Short Communication

Molecular Epidemiology of Human Enterovirus 71 Strains in the Nanchang Region of China in 2011

Xianfeng Zhou*, Guoyin Fan, Wen Xia, Fenglan He, Maohong Hu, Xiansheng Ni, Yue Zhang, and Haiying Chen

Department of Microbiology, Nanchang Center for Disease Control and Prevention, Nanchang, China

(Received September 10, 2012. Accepted December 28, 2012)

SUMMARY: Hand, foot, and mouth disease (HFMD) is caused by enteroviruses, most commonly enterovirus 71 (EV71) and coxsackievirus A16 (CA16). In general, EV71 infection is more likely to induce severe complications and mortality than other enterovirus infections. The present study focuses on the molecular epidemiology of human EV71 strains in the Nanchang region of China in 2011. Overall, 651 specimens (throat or rectal swabs) were collected, and one-step reverse transcriptase-polymerase chain reaction was performed for analysis. Enteroviruses were detected in 62.2% (405/651) of the specimens. EV71, CA16, and other enteroviruses were found in 292, 73, and 40 specimens, respectively. Phylogenetic analysis of the VP1 region of the 8 EV71 strains found in the Nanchang region indicated that these strains belong to the C4 subgenotype. This study shows that the C4 subgenotype strain of EV71 was prevalent in the HFMD cases of Nanchang in 2011, and it reports the first incidence of adults being infected by EV71 in the Nanchang region. Thus, the surveillance of HFMD epidemiology and monitoring of HFMD severity should be continued.

Enterovirus 71 (EV71) and coxsackievirus A16 (CA16) are the principal pathogens of hand, foot, and mouth disease (HFMD). EV71 is of special concern because it is more likely to induce severe complications and mortality than other enteroviruses, and it has become endemic in Southeast Asia for decades (1,2). EV71 was first described in 1969 during an outbreak with central nervous system complications in California (3). Since then, EV71 infections have been associated with several outbreaks with wide clinical manifestations, ranging from mild HFMD to severe neurological complications and death (4). EV71-associated HFMD outbreaks with severe central nervous system complications have frequently been reported, especially in the Asian-Pacific region (5,6). In 2008, an EV71 outbreak in China infected over 25,000 people and caused over 30 deaths (7).

HFMD usually affects children aged below 10 years, and especially those aged below 5 years (8,9). Although both children and adults are at risk of contracting HFMD, few adult cases of HFMD have been reported thus far. In adults, the transmission of EV71 within households is common; however, the manifestation of EV71 infection is usually limited to mild illnesses (10). During our 2011 HFMD surveillance of the Nanchang region, we encountered 2 adult cases of EV71-associated HFMD. This is the first report of adult cases of HFMD in this region.

The present study aims to understand the molecular

epidemiology of human EV71 strains in the Nanchang region. A total of 651 throat or rectal swabs were collected from 651 patients with HFMD symptoms in 5 local hospitals from January 5 to November 23, 2011. Viral RNA was extracted from the clinical samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification was performed using the Qiagen One-Step RT-PCR Kit to amplify the VP1 region of the viral genome. Three sets of primers were used for amplifying a "universal" sequence (EVU), commonly present in all human enteroviruses, and the specific sequences of EV71 and CA16 in order to detect human enteroviruses, EV71, and CA16, respectively (11). The genes in the VP1 region of the 8 representative EV71 strains were amplified using the forward primer VP1-F (5'-aaaggatccggagatagggtggcagatgt-3') and the reverse primer VP1-R (5'-gggaagcttaagagtggatgcgtgtgc-3'). The products were cloned into the pMD19-T vector (Takara, Dalian, China), and subsequently subjected to bidirectional DNA sequencing (Invitrogen Corp., Shanghai, China).

Figure 1 shows the number of cases of HFMD diagnosed each week from January 5 to November 23, 2011. Of the 651 samples, 405 (62.2%) were tested positive for enteroviruses by RT-PCR. EV71, the most prevalent agent, was present in 44.9% (292/651) samples, CA16 in 11.2% (73/651) samples, and other human enteroviruses (EVU-positive only) in 6.1% (40/651) samples. However, the prevalence of EV71 and CA16 did not differ by geographic location in the Nanchang region. The number of positive cases peaked in the middle of May. Two adults were found to be infected by EV71; 1 exhibited clinical symptoms similar to those of an adult with HFMD in Japan (12). Of all the positive cases, 35.1% (142/405) subjects developed complica-

*Corresponding author: Mailing address: Department of Microbiology, Nanchang Center for Disease Control and Prevention, No. 833, Lijing Road, Honggutan District, Nanchang, Jiangxi Province 330038, China. Tel & Fax: +86-791-86363255, E-mail: xfzhou-nccdc@hotmail.com

Fig. 1. Number of positive cases of hand, foot, and mouth disease reported in our lab from January 5 to November 23, 2011.

