PREVALENCE OF ENTEROVIRUSES ASSOCIATED WITH HAND-FOOT-AND-MOUTH DISEASE ISOLATED FROM CHILDREN PATIENTS IN CENTRAL VIETNAM

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Abstract

Introduction: Hand-foot-and-mouth disease (HFMD) is a viral infection of infants and young children worldwide. The disease is caused by enteroviruses A of the family *Picornaviridae* including coxsackievirus A6 (CV-A6), CV-A10, CV-A16 and enterovirus A71 (EV-A71). ***Aims:*** to determine the proportions of EVs and EV-A71 from the children patients with HFMD and the clinical characteristics of HFMD cases in Central Vietnam. ***Methods:*** 97 throat swabs obstained from children patients suspected of HFMD; collected data from the patients by a clinical doctor in a case-recorded form for demographic data, clinical features; detected enteroviruses and enterovirus A71 by the in-house multiplex real-time RT-PCR. ***Results:*** the proportions of EVs and EV-A71 causing HFMD in the children patients in Central Vietnam were 60.8% and 4.1%, respectively. The clinical characteristics of HFMD reported in most cases were fever (95.9%), followed by oral ulcers (50.1%); symptoms of rashes, vomiting and myoclonus were present in 27.8%, 17.5% and 9.3% of cases, respectively; there was no case involving the central nervous system or pulmonary edema. **Conclusion:** From May 2015 to August 2015, there were 60.8% of HFMD cases of infected with enteroviruses in Central Vietnam, only 4.1% of cases infected with enterovirus A71. No case involved the central nervous system or pulmonary edema.

Key words: HFMD, enteroviruses (EVs), enterovirus A71 (EV-A71).

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1. INTRODUCTION

Hand, foot and mouth disease (HFMD) is a viral infection of infants and young children worldwide. The disease is caused by enteroviruses A of the family *Picornaviridae* including coxsackievirus A6 (CV-A6), CV-A10, CV-A16 and enterovirus A71 (EV-A71) [1]. Since 2011, Vietnam has been facing an unprecedented rise in HFMD infections. Even though the disease is known for causing mild illness in most cases, a suddent increase in 2011 saw infections rise to about 112.300. A total of 169 died and in major cities hospitals were overwhelmed. in 2012, cases of HFMD infection continued to escalate, with peaks of infection reaching more than 6.000 cases each week. By the end of August, the Ministry of Health confirmed that close to 75.000 cases of the disease had been seen, with infections in 63 of the 64 provinces in Viet Nam [3].

An availability of a rapid, high-throughput and accurate diagnostic assay that can simultaneously detect and distinguish between EVs and EV-A71 is an ideal aid to patient management and may help outbreak response with regards to predicting the possible level of severity of the outbreak and thereby initiating appropriate public health interventions.

The aims of this study are to determine the proportions of EVs and EV-A71 from the children patients with HFMD and the clinical characteristics of HFMD cases in Central Vietnam.

2. MATERIALS AND METHODS

The study was a cross-sectional survey. Clinical specimens were 97 throat swabs obstained from patients suspected of HFMD with symptoms of feel tired, get a sore throat, or have a fever of around 38°C to 39°C, sores or blisters may appear in or on the mouth and on the hands, feet, and sometimes the buttocks in Pediatrics Department of Hue Central Hospital, Huong Thuy Hospital and Da Nang

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Hospital for Women and Children; from May 2015 to August 2015. Before collecting patient data and specimen, children parents or guardians were asked to give their oral consent to include the children into the study.

Collection of data from the patients by a clinical doctor in a case-recorded form for demographic data (age, gender), clinical features and laboratory data and clinical diagnosis. Throat swabs were collected and placed in the 3ml vial of DB universal viral transport, kept at minus 20°C in freezer until transporting to the laboratory.

Viral acid nucleic extraction from sample: iVAqRNA extraction kit (Viet A Technology Corporation Company) was used for extracting and purifying viral RNA from all the specimens according to manufacturer's protocol.

cDNA from extracted RNA was synthesized with the iStandardiVAcDNA Synthesis kit (Viet A Technology Corporation Company) for the multiplex real-time PCR procedure.

