VIROLOGICA SINICA, August 2011, 26 (4): 221-228
DOI 10.1007/s12250-011-3195-8

Hand Foot and Mouth Disease Due to Enterovirus 71 in Malaysia

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Abstract: Hand foot and mouth disease is a febrile sickness complex characterized by cutaneous eruption (exanthem) on the palms and soles with simultaneous occurrence of muco-cutanous vesiculo-ulcerative lesions (enanthem) affecting the mouth. The illness is caused by a number of enteroviruses with coxsackievirus A16 and enterovirus 71 as the main causative agents. Human enterovirus 71 (EV71) belongs to the species *Human enterovirus A* under the genus *Enterovirus* within the family *Picornaviridae*. EV71 has been associated with an array of clinical diseases including hand foot and mouth disease (HFMD), aseptic meningitis, encephalitis and poliomyelitis-like acute flaccid paralysis.

A large outbreak of HFMD due to highly neurovirulent EV71 emerged in Malaysia in 1997, and caused 41 deaths amongst young children. In late 2000, a recurrence of an outbreak of HFMD occurred in Malaysia with 8 fatalities in peninsular Malaysia. Outbreak of HFMD due to EV71 recurred in 2003 with an unknown number of cases and mortalities. A similar outbreak of HFMD with 2 recorded deaths in young children occurred in peninsular Malaysia in late 2005 and this was followed by a larger outbreak in Sarawak (Malaysian Borneo) with 6 reported fatalities in the early part of 2006. The current on-going outbreak of HFMD started in peninsular Malaysia in epidemiological week 12 of 2010. As with other HFMD outbreaks in Malaysia, both EV71 and CA16 were the main aetiological viruses isolated. In similarity with the HFMD outbreak in 2005, the isolation of CA16 preceded the appearance of EV71. Based on the VP1 gene nucleotide sequences, 4 sub-genogroups of EV71 (C1, C2, B3 and B4) co-circulated and caused the outbreak of hand, foot and mouth disease in peninsular Malaysia in 1997. Two sub-genogroups (C1 and B4) were noted to cause the outbreak in 2000 in both peninsular Malaysia and Sarawak. EV71 of sub-genogroup B5 with smaller contribution from sub-genogroup C1 caused the outbreak in 2003. In the 2005 outbreak, besides the EV71 strains of sub-genogroup C1, EV71 strains belonging to sub-genogroup B5 were isolated but formed a cluster which was distinct from the EV71 strains from the sub-genogroup B5 isolated in 2003. The four EV71 strains isolated from clinical specimens of patients with hand, foot and mouth disease in the Sarawak outbreak in early 2006 also belonged to sub-genogroup B5. Phylogenetic analysis of the VP1 gene suggests that the EV71 strains causing the outbreak in Sarawak could have originated from peninsular Malaysia. Epidemiological and molecular data since 1997 show the recurrence of HFMD due to EV71 in Malaysia every 2 to 4 years. In each of the past outbreaks, more than one sub-genogroup of the virus co-circulate.

Key words: Hand foot and mouth disease; Enterovirus 71; Malaysia

Received: 2011-03-31, Accepted: 2011-06-24

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INTRODUCTION

The naming of hand, foot and mouth disease is a clinical description of a disease that reflects the anatomical distribution and nature of lesions affecting parts of human body. It is a febrile sickness complex characterized by cutaneous eruption (exanthem) on the palms and soles with simultaneous occurrence of vesiculo-ulcerative lesions (enanthem) affecting the muco-cutaneous membrane of mouth (buccal mucosa, tongue, gums and palate). Clinically, the onset of HFMD becomes apparent with enanthema of the buccal cavity followed by exanthema. Intra-oral ulcerative lesions accompanied by papulo-vesicular lesions on the ventral surface of fingers, palms and soles are characteristics of fully developed HFMD. The papulo-vesicular eruptions may also affect buttocks, elbows and knees[1, 26]. However, patients do not need to have all the full clinical manifestations for a case to be classified as HFMD. The disease is normally self-limiting unless associated with rare and more serious manifestations such as meningo-encephalitis, myelitis and acute flaccid paralysis, encephalomyelitis and myocarditis. Classically, the disease is associated with infection by a non-polio enterovirus, most notably cosackievirus A16 (CA16) and more recently enterovirus 71 (EV71)[1, 26]. Outbreaks and epidemics of HFMD associated with these two enteroviruses are well documented. Other enteroviruses, such as coxsackievirus A4, A5, A9, A10, B2, B5 and echovirus 7 have also been linked to isolated outbreaks and sporadic cases of HFMD[1, 26]. However, these enteroviruses are not known to cause outbreaks with serious morbidity and mortality rate with sufficient regularity to warrant vigilant surveillance of these infections. Consequently, outbreaks of HFMD often go unnoticed or unrecorded, especially in developing countries where availability of diagnostic reagents is often limited.

