Short Communication

An Epidemic of Hand, Foot, and Mouth Disease Caused by Coxsackievirus A6 in Osaka City, Japan, in 2017

Daiki Kanbayashi^{1*}, Atsushi Kaida¹, Yuki Hirai¹, Seiji P Yamamoto¹, Ryoko Fujimori², Megumi Okada², Hideyuki Kubo¹, and Nobuhiro Iritani¹

¹Osaka Institute of Public Health, Osaka 543-0026; and ²Osaka City Public Health Bureau, Osaka City Health Center, Osaka 545-0051, Japan

SUMMARY: The second largest epidemic of hand, foot, and mouth disease since 1982 occurred in 2017, which involved 6,173 cases in Osaka City, Japan. The main causative agent was coxsackievirus A6 (CV-A6). Phylogenetic analysis revealed that the detected CV-A6 strains belonged to genetic groups A3 and A4 in clade A.

Hand, foot, and mouth disease (HFMD) is a common childhood disease caused by human enteroviruses (EVs) belonging to the genus *Enterovirus*, family *Picornaviridae* (1). EV-A71 and coxsackievirus (CV)-A16 have been recognized as the main causative agents of HFMD for many years (1). After the emergence of CV-A6 as the main causative agent of HFMD in Osaka City, Japan in 2011, it has subsequently caused large HFMD epidemics every 2 years (2).

Among the structural proteins of EVs, viral protein 1 (VP1) is exposed on a virion's surface, and it has been considered to contain neutralization epitopes (3,4). A phylogenetic tree based on complete VP1 sequences classified CV-A6 strains into 7 distinct genetic clades (A–G); clade A was sub-classified into 4 distinct genetic groups (A1–A4) (2,5). All CV-A6 strains detected in Osaka City between 2011 and 2015 were classified as clade A (2). Genetic group A1 was detected in 2011, whereas genetic group A3 was detected mainly in 2013 and 2015 (2). Additionally, changes in viral diversity have coincided with CV-A6 outbreaks reported worldwide, including the 2008 Finland outbreak and major outbreaks in Asia, Europe, and the United States in subsequent years (6). Although the relationship between genetic diversity and epidemics has been reported previously, the underlying mechanisms of the evolution and the emergence of novel genetic groups remain unclear because analyses of antigenicity and molecular epidemiology remain insufficient worldwide.

Totally, 6,173 cases of HFMD were reported from approximately 60 pediatric sentinel sites in Osaka City in 2017, marking this outbreak as the second largest HFMD epidemic since the introduction of the National Epidemiological Surveillance of Infectious Diseases in 1982 in Osaka City (Fig. 1). Among patients with HFMD in 2017, 48.2% (2,973/6,173), 80.6%

(4,974/6,173), and 93.0% (5,738/6,173) were < 2, < 4, and < 6 years old, respectively. Surveillance was conducted in 2017, wherein 13 throat-swab, 12 feces, 2 nasal mucus, 1 airway mucus, and 1 cerebrospinal fluid samples were collected from 29 patients with HFMD. In total, 62.1% (18/29) of patients were < 2 years old, potentially indicating a susceptible population for EV infection. EV genomes were screened using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) (7). EV-positive specimens were further analyzed via semi-nested RT-PCR targeting VP1 (8). Complete VP1 sequences were determined from CV-A6-positive specimens (2). Of the 29 specimens, 24 (82.8%) were positive for EVs, including 18 (62.1%) for CV-A6 (10 throat-swab, 7 feces, and 1 airway mucus), 4 (13.8%) for EV-A71 (3 feces, and 1 throat-swab), 1 (3.4%) for echovirus (Echo) type 3 (1 feces), and 1 (3.4%) for Echo type 7 (1 feces). Among 18 CV-A6-positive specimens, 17 complete VP1 sequences were identified in 2017;

Fig. 1. Yearly distribution of HFMD in Osaka City, Japan from 1982 to 2017. HFMD patients were identified in accordance with the diagnostic criteria of the Ministry of Health, Labour and Welfare of Japan, which include a vesicular rash on the hands, feet, and oral mucosa. Clinically diagnosed HFMD patients are weekly reported to the Infectious Disease Surveillance Center from the pediatric sentinel sites in Osaka City since 1982.

Received December 13, 2018. Accepted April 4, 2019.

J-STAGE Advance Publication April 26, 2019.

DOI: 10.7883/yoken.JJID.2018.532

*Corresponding author: Mailing address: Osaka Institute of Public Health, 8-34 Tojo-cho, Tennouji-ku, Osaka 543-0026, Japan. Tel: +81-6-6771-3147, Fax: +81-6-6772-0676, E-mail: kanbayashi@iph.osaka.jp