Amplification assays for detection of Enteroviruses and Enterovirus A71: the in-house multiplex real-time PCR for detecting simultaneously enteroviruses and enterovirus A71-associated with HFMD was carried out with the primers and probes as described in previous protocol of Oxford University Clinical Research Unit, Centre for Tropical Medicine [7] with some modifications. Real-time PCR was done using the iPreniumiVApPCR Mastermix (Viet A Technology Corporation Company) and was performed in a StratageneMx 3000 machine (USA). The reaction was performed in a final volume of 25 µl. PCR program condition was initial denaturation at 95°C/2min, 45 cycle of (95°C/15sec, 53°C/1min, 72°C/15 sec) and cooling at 37°C/1sec.

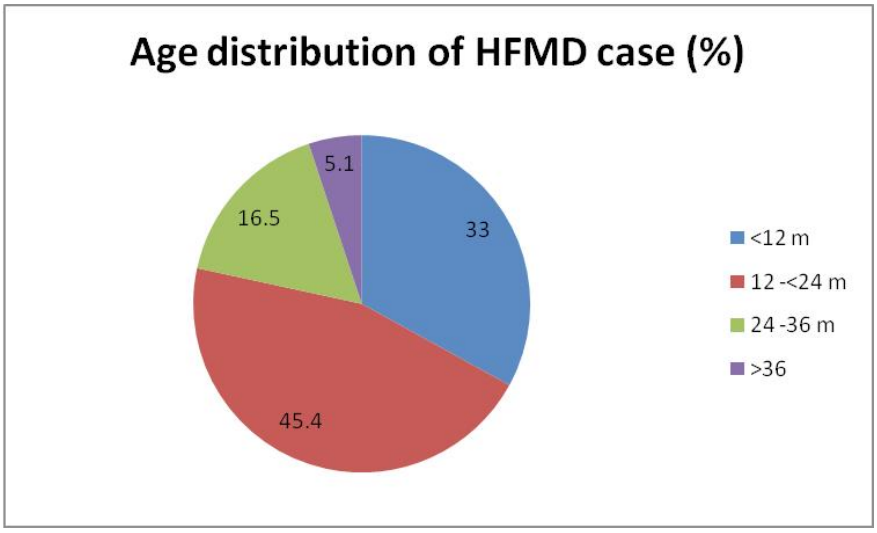
Statistical analysis was performing using Medcalc software.

3. RESULTS

3.1. Age distribution of HFMD cases

Among of 97 HFMD patients there were 59 cases (60.8%) infected with EVs, including 4 cases (4.1%) infected with EV-A71.

Figure 1. Age distribution of HFMD cases

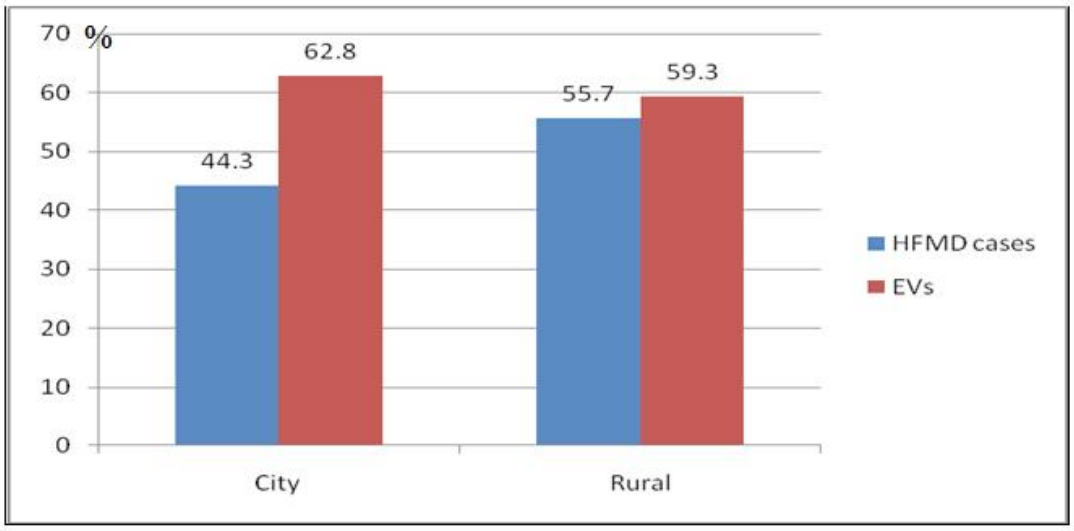


The age of the patients ranged from 2 months to 53 months; 78.4% of the patients were less than 2 years of age (33% for the age <12 months and 45.4% for the age from 12 to less than 24 months; the ages of the patients 24-36 months and >36 months were 16.5% and 5.1%, respectively. The mean age was 17.4 months. There was significant difference of percentages of HFMD cases between the group of children aged younger than 24 months and the group children aged greater than or equal to 24 months (78.4% vs 21.6%), ($p<0.05$).

