Hand, foot and mouth disease in Guangdong, China, in 2013: new trends in the continuing epidemic

J. Lu[1,2,*], H. Zeng[1,*], H. Zheng[1], L. Yi[1,2], X. Guo[1], L. Liu[1], L. Sun[1], X. Tan[1], H. Li[1], C. Ke[1] and J. Lin[1]

1) *Guangdong Provincial Center for Disease Control and Prevention and* 2) *Guangdong Provincial Institution of Public Health, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, China*

Abstract

Millions of incidents of hand, foot and mouth disease occur annually in China, with EVA71 and CVA16 as two major causative pathogens. A provincial surveillance system has been implemented in Guangdong for almost 5 years to analyze the aetiological spectrum and epidemic changes. An unusual enterovirus type, CVA6, was identified as the predominant serotype associated with an HFMD epidemic from late 2012 to 2013. In contrast to virus strains isolated before, all CVA6/CHN/2012–2013 strains segregated into one major genetic cluster. This study suggested that one cluster of circulating CVA6 strain had emerged as a new and major cause during a continuing HFMD epidemic in Guangdong, China.

Keywords: Coxsackievirus A6, enterovirus, foot and mouth disease, hand, molecular epidemiology, phylogenetic analyses
Original Submission: 14 November 2013; **Accepted:** 20 November 2013
Editor: L. Kaiser
Article published online: 16 January 2014
Clin Microbiol Infect 2014; **20:** O442–O445
10.1111/1469-0691.12468

Corresponding author: H. Li, Guangdong Provincial Center for Disease Control and Prevention, No.160, Qunxian Road, Dashi Town, Panyu District, Guangzhou City, Guangdong Province, China
E-mail: gzli_hui@tom.com

*These authors contributed equally to this article.

Introduction

Hand, foot and mouth disease (HFMD), is a major public health problem in China, resulting in millions of clinical cases and hundreds of deaths each year. Outbreaks of HFMD were mainly caused by two types of *enterovirus A* species, enterovirus A71 (EVA71) and coxsackievirus A16 (CVA16), with differing ratios [1–3]. Sporadic cases associated with another coxsackievirus, such as CVA4, CVA5, CVA6, CVA10 and CVB2 to B5, were occasionally reported [4,5].

Guangdong (GD), as the most populous province in China, has been suffering from a serious HFMD epidemic [6]. The number of reported cases was up to 330 645 in 2012 and Guangdong ranked first among 31 provinces in China. Due to the severity of the HFMD epidemic, a provincial web-based surveillance system has been established since 2008 [6]. Medical institutions at all levels in GD were responsible for reporting clinical cases and collecting specimens. HFMD cases were identified according to the Ministry of Health diagnostic criteria (http://www.nhfpc.gov.cn/jkj/index.shtml). Centres for disease control and prevention (CDCs) at the city level run the real-time PCR (RT-PCR) for detecting the presence of the common (universal) sequence of enterovirus and the specific sequences of EVA71 and CVA16 [6]. Positive results were reported to GD CDC. In this study, we described the provincial surveillance results for HFMD from January 2008 to August 2013. The HFMD aetiological spectrum and the epidemic changes were analysed to assess the relative frequency of enterovirus infections and to provide information for future surveillance studies and vaccine development.

A total of 1 248 700 HFMD cases were reported from January 2008 to August 2013; 27 961 EV-positive samples were sent to GD CDC for verifying and further classification. As illustrated in Fig. 1(a), EVA71 and CVA16 have co-circulated as the two most frequent EV types causing repeated HFMD outbreaks in GD from 2008 to 2012. EVA71, as the most predominant EV, represented about 40% of EV-positive samples, except for in 2009. A similar trend was also observed at a national level [3,7]. Notably, the changing aetiology was observed in 2013. A large percentage of non-EVA71 and non-CVA16 EVs was detected during continuing surveillances. Retrospective EV serotype screening was carried out on archived EV-positive samples by using CODEHOP PCR [8]. The PCR products (*c.* 350–400 nt) were sequenced and the virus serotype was determined according to a previously described molecular typing method [9]. From the primary results, CVA6 was the most isolated serotype in the new HFMD epidemic in GD.

