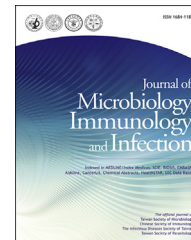




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Brief Communication

# Updates on the molecular epidemiology of Enterovirus D68 after installation of screening test among acute flaccid paralysis patients in Taiwan



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**Abstract** In response to acute flaccid paralysis (AFP) in association with Enterovirus D68 (EV-D68) infection, Taiwan Centers for Disease Control began to screen EV-D68 infection among each AFP patient since July 2015 and detected the first case in August 2016. This article updated the molecular epidemiology trends of EV-D68 from the national surveillance data. Copyright © 2018, Taiwan Society of Microbiology. Published by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Enterovirus D68 (EV-D68) is an emerging infectious disease accounted for a large respiratory illness outbreak in North America in 2014 and known to be associated with a cluster of acute flaccid myelitis (AFM) and acute flaccid paralysis

(AFP).<sup>1</sup> Owing to the emergence of novel pathogen associated with AFP, additional oropharyngeal specimens have been requested by Taiwan Centers for Disease Control (TCDC) among every suspected AFP case for screening EV-D68 infection since July 2015. We detected a case of acute flaccid myelitis (AFM) associated with EV-D68 infection in August 2016. A new subclade B3 of EV-D68 circulated dominantly in Taiwan was reported before.<sup>2</sup> Infection with the B3 clade associated with respiratory or neurological complications was also described in Hong Kong, Sweden, the Netherlands, and Italy.<sup>3–6</sup> To update the molecular

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epidemiology trends of EV-D68 infections, we report the surveillance results of EV-D68 in Taiwan and compare the phylogenetic analysis data between EV-D68 strains detected in 2016 and those circulated in 2014.

There are three surveillance systems to monitor patients with EV-D68 infections in Taiwan. One is the laboratory-based surveillance system named Taiwan Virology Reference Laboratory Network (TVRLN), which has been set up by TCDC since 1999. Throat swabs or rectal swabs from patients with respiratory infection or suspected enterovirus infections were collected by designated physicians in every township (N = 368), and the viruses were isolated following the standard protocols as previously described.<sup>7</sup> The annual average number of samples submitted to TVRLN was 10,000–17,000 from 2007 to 2016. The other is AFP surveillance system which was set up for polio eradication as guided by World Health Organization. For laboratory diagnosis, two stool specimens should be collected 24–48 h apart and within 14 days of the onset of paralysis. In July 2015, in respond to the outbreak of AFM cases associated with EV-D68 infections in the United States, TCDC had requested the clinicians to collect an additional oropharyngeal specimen for analysis. The third surveillance system is relied on clinicians to report suspected enterovirus infection with severe complications (EVSC), which is on the basis of Notifiable Disease Surveillance System. Because EV-D68 could not be identified by using commercial immunofluorescence assay kits yet, only molecular methods and sequence analysis could be used for EV-D68 identification. The molecular method we use for enterovirus detection was Consensus-Degenerate Hybrid Oligonucleotide Primer reverse transcription polymerase chain reaction (EV CODEHOP RT-PCR).<sup>8</sup>

TCDC has identified 92 isolates of EV-D68 from 2007 to 2016 by TVRLN. Among these cases with laboratory-confirmed EV-D68 infection, the ratio of male to female patients was 1.24 and the median age was 6-year-old (0–86). All presented mild upper respiratory tract infection. All isolates were yielded from oropharyngeal specimens. In addition, TCDC had collected 66 oropharyngeal specimens from AFP cases during July 2015 to 2016. The median time from the flaccid/paralysis onset to the clinical specimens collection for EV CODEHOP RT-PCR was four days. Nine specimens yielded positive results including one EV-D68, one CV-A4, one CV-B4, two CV-A6, and four EV-A71. The only distinctive clinical feature of the EV-D68 positive patient was AFP preceded by upper respiratory tract infection illness, such as cough and rhinorrhea, rather than

hand-foot-mouth disease or herpangina symptoms seen in others. And none EV-D68 had been detected among EVSC cases before 2017 since the molecular typing for enterovirus installed in 2007.