Human enterovirus 71 (EV71) belongs to the species *Human enterovirus A* under the genus *Enterovirus* within the family *Picornaviridae*. It is a small non-envelope virus of 28 to 30 nm in diameter with a capsid enclosing a core of a single-stranded, positive-sense RNA approximately 7.5 kilobases in size. The viral capsid is icosahedral in symmetry and composed of 60 identical units (protomers) each consisting of four structural proteins, VP1-VP4. The complete nucleotide sequences of the EV71 prototype strain BrCr and a number of other strains have been determined [5]. The single open reading frame (ORF) encodes a polyprotein of 2194 amino acids and is flanked by an untranslated region (TR) of about 700 nucleotides at the 5' end and a variable length of less than 100 nucleotides with a poly-A tract at the 3' end. The polyprotein is subdivided into three regions, P1, P2 and P3. P1 encodes four viral structural proteins 1A-1D (VP4, VP3, VP2, VP1); P2 and P3 encode seven ono-structural proteins 2A-2C and 3A-3D. The roles and functions of the 11 individual EV71 proteins are thought to be identical to that determined for poliovirus and other non-polio enteroviruses[5, 26]. EV71 has been associated with an array of clinical diseases including hand, foot and mouth disease (HFMD), aseptic meningitis, encephalitis and poliomyelitis-like acute flaccid paralysis. The virus was first isolated from a child with aseptic meningitis in California, USA and subsequently characterized as a new serotype of the genus *Enterovirus*[30]. In the years following its initial isolation, outbreaks of

HFMD with complications due to the virus were described in various parts of the world [3, 11, 13, 19]. The neurovirulence of EV71 gained global attention in an outbreak in Bulgaria which caused 705 cases with CNS involvement and 149 cases of poliomyelitis-like disease with 44 deaths in 1975[14, 31]. A similar outbreak followed in Hungary in 1978 resulted in many cases of poliomyelitis-like disease and 47 deaths[27]. Subsequently, several milder epidemics of CNS disease associated with EV71 have been reported in New York, Hong Kong, Australia and Philadelphia [8, 12, 15, 29]. In Japan, two epidemics of EV71 occurred with most cases characterized by HFMD and a low incidence of CNS disease [32, 18, 14]. The largest outbreak recorded thus far occurred in Taiwan in 1998 with more than 100000 cases of HFMD and 78 died of acute brainstem encephalomyelitis with neurogenic cardiac failure and pulmonary oedema [7, 20, 33, 36].

HAND FOOT AND MOUTH DISEASE IN MALAYSIA

In Malaysia, the prevalence, incidence, aetiology and disease burden of HFMD were essentially unknown before May 1997. Interest in the disease and followed by the development of an in-place surveillance system for the disease started following a large outbreak of HFMD in 1997associated with a number of deaths in children due to acute infection of the brain stem [22, 23]. The first documented outbreak of HFMD in Malaysia occurred in April 1997, initially in the state of Sarawak (East Malaysia in Borneo Island) and subsequently spread to peninsular Malaysia by June[34]. The actual number of children who developed HFMD in the outbreak was not known but a total of 4253 cases of HFMD (2113 from Sarawak and 2140 from peninsular Malaysia) were reported by June 1997[34]. Children aged 4 years and below comprised more than 80% of the HFMD cases with more than 50% of the affected children of aged less than 2 years. As with findings in other parts of the world, HFMD in Malaysia essentially affected children of very young age, particularly those between 1 and 2 years of age. In the 1997 outbreak, 41 deaths (29 from Sarawak and 12 from peninsular Malaysia) were recorded. The mean age of the children who succumbed to the illness was 1.6 years (range 7 months to 6 years) with about 80% below 2 years old[23, 34]. All fatal cases developed signs of shock, including pallor, cold extremities with poor peripheral circulation, delayed capillary refill, and weak peripheral pulses at or soon after admission. Pulmonary oedema was present in many cases as the terminal event and nearly all the fatal cases had sinus tachycardia but no cardiac arrhythmia noted. Echocardiograms obtained on most patients showed a poorly contractile globular left ventricle with low output (ejection fraction under 60%). In most cases, children succumbed to the illness with 6 six days of onset of illness, and death usually ensued within 24 hours following the onset of cardiac instability. Several of the fatal cases also had signs of central nervous system involvement (15% had acute flaccid paralysis and 45% had seizures)[22, 23]. Four fatal cases from peninsular Malaysia had full post-mortem performed and all showed similar pathologic findings. The main histopathological findings were in the brainstem and spinal cord. The entire brainstem including dorsal nucleus of the vagus, tractus solitarius, and reticular formation were inflamed to varying degrees. Inflammation was also present in the pons, midbrain and diencephalons. Severe and