The percentages of HFMD cases with enteroviruses in the group of children aged younger than 24 months and the group children aged greater than or equal to 24 months were 64.5% and 47.6%, respectively ($p>0.05$). EV-A71 infection was only found in the group children aged greater than or equal to 24 month (19%).

3.2. Geographical regions distribution of HFMD cases

Figure 2. Geographical regions distribution of HFMD cases



The percentages of HFMD in the group of patients from city and the group of patients from rural were 44.3% and 55.7%, respectively. There was significant difference of proportion of cases of HFMD cases between two age groups of patients (p>0.05).

The percentages of HFMD cases with enteroviruses in the group of patients from city was 62.8% and in the group of patients from rural was 59.3% (p>0.05). EV-A71 infection was only found in the group of rural children (7.4%).

3.3. Gender distribution of HFMD cases

Table 1. Gender distribution of HFMD cases

HFMD cases	Male	Female	Sex ratio (m:f)
Infected with EVs	62.6 %	37.3%	*1.68:1*
Infected with EV-A71	75%	25%	*3:1*
All HFMD patients	57.7%	42.3%	1.36:1

In this study, there was a male predominance in HFMD cases. Males were 57.7% and females 42.3% with a male-to-female ratio of 1.36:1. The ratio of male and female of EVs infection and EV-A71 infection were 1.68:1 and 3:1, respectively.

3.4. Clinical characteristics of HFMD patients

Fever was reported in most cases 95.9%, followed by oral ulcers (50.1%) which was markedly higher than other symptoms. No case involving the central nervous system or pulmonary edema have been reported.

Table 2. Clinical characteristics of HFMD patients

Symtoms recorded at admission in terminal hospital	
Fever	95.9
Oral ulcers	50.1
Vesicular erythema (rash) (over hands/feet)	27.8
Vomiting	17.5
Myoclonus	9.3

4. DISCUSSION

HFMD is caused by enteroviruses including enterovirus A71. Indeeded, there were 60.8% of cases of infected with by enteroviruses and 4.1% of cases infected with enterovirus A71 in this study. The true incidence was probably much higher because many patients could have presented with only mild HFMD and been managed in the out-patient setting and not tested. The percentage of cases infected with enteroviruses in this study was the same in the Republic of Korea from 2008 to 2009 (62.2%). In Ko-

rea, the most common pathogen was coxsackievirus A10, whereas EV-A71 was not detected in patients with HFMD or herpangina in 2008 but in 2009, however, EV-A71 was detected most commonly (55.2%) [5], higher than our result (4.1%). The percentage of cases infected with enterovirus A71 in this study was also lower than references such as the outbreak in Zunyi, China between 2012 and 2014 (9%) [9] and in Ho Chi Minh city in 2005 (42.1%) [8]. There was no outbreak of HFMD and no severe HFMD case was reported in Central Vietnam during the course of study, these explaned why the percentage of HFMD cases with EV-A71 in our result was lower than in others.

Our study showed that the ages of patient were taken into consideration, most of the HFMD patients were aged less than 24 months (78.4%). Lower numbers of HFMD cases were observed in the age group ≥24 months (21.6%). In the previous study of Nguyen et al., 87% of children who died from HFMD in Vietnam in 2011 were 3-year-old or younger [4]; the proportion of our HFMD children aged 3-year-old or younger was 94.6%. The number of HFMD cases decreased as age decreased, except for the age group <12 months. The incidence of HFMD varied greatly with age, with highest rates in children aged from12 to less than 24 months (figure 1). It is the same with the outbreak in Zunyi, China from January 1, 2012 to November 11, 2014 (figure 2) [9].