The phylogenetic analysis based on viral protein (VP) 1 region (927 nucleotides) of EV-D68 displayed three major clades, A, B, and C.<sup>9</sup> Clade A and B could be further divided into subgroups, subclade A1 and A2, and subclade B1, B2, and B3, respectively.<sup>2</sup> The genotyping results collected from 2007 to 2016 (n = 93, 92 from TVRLN, 1 from AFP surveillance system) showed that Taiwan EV-D68 can also be classified as either clade A or B (Table 1). A phylogenetic investigation was conducted with whole VP1 sequences of some EV-D68 by the neighbor-joining method using MEGA program (version 6, <http://www.megasoftware.net>) with a bootstrap value of 1000 to construct a phylogenetic tree (Fig. 1). Based on the EV-D68 sequence data, subclade A1, A2 and B1 were the prevalent genotypes from 2007 to 2013 in Taiwan. However, most EV-D68 strains detected after 2014 in Taiwan switched to subclade B3, except one was subclade A2 in 2016. The nucleotide similarities of EV-D68 subclade B3 between other subclade B (B1 and B2) and subclade A ranged from 92.2 to 97.7%, and 86.4–88.0%, respectively.

Previous reports have shown the EV-D68 isolates from 2007 to 2014 in Taiwan belonged to Cluster 1 (now designated as subtype B) and Cluster 3 (now designated as subtype A), indicating various clades of EV-D68 co-circulated in Taiwan.<sup>10</sup> Nevertheless, it was not known whether EVD-68 infection was in association with those AFP cases until oropharyngeal specimens were routinely submitted to Taiwan CDC since July 2015. This AFP case associated with EV-D68 infection highlight the importance of screening EV-D68 infection among AFP cases. And EV-D68 subclade B3 infection association with AFP in this study raised some concern about how does viral molecular evolution impact its neurological complication in patients.

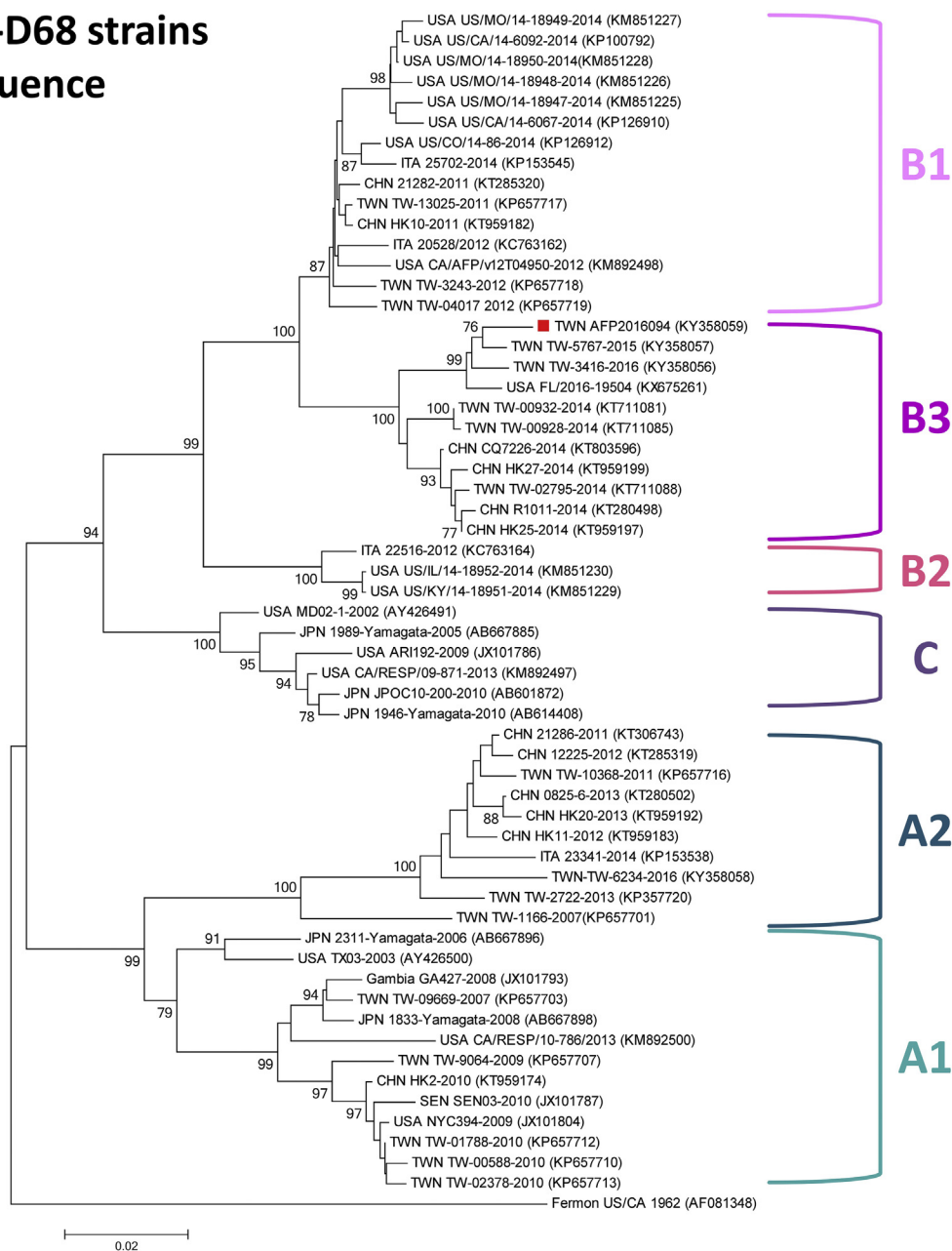
During the outbreak in the United States, 2014, most AFM cases associated with EV-D68 infections belonged to subclade B1.<sup>1</sup> The emerging of EV-D68 subclade B1 was also observed in parts of European countries.<sup>2</sup> Then EV-D68 subclade B3 had been found in Hong Kong, China, and Taiwan since 2014 and emerged worldwide in 2016, such as the Netherlands, and Sweden.<sup>4,5</sup> The molecular difference of subclade B3 distinguished from other B clades had been compared in the previous study but the clinical difference of subclade B3 from others haven't been well characterized yet.<sup>2</sup>