widespread inflammation was noted in the grey matter with inflammatory changes that consisted of peri-vascular culfing by mononuclear cells predominantly and neutrophils occasionally. Cluster of neurons showed degeneration and necrosis with neuronophagia. Microglial nodules were prominent which appeared to form microabscesses. Mild meningitis was noted throughout the CNS but there was no evidence of cerebritis though focal areas of inflammation were noted in the white matter of cerebellum. All cases had histological evidence of pulmonary oedema and the myocardia did not show any inflammation [22, 23]. Both CA16 and EV71 were the major viruses isolated from cases of HFMD but only EV71 was isolated from fatal cases. Other types of enteroviruses, adenovirus and human herpesvirus 1 were isolated in a small proportion of cases of HFMD [16].

A similar outbreak of HFMD occurred in Malaysia during the latter part of 2000. 8 deaths were reported in peninsular Malaysia with unspecified number of deaths recorded in East Malaysia [16]. The presenting clinical features of children who succumbed to the disease were no different from those seen in the 1997 outbreak. In addition to CA16 and EV71, echovirus 7 (Eo7) was also isolated from cases of HFMD seen at the University of Malaya Medical Centre during the outbreak [16, 21]. Eo7 has previously been implicated as a cause of mild febrile exanthematous diseases in children but has not been reported in association with an HFMD outbreak. During the 2000 HFMD outbreak, besides EV71, Eo7 was also isolated from a few fatal cases of encephalomyelitis. The significance of Eo7 isolation from these fatal cases and its association with EV71 need further study although there have been several earlier reports of fatal infections due to the virus [2, 17, 24, 35].

Malaysia experienced a smaller outbreak of HFMD in 2003 which appeared to begin in Sarawak and subsequently spread to peninsular Malaysia, a temporal pattern of spread that was similar to the HFMD outbreak in 1997. Again, both EV71 and CA16 were the main causative viruses isolated [6, 9, 25, 28]. On the contrary, the outbreak of HFMD in 2005 started in peninsular Malaysia and later spread to Sarawak in early 2006. The outbreak started in late February with 2 599 cases of HFMD reported in peninsular Malaysia. Eight young children below 5 years old succumbed to the illness, 2 from peninsular Malaysia and 6 from Sarawak. Based on the clinical samples submitted to the National Public Health Laboratory for virological investigation, both EV71 and CA16 were the main aetiological viruses isolated but the isolation of CA16 appeared to precede the isolation of EV71 as the aetiological agent of HFMD (Fig. 1). During the same period, EV71 was also isolated from the blood and stool specimens of a 15 months child who succumbed to febrile illness with clinical presentation of brainstem encephomyelitis with neurogenic pulmonary oedema but without any cutaneous and/or muco-cutaneous manifestations of HFMD (Unpublished data). In 2007, an outbreak of HFMD was only noted in peninsular Malaysia. The outbreak was almost purely due to CA16 though EV71 was isolated from 18 of the 5380 patients' clinical samples submitted for laboratory study. The current on-going outbreak of HFMD started in peninsular Malaysia in epidemiological week 12 (Fig. 2). Up to epidemiological week 33, clinical samples (oral swab or rectal swab) from 1647 patients were submitted to the National Public Health Laboratory for virological confirmation. One hundred

and forty eight isolates of CA16 and 123 isolates of EV71 were isolated. As with other HFMD outbreaks in Malaysia, both EV71 and CA16 was the main aetiological viruses isolated. In similarity with the HFMD outbreak in 2005, the isolation of CA16 preceded the appearance of EV71 (Fig. 2).