Fig. 2. Phylogenetic analysis of 8 EV71-positive specimens NC399/NC/CHN/2011–NC511/NC/CHN/2011 (accession nos. KC007533–KC007540) detected in the present study and reference strains (accession nos. JF519717, JN944895, JF918554, JN944886, HM212466, HM 212458, GU198376, FJ496153, EU753369, HM212446, FJ556874, and FJ151502 for C4; AB115494 for C3; AF116819 and AF135947 for C2; AF009549 for C1; AF135885, AF135873, AF376066, and AF376117 for B; AB204853 for A).

tions. Twenty-one patients (5.2%, 21/405) infected by EV71 developed serious complications such as encephalitis and aseptic meningitis.

Phylogenetic analysis of EV71 was conducted using

the BLAST/FASTA program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The genetic relationship between the strains in this study and the reference strains was analyzed by the MEGA 4 software, using the genomic sequences of the VP1 region. The phylogenetic tree was constructed using the neighbor-joining method (Fig. 2). The genotype analysis revealed that the EV71 strains of the Nanchang region belonged to the C4 subgenotype, commonly found in China in 2008, and that they varied distinctly from the strains reported by Liu et al. in 2011 (11). On the basis of this study, the EV71 strains prevalent in the Nanchang region were found to be genetically similar to the C4 strains of EV71 prevalent in Fuyang, a neighboring city where the HFMD outbreak occurred in 2008 (7).

In summary, the present study showed that in 2011, the EV71 C4 virus was the most prevalent causative agent of HFMD in children of the Nanchang region. The C4 strains have been circulating in China since 2008, threatening the health of children. Thus, more effective surveillance and highly targeted intervention mechanisms should be developed for the control and prevention of HFMD and enterovirus infections in at-risk individuals.

Conflict of interest None to declare.

REFERENCES

- Qiu, J. (2008): Enterovirus 71 infection: a new threat to global public health? *Lancet*, 7, 868–869.
- Lee, T.C., Guo, H.R., Su, H.J., et al. (2009): Diseases caused by enterovirus 71 infection. *Pediatr. Infect. Dis. J.*, 28, 904–910.
- Schmidt, N.J., Lennette, E.H. and Ho, H.H. (1974): An apparently new enterovirus isolated from patients with disease of the central nervous system. *J. Infect. Dis.*, 129, 304–309.
- Wong, S.S., Yip, C.C., Lau, S.K., et al. (2010): Human enterovirus 71 and hand, foot and mouth disease. *Epidemiol. Infect.*, 138, 1071–1089.
- Shekhar, K., Lye, M.S., Norlijah, O., et al. (2005): Deaths in children during an outbreak of hand, foot and mouth disease in Peninsular Malaysia—clinical and pathological characteristics. *Med. J. Malaysia*, 60, 297–304.
- Chan, K.P., Goh, K.T., Chong, C.Y., et al. (2003): Epidemic hand, foot, and mouth disease caused by human enterovirus 71, Singapore. *Emerg. Infect. Dis.*, 9, 78–85.
- Yang, F., Ren, L., Xiong, Z., et al. (2009): Enterovirus 71 outbreak in the People's Republic of China in 2008. *J. Clin. Microbiol.*, 47, 2351–2352.
- Chatproedprai, S., Theanboonlers, A., Korkong, S., et al. (2010): Clinical and molecular characterization of hand-foot-and-mouth disease in Thailand, 2008–2009. *Jpn. J. Infect. Dis.*, 63, 229–233.
- Iwai, M., Masaki, A., Hasegawa, S., et al. (2009): Genetic changes of coxsackievirus A16 and enterovirus 71 isolated from hand, foot, and mouth disease patients in Toyama, Japan between 1981 and 2007. *Jpn. J. Infect. Dis.*, 62, 254–259.
- Chang, L.Y., Tsao, K.C., Hsia, S.H., et al. (2004): Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. *JAMA*, 291, 222–227.
- Liu, M.Y., Liu, W., Liu, J., et al. (2011): Characterization of an outbreak of hand, foot, and mouth disease in Nanchang, China in 2010. *PLoS One*, 6, e25287. Doi:10.1371/journal.pone.0025287.
- Akiyama, K., Imazeki, R., Yoshii, F., et al. (2008): An adult case of hand, foot, and mouth disease caused by enterovirus 71 accompanied by opsoclonus myoclonica. *Tokai J. Exp. Clin. Med.*, 33, 143–145.