

Laboratory Based Surveillance of Hand, Foot and Mouth Disease in Four Provinces of Northern Thailand (2012-2016)

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Authors' contributions

This work was carried out in collaboration between all authors. Author PV designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PV and ST managed the analyses of the study. Author SC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Hand, foot and mouth disease (HFMD) is a common childhood exanthema, characterized by a brief febrile illness, sore in the mouth and vesicular lesions on the hands, feet and mouth. HFMD is caused by enteroviruses, mainly enterovirus 71 (EV71) and Coxsackievirus A16 (CA16). An outbreak of HFMD was reported in Thailand in 2012 with incidence of 70.48 per 100,000 populations. Endemicity of these viruses across Thailand has been suspected.

Methodology: A total of 134 stool specimens of suspected HFMD patients from four Northern provinces; Chiangmai, Lampang, Lamphun and Mae Hong Son were analyzed from 2012 to 2016 using RT-PCR based detection method.

Results: Enteroviruses were detected in 74 specimens (55.2%), of which 27 were of CA16 (36.5%), 24 of EV71 (32.4%) and remaining 23 of other enteroviruses (31.1%). These results confirmed circulation of EV71 and CA16 in this region and causing HFMD. The young children below five years were predominant in the study group.

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Conclusion: Laboratory based surveillance confirms the endemicity of enteroviruses in this defined geographical area and occurrence of such cases should be suspected and confirmation undertaken.

Keywords: Hand foot and mouth disease; laboratory surveillance; Northern Thailand.

1. INTRODUCTION

Hand, foot and mouth disease (HFMD) is a common childhood exanthema, characterized by a brief febrile illness and vesicular lesions on the hands, feet and mouth and is caused by enteroviruses [1,2]. The two major causative agents of HFMD are enterovirus 71 (EV71) and Coxsackievirus A16 (CA16) [3,4]. Other members of enteroviruses, such as Coxsackievirus A4, Coxsackievirus A6, Coxsackievirus A10, Coxsackievirus B3, Coxsackievirus B5 and Echovirus 19, have also been associated with HFMD, but, in these cases, patients generally present with mild symptoms [5,6]. EV 71 infection has been associated with higher incidence of severe cases manifesting with acute neurological manifestations and death [7,8].

Between 2007-2011, Thailand reported approximately 12000 to 18000 HFMD cases annually with the average incidence of 20.2 cases per 100,000 population [9,10]. An extensive outbreak of HFMD occurred in 2012 with an incidence of estimated 62.7 per 100,000 population. This outbreak affected several parts of Thailand. The incidence rate per 100,000 population in the central part was 61.11 while it was 60.01 in the northern region [11]. During the same period, Cambodia reported more than 60 deaths within 3 months [12].

To undertake surveillance of HFMD in four Northern provinces of Thailand, the Regional Medical Sciences center at Chiangmai (RMSC 1 CM), Department of Medical sciences, Ministry of Public Health, Thailand established RT-PCR diagnostic services for HFMD. The present study was aimed at analyzing causative agents of HFMD from suspected patients in 4 provinces of Northern Thailand; Chiangmai, Lampang, Lamphun and Mae Hong Son province from September 2012 to December 2016 as part of laboratory based surveillance of HFMD.

2. MATERIALS AND METHODS

2.1 Specimen

Stool specimens were collected from suspected HFMD patients in hospitals of four provinces of

Northern Thailand viz. Chiangmai, Lamphun, Lampang and Mae Hong Son province (Fig. 1) from September 2012 to December 2016 and transported to RMSC 1 CM. A total of 134 stool specimens were analyzed for enteroviruses infection by RT-PCR.

2.2 Specimen Preparation

One gram of each stool specimen was initially subjected to 5 ml of phosphate buffered saline (PBS) of pH 7.4, containing CaCl₂ and MgCl₂, and 100 µl of chloroform. These were mixed and shaken vigorously for 20 min by shaker, followed by centrifugation at 1500x g for 20 min. Stool supernatant (140 µl) was aspirated in tube, to which was added 14 µl of 1 mg/mL proteinase K. The mixture was incubated at 37°C for 30 min.

2.3 RNA Extraction

Viral RNA was extracted from stool supernatant using spin column of QIAamp viral RNA Mini Kit, (QIAGEN, Hilden, Germany) according to manufacturer's instruction. The eluted RNA was kept in -70°C until use.

2.4 RT-PCR Assay

Enteroviruses detection was performed using RT-PCR according to protocol previously description [13]. Specific primers included 1 pair of pan-enterovirus primers for 5' untranslated region (5'UTR) which amplified all known enteroviruses genomes by single RT-PCR. Multiplex PCR followed. Two pairs of specific primers for VP1 gene of EV71 and CA16 were amplified for enteroviruses typing by multiplex RT-PCR. The size of the resulting bands was identified as 440 base pair of pan-enterovirus for enteroviruses group, 264 base pair for EV 71 and 550 base pair for CA16.