In between outbreaks, EV71 was also known to be isolated intermittently but with low numbers of isolates, by the National Public Health Laboratory since it started to offer diagnostic virological services in 2004. Based on this passive surveillance of HFMD, the number of CA16 and EV71 isolated in the years from 2006 to 2009 is shown in Table 1. The data suggests that both these two enteroviruses causing HFMD underwent low endemic circulation in between outbreaks and led to severe outbreaks when the susceptible paediatric population reached a critical level in an environment for sustained explosive transmission.

Table 1. The number of Coxsackievirus A16 and Enterovirus 71 isolated from clinical samples of respective number of cases of Hand foot and mouth disease (HFMD) submitted to National Public Health Laboratory for laboratory investigation for the years 2006 to 2009.

Year	Number of cases of HFMD	Number of virus isolated	
		Enterovirus 71	Coxsackievirus A16
2006	210	4	21
2007	5380	18	399
2008	450	49	1
2009	381	26	58

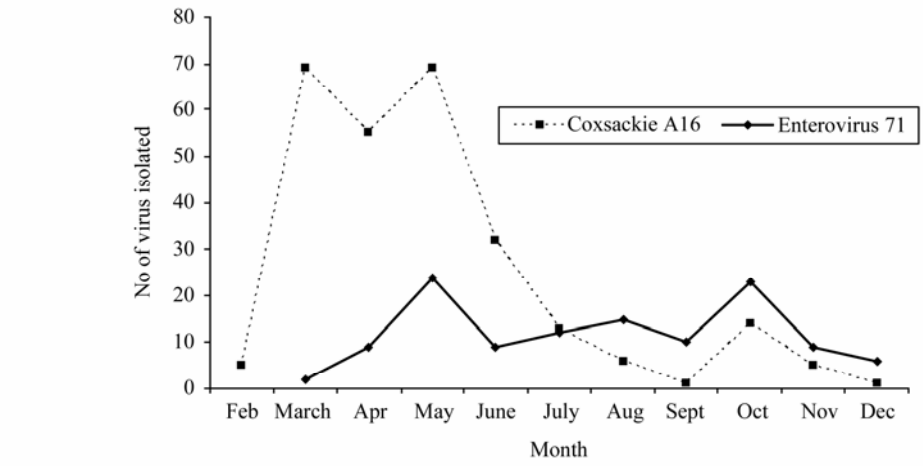

Fig. 1. Temporal relationship on the isolation of coxsackievirus A16 and enterovirus 71 during the outbreak of hand foot and mouth disease in 2005.

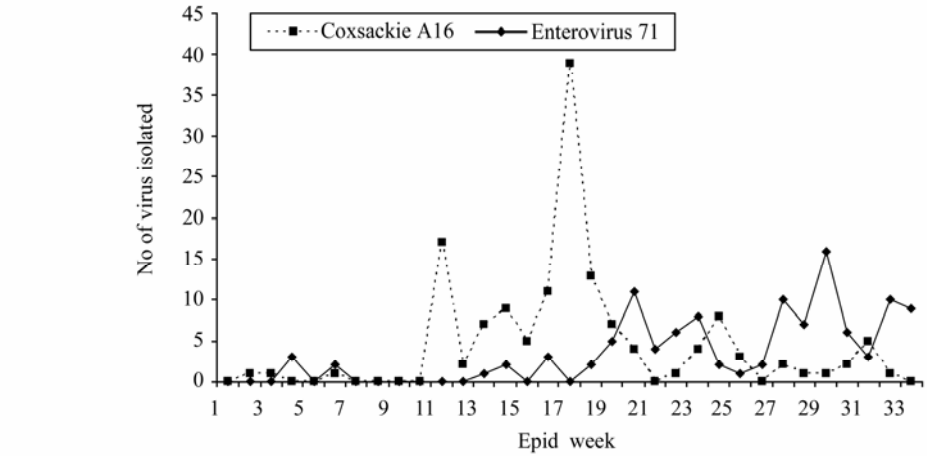

Fig. 2. Temporal relationship on the isolation of coxsackievirus A16 and enterovirus 71 during the outbreak of hand foot and mouth disease in 2010.

