GENETIC CHARACTERIZATION OF ENTEROVIRUS 71 FROM A SIBLING OF A FATAL CASE OF HAND, FOOT AND MOUTH DISEASE IN JAKARTA, INDONESIA

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Abstract. Enterovirus 71 (EV71), a member of the picornaviridae family, is a main cause of hand, foot and mouth disease (HFMD), some subgenogroups have been associated with serious complications, such as encephalitis, meningitis, delayed neurological development, reduced cognitive function and a poliomyelitis-like paralysis. We reported here the findings of subgenogroup analysis of enterovirus A-71 in a sibling of a fatal case of HFMD in Jakarta, Indonesia and found it to be subgenogroup B5.

Keywords: Enterovirus 71, hand, foot and mouth disease, Jakarta, Indonesia

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INTRODUCTION

Enterovirus A71 (EV71) is a non-enveloped positive ssRNA virus belonging to the Family Picornaviridae, genus Enterovirus (Puenpa *et al*, 2019). The virus is associated with hand, foot and mouth disease (HFMD) whose symptoms may include fever, oral, sole and palm lesions (Yusof *et al*, 2011). EV71 is the second most common causative agent of HFMD after coxsackievirus A16 (CV-A16) (Guerra *et al*, 2021; Yi *et al*, 2017). In severe cases HFMD may result in neurological complications, such as encephalitis, meningitis, delayed neurological development, reduced cognitive function and poliomyelitis-like paralysis (Lee, 2016). With

the great reduction in polio due to vaccinations, EV71 appears to have entered the niche left by polio virus (Ong and Wong, 2015). It is a highly transmissible enterovirus resulting in hospitalizations of hundreds of children worldwide (Puenpa *et al*, 2019). The virus is endemic to large parts of Southeast Asia with cyclic outbreaks occurring every 2-3 years (Geoghegan *et al*, 2015).

In 1957, the first HFMD case was reported in New Zealand, and the first EV71 was isolated in 1969 in the United States from a 9-month old child with encephalitis (Chong *et al*, 2015). Southeast Asian countries have experienced a surge in EV71 infections (Gonzalez *et al*, 2019; Sabanathan *et al*, 2014). In Indonesia, EV71 was implicated as the causative agent in a 2016 HFMD outbreak in Banjarmasin, South Kalimantan (Wiyatno *et al*, 2018).

We report here the findings of subgenogroup analysis of EV71 subgenogroup B5 in a sibling of a fatal case of HFMD in Jakarta, Indonesia.

Case report of sibling who expired

A 4½ year old girl presented to Hermina Jatinegara Hospital, Jakarta, Indonesia with a 3-day history of fever, vomiting and mouth ulcers causing the child to be unable to eat. She was previously healthy and her past medical history revealed no major medical problems. Her temperature on admission was 38°C but her other vital signs were normal. Her lips were dry; she had no sunken eyes; normal skin turgor and her capillary refill time was 2 seconds consistent with only mild dehydration. She had ulcers was on her lips and palate. She had no dyspnea, no rashes and no meningeal signs. The rest of the physical examination was unremarkable. She was diagnosed as having a common cold, herpangina and mild dehydration due to inadequate fluid intake. The laboratory results revealed a hemoglobin level of 15.2 g/dl, a white blood cell count of 21,340/µl, a platelet count of 513,000/µl, with 65% neutrophils, 28% lymphocytes, 5% monocytes 0% basophils, 2% eosinophils and 0% bands.

She was admitted, started on intravenous fluid, given antipyretics and corticosteroid mouth gel. Three hours later, she developed tachycardia with a heart rate of 155 beats per minute, tachypnea with a respiratory rate of 42 times per minute, nasal flaring but no chest retractions. Her oxygen saturation was 96% on 1 liter of oxygen/minute by nasal cannula. She became lethargic, had a generalized tonic clonic seizure, and developed a distended abdomen.