CODEHOP PCR is not always as sensitive as specific RT-PCR for detecting certain serotypes of EV [10]. To improve the detection rate, CVA6-specific real-time RT-PCR was performed by using a commercial kit (JC20106, bioPerfectus technologies, Jiangsu, China). The specificity of this kit was firstly confirmed in the laboratory. One hundred

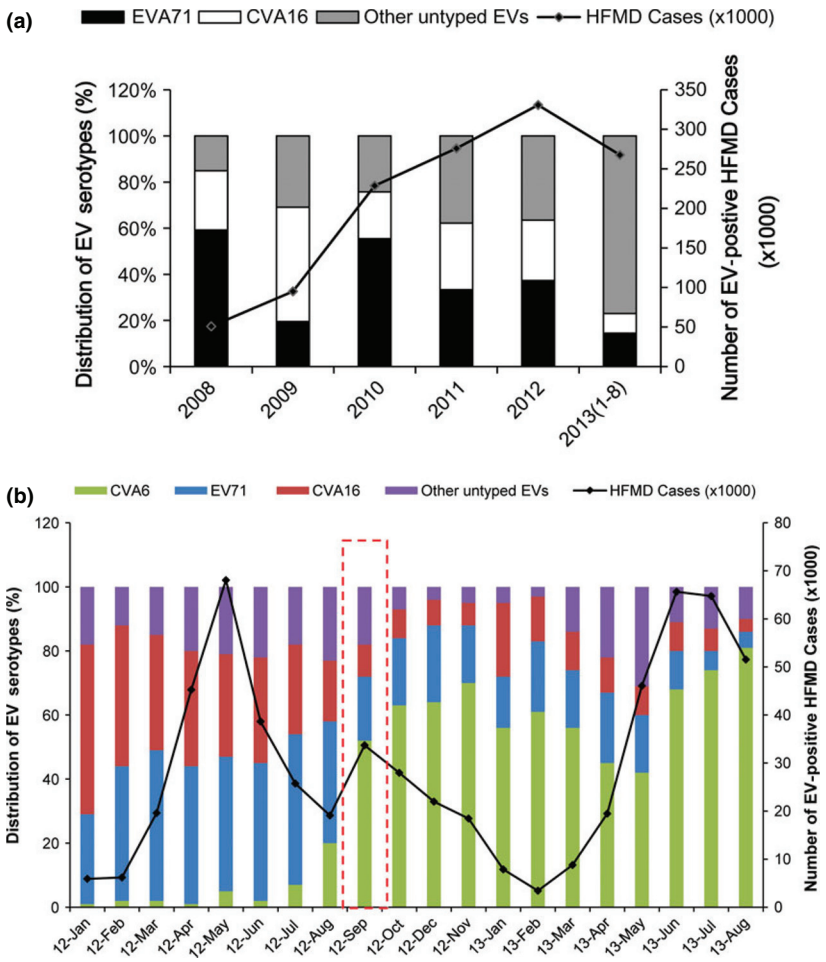

FIG. 1. (a) Enterovirus-assoicated HFMD cases and enterovirus distribution in Guangdong Province, China, from 2008 to August 2013. The continuous line describes the total number of HFMD cases reported each year in Guangdong; the histogram shows the percentage of EVA71, CVA16 and other untyped enterovirus isolates in EV-positive samples. (b) Monthly distribution of CVA6, EVA71, CVA16 and other untyped enteroviruses in Guangdong Province, China, from 2012 to August 2013. The continuous line describes the total number of HFMD cases reported each month in Guangdong; the histogram shows the distribution of EVA71, CVA16, CVA6 and other untyped enterovirus isolates in each month. The high ratio of CVA6, which began to be detected in September of 2012, is highlighted with a dashed border.

EV-positive samples were randomly selected during each month from January 2012 to August of 2013 and specific RT-PCR for EVA71, CVA16 and CVA6 was performed to analysis the distribution of each EV. As shown in Fig. 1(b), EVA71 and CVA16 were detected as the predominant viruses (>60%) from January to August 2012 and the total reported HFMD cases attained a peak in May. In contrast, CVA6, a rare viral pathogen in previous epidemics, was also detected during July 2012 and replaced EVA71 and CVA16 as the major HFMD-associated pathogen from September 2012. In addition, this new outbreak of CVA6 infection may contribute to the second peak of the HFMD epidemic during September 2012. This epidemic change became more obvious during 2013. In total, of 800 enterovirus-positive samples collected from January to August of 2013, 483 (60.3%) were caused by CVA6, 119 (14.8%) by EVA71, 89 (11.1%) by CVA16, and 109 (14.1%) by other untyped enteroviruses. The peak detection of CVA6 occurred during June and July, coinciding with the HFMD summer pandemic. All these findings suggested that CVA6 was the major circulating EV and responsible for the continuing HFMD epidemic in 2013.

Direct sequencing was performed on the full VP1 region of 69 randomly selected CVA6-positive samples (Genbank Accession Number: KF734951-KF734960 and KF836548-KF836606). Maximum likelihood (ML) trees were estimated by using the best-fit Kimura 2-parameter + I model of nucleotide substitution in Mega 5.0 [11]. A phylogenetic tree drawn on the basis of 55 representative sequences segregated CVA6 strains into six