3. RESULTS

A total of 134 stool samples were analyzed, of which 74 (55.2%) were positive for RNA of enteroviruses and further enteroviruses typing were determined EV71 and CA16 by multiplex

RT-PCR. CA16 virus found in 27 cases (36.5%), in 23 cases EV71 (32.4%) was detected and other enteroviruses were seen in 23 cases (31.1%).

The distribution of 74 confirmed HFMD by gender, age group and province has been shown in Table 1. The proportion male to female was 1.3:1, The under 5 years of age group was found highest and constituted 90.5% of the group, followed by age 5-9 years group 8.1% and more than 30 years group 1.4%. The confirmed HFMD cases were distributed 68.9%, 17.6%, 9.5% and 4.1% in Chiangmai, Lampang, Mae hong son and Lamphun province, respectively.

The distribution of enteroviruses types EV71 and CA16 were predominant in age group under 5 years with figures of 40.3% for CA16 and 32.8% for EV71. In Chiangmai province, EV71 was predominantly (41.2%) circulating virus. In Lampang province has shown other enteroviruses, CA16 and EV71 as 46.2%, 30.8% and 23.1%, respectively. CA16 and other enteroviruses were found 71.4% and 28.6% in Mae hong son province, while Lamphun province had found only CA16 virus.

The distribution of enteroviruses infection and enteroviruses typing by year are shown in Fig. 2. The annual infection rate was 79.2%, 60.9%, 64.9%, 40.9% and 28.6%, respectively. The EV71 detection was highest (78.9% and 42.8%) in 2012 and 2013, followed by CA16 and other enteroviruses. In 2014, the predominant virus was CA16 (66.7%) and EV71 was not detected. Moreover in 2015, the predominant viruses (77.8%) belonged to other enteroviruses. In 2016, we found predominant CA16, followed by other enteroviruses and EV71, respectively.

4. DISCUSSION

Our study has shown that the infection rate for HFMD in four North Thailand provinces was decreasing from 79.2% in 2012 to 28.6% in 2016. Two major causative agents of HFMD in this geographical area were CA16 as 36.5% and EV71 32.4%. In confirmed HFMD cases, the proportion of male was higher than female, which is in consonance with the previous studies [14,15]. The age group of major affected population was of children under 5 years. This is also in conformation with similar earlier studies [14,15,16,17].

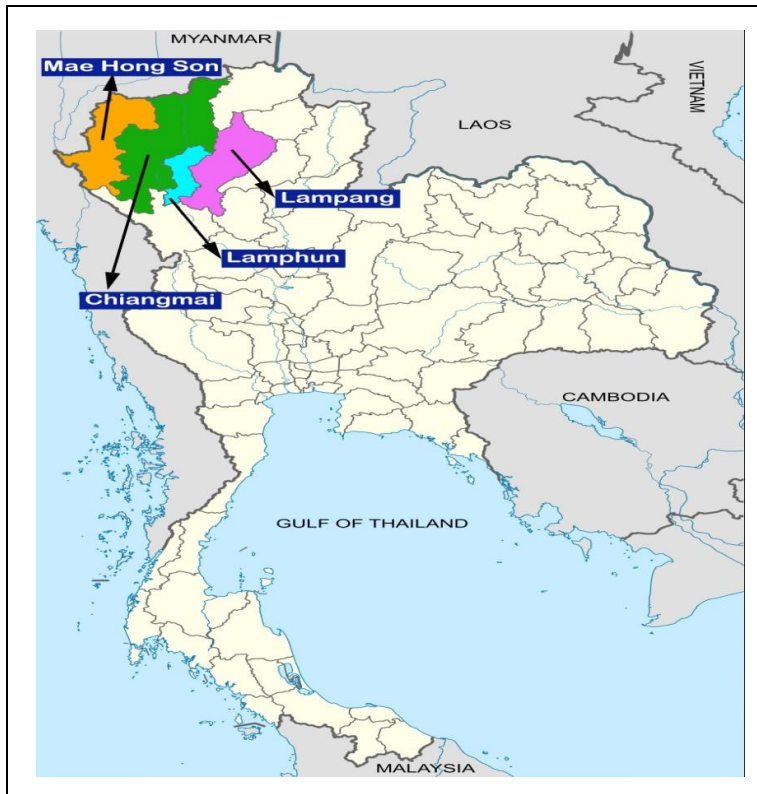


Fig. 1. Map of 4 provinces of Northern Thailand

Table 1. Distribution characteristics of HFMD by gender, age and province and the RT-PCR results for confirmed case, from 2012 to 2016