She was transferred to the pediatric intensive care unit where she was diagnosed with having sepsis and was started on Cefotaxime at a dose of 200 mg/kg/day. Ten hours later, she developed apnea and cardiac arrest and responded to resuscitation. At the time of intubation, she had blood stained sputum. Her electrocardiogram

revealed atrial tachycardia with a 1:1 atrioventricular conduction with amiodarone. Further laboratory testing revealed a C-reactive protein (CRP) of 47 mg/dl, an aspartate aminotransferase (AST) of 36 U/l, an alanine aminotransferase (ALT) of 21.7 U/l, an albumin level of 3.8 g/dl, a blood urea nitrogen (BUN) of 32.1 mg/dl, a creatinine level of 0.51 mg/dl and a blood gas showing a pH of 7.442, a partial pressure of carbon dioxide (pCO_2) of 25.2 mmHg, a partial pressure of oxygen (pO_2) of 50 mmHg, a bicarbonate (HCO_3) of 17.2 mmol/l, a base excess (BE) of -7.1 mmol/l and an oxygen saturation of 87.4%. The patient developed bradycardia following the cardiac arrest and expired 12 hours after admission. Specimens were not collected for viral screening due to her rapid deterioration.

Case report of subject from whom enterovirus isolate was obtained

The day after the above-described patient died her sibling was brought to the emergency department of the same medical institution with a 4-day history of fever. The sibling was aged 3½ years. Her past medical history was negative for any major medical problems. On physical examination her temperature was 36°C and the rest of her vital signs were also normal. She had multiple mouth ulcers and a papular rash on her back, her palms and her soles. She was diagnosed with having HFMD disease. Initial laboratory testing revealed a hemoglobin level of 13 g/dl, a white blood cell count of 7500/µl, a platelet count of 333,000/µl, 69% neutrophils, 22% lymphocytes, 9% monocytes, 0% basophils, 0% eosinophils and 0% bands and her CRP level was 75 mg/l. Rectal and nasopharyngeal swabs for polymerase chain reaction (PCR) testing were obtained to test for enterovirus. The PCR testing was preformed following the conventional method (Wiyatno *et al*, 2018). Swab specimens were also tested for paramyxovirus and herpesvirus groups by DNA; both were negative. Phylogenetic analysis of the viral protein 1 (VP1) region confirmed the isolate to be EV71 subgenogroup B5 (GenBank accession number: MT431549) (Fig 1). She was discharged after 2 days hospitalization.

Although not confirmed by viral testing, the infection in the first case described above was most likely caused by EV71, the same as the younger sibling.

This study was approved by the Eijkman Institute Research Ethics Commission (number 66, November 2013; amended in 2017).

DISCUSSION

Several Asia-Pacific countries have reported yearly outbreaks of HFMD and EV71 has been implicated in the majority of severe cases (Head *et al*, 2020). EV71 is contagious with greater pathogenic potential than other group of enteroviruses (Yi *et al*, 2017). EV71 infection commonly presents

