are conflicting. Another important issue pertaining to manufacturing processes of inactivated whole virus vaccine is that no international reference standards for potency assays and quantification of EV71 vaccine antigens exist [78]. Establishment of such standards is urgent in Asia, where the vaccine will likely be used soonest and most extensively.

9 Concluding Remarks and Unsolved Problems

The emergence of EV71 in the Asia-Pacific region over the last 15 years has had a major public health impact. Molecular epidemiological studies suggest that some subgenogroups appear to have massive potential for explosive epidemics, while others circulate in a more indolent pattern. However, the biological determinants of these differences are poorly understood. The epidemiological differences observed between EV71 in the Asia-Pacific region and the strains found in Europe and USA also represent an unsolved puzzle. There are no reliable and easy-to-use clinical tools to predict who will develop neurological complications and which patients with CNS involvement are at risk of disease progression. The virological and host determinants of the wide-ranging clinical phenotypes in those infected remain unclear. There are relatively good animal models of neurological disease caused by EV71, but there is an urgent need for an animal model of cardiorespiratory dysfunction to advance

understanding of its pathogenesis. Despite lack of solid evidence for its efficacy, the wide use of IVIG for severe EV71 infection in many Asian countries will make efficacy trials difficult. There is still no specific antiviral drug available for EV71 infection although the determination of its crystal structure and identification of several EV71 receptors should accelerate drug discovery. An inactivated whole virus vaccine is nearing clinical availability, but important steps must be taken to ensure that it ultimately reaches the populations in greatest need. Public health intervention and control measures of EV71 epidemics so far have been empirical and generic, not stringently evidence based. Because they have significant socioeconomic impact, further research is needed on the transmission dynamics of the virus and which of these public health intervention strategies most effectively limit the havoc wreaked by future EV71 outbreaks.

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Suggested Reading

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