Factor	Description	Stool		Positive enteroviruses	Enteroviruses by RT-PCR					
		sample	n		EV71	CA16	Others			
		n	n	%	n	%	n	%	n	%
2012-2016	Patient	134	74	55.2	24	32.4	27	36.5	23	31.1
Gender	Male	67	42	56.8	13	31.0	18	42.9	11	26.2
	Female	67	32	43.2	11	34.4	9	28.1	12	37.5
Age group (year)	0-4	112	67	90.5	22	32.8	27	40.3	18	26.9
	5-9	15	6	8.1	2	33.3	0	0.0	4	66.7
	10-19	4	0	0.0	0	0.0	0	0.0	0	0.0
	20-29	1	0	0.0	0	0.0	0	0.0	0	0.0
	≥ 30	2	1	1.4	0	0.0	0	0.0	1	100.0
Province	Chiangmai	103	51	68.9	21	41.2	15	29.4	15	29.4
	Lampang	18	13	17.6	3	23.1	4	30.8	6	46.2
	Lamphun	4	3	4.1	0	0.0	3	100.0	0	0.0
	Mae Hong Son	9	7	9.5	0	0.0	5	71.4	2	28.6

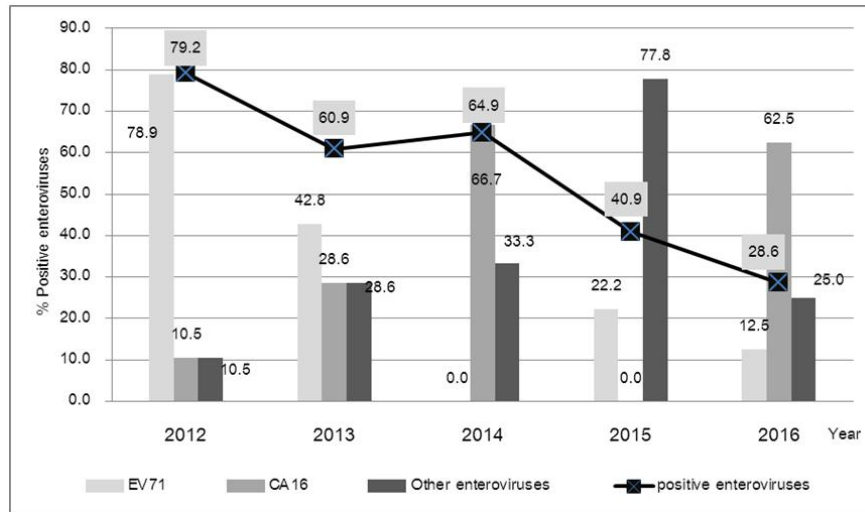


Fig. 2. Proportionate positivity of enteroviruses infection and typing EV71, CA16 and other enteroviruses by years studied

In 4 Northern provinces, different types of enteroviruses were circulating at different times as has been shown earlier in some studies from Thailand [16,17]. From September 2012 to December 2012, EV71 was predominant in this region, whereas a study in Bangkok and Khonkaen found CA6 became predominant from January to October 2012 after replacing EV71 as the predominant type during the previous year [16].

In 2013, there was no large scale outbreak but EV71 was most frequently (30%) isolated in a study at King Chulalongkorn Memorial hospital [17]. Thailand epidemiology surveillance report in 2014 has shown high HFMD morbidity rate 97.36

per 100,000 populations [18]. In another study at Thai National Institute of Health EV71 was observed to be the predominant type [19].

Several other members of enteroviruses were found in this study with variable percentage of detection. In the southern part of Thailand during 2013-2015, 30.09% belonged to category of other enteroviruses [20]. We therefore considered adding others serotyping which high prevalence in Thailand and other country such as CA6, CA8 and CA10 [11,16,21,22].

In 2016, the infection rate was low in 4 Northern provinces, CA16 was till predominant. While this study had smaller number of specimens,

continuous laboratory based surveillance is being pursued by this Institute to elucidate epidemiological data on this emerging infectious disease.

5. CONCLUSION

Laboratory plays an important role in confirmation of diagnosis of disease, as well as surveillance of two major causative agents of HFMD; CA16 and EV71 that are continuously circulating in four Northern Thai provinces and may cause sporadic or outbreak situation. Young children under 5 years old were still the highest affected population. Appropriate hygiene in nurseries, kindergartens and schools should be implemented vigorously. As we continue to elucidate more laboratory data in future, it will strengthen surveillance and generate evidence for initiating public health actions to fulfill the health promotion, protection and control effectiveness in Northern Thailand against HFMD.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was permission and approved by the director of the Regional Medical Sciences Center 1 Chiangmai.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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