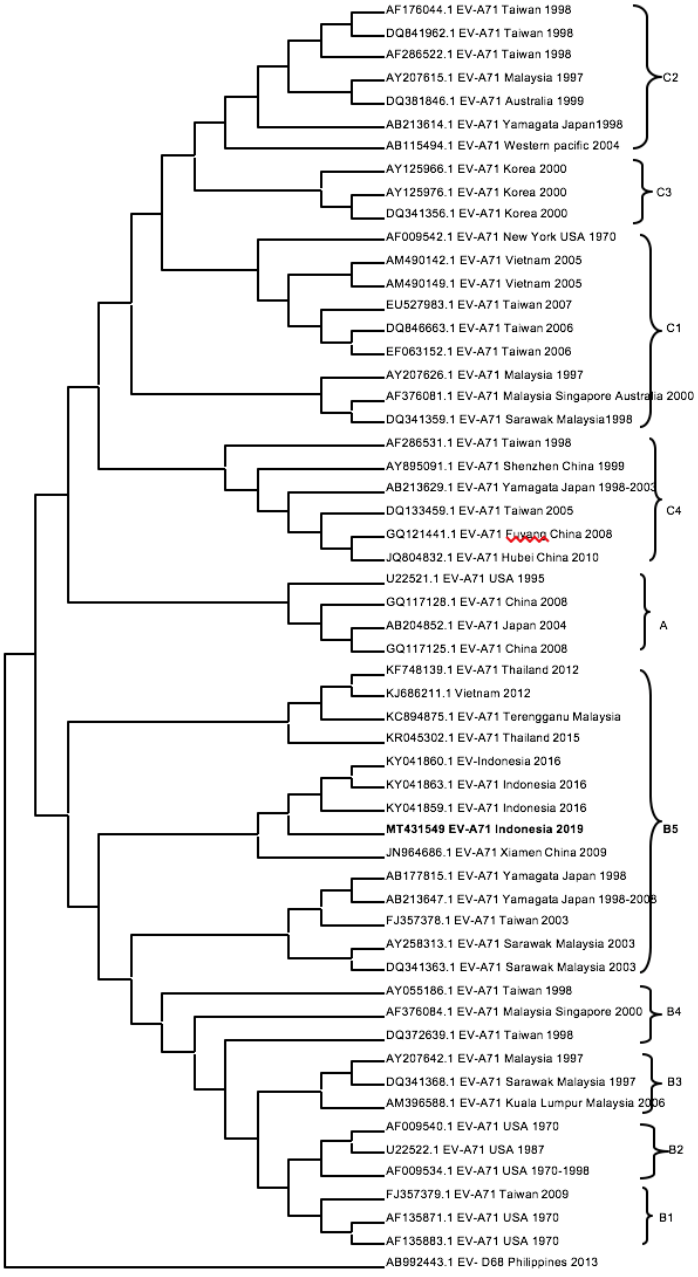

Fig 1 - The phylogenetic tree of 761-bp of EV71 VP1 region isolated from a three-year-old girl suffering from HFMD

The Enterovirus D68 VP1 gene was used as an outgroup. The GeneBank accession numbers are written for each EV71 gene strain.

with fever, blister sand a rash; it can pass through the blood-brain barrier causing encephalitis; it can cause cardiopulmonary disease and death (Li and Lao, 2017). The mechanism for the neurotropism seen with EV71 is unclear.

In our study, we did not collect a specimen from the patient who died from HFMD but did obtain a specimen from a younger sibling with HFMD and identified it as EV71 genogroup B5. Sequencing of this specimen showed it had a high sequence similarity to specimens isolated in Xiamen, China in 2009, Taiwan in 2012 and Thailand in 2015 (Fig 1) and found tt was in the same cluster as a previous EV71 strain reported in Banjarmasin, South Kalimantan, Indonesia in 2016 (Wiyatno *et al*, 2018). The clinical manifestations of these various patients differed.

EV71 subgenogroup B5 has been reported to be the most prevalent subgenogroup in Southeast Asia (Ooi *et al*, 2009). EV71 subgenogroup B5 was reported from the Asia-Pacific region for the first time in 1997 and became a predominant strain in Malaysia in the early 2000s (Cardosa *et al*, 2003). EV71 subgenogroup B5 has a higher incidence in young children and caused large, nationwide outbreaks in Singapore during 2006 and 2008, in Taiwan in 2007-2012, in Vietnam in 2013 and 2018 and in Thailand in 2006-2017 (Puenpa *et al*, 2019).

In the last decade, EV71 subgenogroups B5 and C4 have replaced other subgenogroups as the predominant strains in the Asia-Pacific region (Noisumdaeng *et al*, 2019; Puenpa *et al* 2019). Since 2008, subgenogroup C4 has caused major outbreaks in China, South Korea, Cambodia, Vietnam, Malaysia and Australia, with almost 12.5 million cases resulting in 3,500 deaths (Puenpa *et al*, 2019). Subgenogroup C4 is associated with a higher incidence of Central Nervous System (CNS) infections and a longer duration of spread (Fu *et al*, 2020; Nhan *et al*, 2020). Subgenogroup B5 has also been reported to cause CNS infections (Ooi *et al*, 2007). EV71 can recombine with other viruses gaining new traits (Simmonds, 2010; Yip *et al*, 2013). Further studies are needed to determine if C4 and B5 genetic recombination is the etiology of the more frequently reported encephalitis cases due B5 or not (Huang *et al*, 2014). Some mutations of EV71 subgenogroup B5 have been reported to be associated with greater virulence (Chua and Kasri, 2011; Chang *et al*, 2021). EV71 subgenogroups B5 and C4 have been reported to have similar antigenic variations which may increase their transmission efficiency (Luo *et al*, 2015; Roberts *et al*, 2019). The E145Q mutation in the VP1 gene, associated with acute flaccid paralysis (Huang *et al*, 2012), was not present in our isolate.

There is limited data regarding EV71 circulation in Indonesia. The prevalence and clinical manifestations

are unclear. Two EV71 vaccines were approved in 2015 in China that have been reported to prevent neurologic complications (Yi *et al*, 2017). Studies of the epidemiology of EV71 in Indonesia are needed to better understand this disease and its trends.

We reported here the findings of subgenogroup analysis of EV71 in a sibling of a fatal case of HFMD in Jakarta, Indonesia and found it to be subgenogroup B5.

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CONFLICTS OF INTEREST DISCLOSORE

The authors declare no conflicts of interest.

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