Figure 3. Age distribution of HFMD cases in Central Vietnam (in this study)

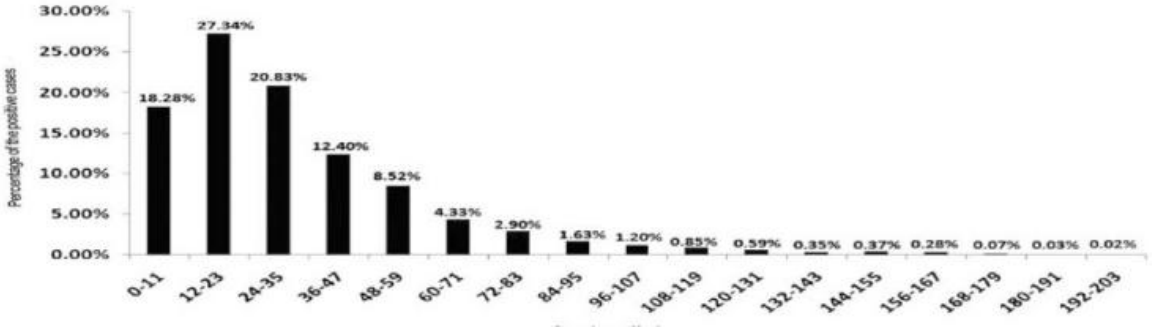
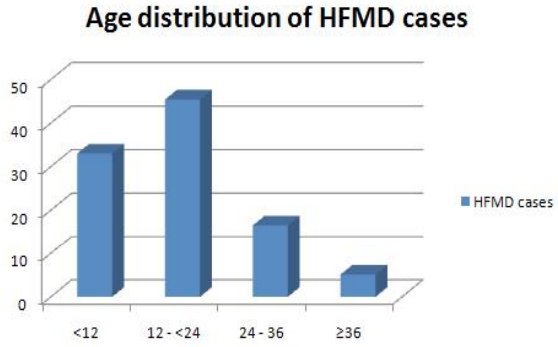


Figure 4. Age distribution of HFMD cases in Zuny1, China [9]

The ratio of male to female of HFMD cases in our study was 1.36, the same in Singapore (1.3) [6] but lower than in outbreaks in other countries such as in China (1.7) [9], in Malaysia (1.9) [2] and also in Vietnam (2.2) [4]. This indicated that the HFMD is more common in male than female but there is no plausible explanation for this skewed ratio.



The exposure history of the cases, those who attended day care or had known contact with other HFMD cases, was poorly recorded and did not indicate any particular source of transmission. HFMD is known to spread through direct contact with mucus, saliva or feces of an infected person. Therefore, health education including how to prevent the spread of HFMD for everyone, even young children plays an important role.

Our results indicated that fever was reported in most cases (95.9%), followed by oral ulcers (50.5%) which were markedly higher than other symptoms; no case of central nervous system was reported. Proportions of the cases having fever and oral ulcer in this study were slightly lower than in a study in Sarawak, Malaysia (fever 100% and oral ulcer 66%) [2]. In our study, symptoms of rashes, vomiting and myoclonus were present in 27.8%, 17.5% and 9.3%, respectively. Shah et al. showed that in the outbreak of HFMD in late year 2000 in Singapore, symptoms of vomiting were present in 37.2% of the cases; oral ulcers were found in 96.1%, rashes over hands in 87.6%, over feet in 86.8% and over buttocks in 54.3% [6]. In Vietnam in 2011, of the 169 deaths from HFMD, symptoms at admission included fever (98%), myoclonus (66%), vomiting (53%), oral ulcers

(50%) and vesicular erythema (50%) [4]. Taken together, warning signs of HFMD could be considered as high fever, persistent vomiting with or without oral ulcers and vesicular erythema.

5. CONCLUSION

By using the in-house multiplex real-time PCR, the proportions of EVs and EV-A71 causing HFMD in the children patients in Central Vietnam were 60.8% and 4.1%, respectively. The clinical characteristics of HFMD reported in most cases were fever (95.9%), followed by oral ulcers (50.1%); symptoms of rashes, vomiting and myoclonus were present in 27.8%, 17.5% and 9.3% of cases, respectively; there was no case involving the central nervous system or pulmonary edema.

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