Upsurge of Coxsackievirus A6 in Osaka City, Japan

Fig. 2. Maximum likelihood phylogram based on complete coxsackievirus A6 (CV-A6) viral protein 1 nucleotide sequences (915 nucleotides). A phylogenetic tree was constructed using 69 strains, including 42 that were detected in Osaka City between 2011 and 2017 and 27 reference strains retrieved from GenBank, using MEGA version 7.0 (<http://www.megasoftware.net>) and the Tamura-Nei model. Initial trees for the heuristic search were obtained automatically by applying the neighbor-joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with the superior log likelihood value. The numbers at the nodes indicate the bootstrap support values, expressed as a percentage of 1,000 replicates (values smaller than 75 were omitted). The scale bar indicates genetic distances. Black and white circles indicate the CV-A6 strain detected in Osaka City in 2017 and the CV-A6 strains detected in Osaka City from 2011 to 2015, respectively. White squares indicate the CV-A6 strains detected in the rest of Japan. Each strain ID consists of a three-letter abbreviation of the country and the year of detection. The accession number is provided at the end of the strain ID in parentheses. ESP, Spain; FIN, Finland; FRA, France; JPN, Japan; CHN, China; TWN, Taiwan; USA, United States of America.

and deposited in GenBank under the accession numbers LC419996–LC420012. All 17 CV-A6 strains were classified as clade A (Fig. 2). These strains were further divided into genetic groups A3 (64.7%, 11/17) and A4 (35.3%, 6/17). The complete VP1 sequences of genetic group A3 detected in 2017 exhibited 94.6%–99.3% and 98.3%–100% nucleotide and amino acid identity to genetic group A3 strains detected in Osaka City in 2013 and 2015, respectively. Additionally, the upsurge of genetic group A4, which differs from the conventional genetic groups, was confirmed in 2017 since its initial detection in 2009 in Japan and reemergence in 2015 in Osaka City. CV-A6 genetic group A4 was one of the major epidemic strains in 2017. Comparison of the complete VP1 sequences of CV-A6 strains detected in 2017 and the strains detected in 2009 and 2015 revealed 93.2%–96.0% and 98.0%–98.7% nucleotide and amino acid identity, respectively. All CV-A6 strains classified into genetic group A4 in Osaka City in 2017 possess a Ser137Asn amino acid substitution, which is located in a region presumed to be highly exposed in the complete amino acid sequences of VP1, compared with genetic group A3 strains in Osaka City in 2013 and 2015.

We found that CV-A6 was responsible for the HFMD epidemics which occurred in Osaka City every 2 years since 2011, when CV-A6 appeared as the main causative agent of HFMD in Osaka City, until 2017. The epidemiology of Japan revealed a similar trend (9). Population immunity and antigenic differences may be important factors in the recent HFMD epidemics caused by CV-A6 because strains with amino acid mutations in antibody-accessible regions on the surface of VP1 or with different genetic backgrounds emerged and caused large epidemics. The emergence and predominance of CV-A6 strains with different genetic backgrounds and amino acid mutations may be induced by immunological pressure in an effort to escape host immunity. Continuous monitoring of CV-A6 activity and antigenicity analysis will clarify these issues. Additionally, genetic group A4 has been responsible for large-scale HFMD outbreaks since 2013 in China (10). Thus, this genetic group may be spreading across Asia.

The accumulation of knowledge about this emerging pathogen may provide insights into the prevention and control of HFMD and vaccine development.

This work was approved by the ethics committee of the Osaka Institute of Public Health (No. 1709-08).

Acknowledgments We thank Mr. Kaoru Takino, Dr. Takayuki Nishio, Dr. Jun Ogasawara, the staff of Osaka Institute of Public Health and Osaka City Health Center, and the pediatricians who collected clinical specimens for supporting our work. This study was partially supported by JSPS KAKENHI Grant Number 26860453, 18K17367 to KD. We would also like to thank enago for the English language review.

Conflict of interest None to declare.

REFERENCES

1. Pallansch MA OM, Whitton JL. Enterovirus: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, editors. *Fields Virology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2013. p.490-530.
2. Kanbayashi D, Kaida A, Yamamoto SP, et al. Impact of coxsackievirus A6 emergence on hand, foot, and mouth disease epidemic in Osaka city, Japan. *J Med Virol*. 2017; 89:2116-21.
3. Xu L, Zheng Q, Li S, et al. Atomic structures of coxsackievirus A6 and its complex with a neutralizing antibody. *Nat Commun*. 2017; 8:505.
4. Li R, Zou Q, Chen L, et al. Molecular analysis of virulent determinants of enterovirus 71. *PLoS One*. 2011; 6:e26237.
5. Zeng H, Lu J, Zheng H, et al. The epidemiological study of coxsackievirus A6 revealing hand, foot and mouth disease epidemic patterns in Guangdong, China. *Sci Rep*. 2015; 5:10550.
6. Anh NT, Nhu LNT, Van HMT, et al. Emerging coxsackievirus A6 causing hand, foot and mouth disease, Vietnam. *Emerg Infect Dis*. 2018; 24:654-62.
7. Kaida A, Kubo H, Takakura K, et al. Associations between co-detected respiratory viruses in children with acute respiratory infections. *Jpn J Infect Dis*. 2014; 67:469-75.
8. Perera D, Shimizu H, Yoshida H, et al. A comparison of the VP1, VP2, and VP4 regions for molecular typing of human enteroviruses. *J Med Virol*. 2010; 82:649-57.
9. Pons-Salort M, Grassly NC. Serotype-specific immunity explains the incidence of diseases caused by human enteroviruses. *Science*. 2018; 361:800-3.
10. Song Y, Zhang Y, Ji T, et al. Persistent circulation of coxsackievirus A6 of genotype D3 in mainland of China between 2008 and 2015. *Sci Rep*. 2017; 7:5491.