Enterovirus 71 (EV71) is divided into three distinct genogroups (A, B, C) and 10 sub-genogroups (A, B1-5, C1-4) based on the estimation of the phylogenetic relationship of VP1 gene nucleotide sequences [4]. Based on the analysis of the complete VP1 gene nucleotide sequences of a number of EV71 isolated in Malaysia, 4 sub-genogroups of EV71 (C1, C2, B3 and B4) were co-circulating and caused the outbreak of hand foot and mouth disease in peninsular Malaysia in 1997 where as 2 sub-genogenogroups (B3 and C1) were co-circulating in Sarawak. Two subgenogroups (C1 and B4) appeared to cause the outbreak in 2000 in both peninsular Malaysia and Sarawak. A new subgenogroup B (B5) with minor contribution from subgenogroup C1 were the EV71 strains causing the 2003 HFMD outbreak in Sarawak but the status of the EV71 strains causing the outbreak in peninsular Malaysia were not analyzed. In the 2005 outbreak, besides EV71 strains of subgenogroup C1, EV71 strains belonging to subgenogroup B5 were isolated but formed a cluster which was distinct from EV71 strains of the subgenogroup B5 isolated in 2003 [6, 25, 9]. The four EV71 strains isolated from clinical specimens of patients with hand foot and mouth disease in the Sarawak outbreak in early 2006 also belonged to subgenogroup B5. Phylogenetic analysis of the VP1 gene sequences showed that the four Sarawak EV71 isolates belonged to the same cluster as the EV71 strains that were isolated in peninsular Malaysia as early as May 2005. The data suggested that the EV71 strains causing the outbreak in Sarawak in 2006 could have originated from peninsular Malaysia[9].

COMMENTS

Including the present on-going outbreak, Malaysia has experienced 5 outbreaks of HFMD caused by EV71 since its first recorded emergence in 1997. Past epidemiological data shows that outbreak of HFMD due to EV71 in Malaysia recurred every 2 to 4 years. Based on the available passive surveillance data from 2006, it shows that EV71 underwent low endemic circulation in between outbreaks. Demographic changes in Malaysia such as population growth, urbanization, the increasing reliance on communal child-care (nurseries) by working parents and close congregation of young children in kindergarten, may have contributed to the endemic circulation of EV71. In each of the past outbreaks of HFMD in Malaysia, more than one sub-genogroup of EV71 was co-circulating. With the exception of the EV71 sub-genogroup C1 which was consistently isolated in all four previous outbreaks, the sub-genogroup B viruses differed from one outbreak to the next. The practical significance of this report demonstrates that different sub-genogroups of EV71 could co-circulate and cause an outbreak. Molecular data suggests the sub-genogroup B viruses could have undergone accumulated mutations and genetic evolution in Malaysia in between outbreaks. Thus, continuous virus surveillance and genetic analysis of EV71 isolated in Malaysia, especially in between outbreaks, is necessary and crucial and can lead to a better understanding of its genetic evolution, transmission and possible future control and prevention.

To date, there is no effective antiviral drug available for the treatment of severe EV71 infections, neither is there a vaccine available for the prevention and control of HFMD outbreaks and their associated complications. Thus, the only current means of preventing EV71 infection is through avoidance of

contact between infected and susceptible individuals and implementation of strict personal hygiene, especially during epidemics. Indeed, even if these actions are to have any effect, it is imperative that an adequate surveillance of EV71 activity is maintained in the community to provide some form of early warning of impending outbreaks. Realistically, it is not easy with close and intense mixing of young children in nurseries and kindergartens, regardless of the further complications introduced by those who are excreting the virus without symptoms of infection. Previous studies in USA have shown that even the high standard of hygiene practiced in developed countries are not sufficient to prevent the transmission of enteroviruses between children and thus susceptible young children remain vulnerable to epidemics of HFMD due to EV71 and its associated morbidities and mortalities. From the public health perspective and based on the successful experience of the poliomyelitis control and eradication programme, an effective live-attenuated EV71 vaccine is urgently needed to control EV71 epidemics and reduce its health and socio-economic impact.

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