**Table 1** Genotyping of Taiwan EV-D68 isolates, 2007–2016.

Subclade	Number of isolates by year									
	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
A1	10		1	16	1		3			
A2	2		1	2	3					1
B1					7	2				
B2										
B3								8	16	11
B <sup>a</sup>	5	3								

<sup>a</sup> The clade B strains before 2009 did not belong to B1, B2 or B3. The subclades were categorized by partial VP1 sequence (340 nucleotides).

## Phylogeny of EV-D68 strains by VP1 gene sequence



**Figure 1.** Phylogenetic analysis of EV-D68 strains. The analysis was performed by using MEGA version 6 software (<http://www.megasoftware.net/>) with neighbor-joining method and evaluated with 1000 bootstrap replicates along branches. Scale bars indicate nucleotide substitutions per site. The strain caused in AFP case was labeled with square located in the subclade B3. Viruses were identified by country/strain/year of isolation/GenBank accession number. CHN, China; GAM, Gambia; ITA, Italy; JPN, Japan; HK, Hong Kong; NED, the Netherlands; SEN, Senegal; TW, Taiwan; USA, United States.

Intriguingly, severe neurological manifestations including AFM were occasionally seen among subclade B3 infected patients.<sup>4–6</sup> Epidemiological studies showed that one in 25 and three in 74 patients with subclade B3 infection associated with severe neurological symptoms were found in the Netherlands and Sweden, respectively, approximately 4% in both cohorts.<sup>4,5</sup> And one AFM in 37 patients ( $n = 8$  in 2014,  $n = 16$  in 2015,  $n = 13$  in 2016) with subclade B3 infection from our surveillances, approximately 2.7% (Table 1). The neurological complications rate associated with subclade B3 infection in our cohorts was

roughly close to the rate mentioned above. Comparing the variation of amino acid sequences between the EV-D68 isolates from the AFP patient and those subclade B3 from other patients without neurological complications, there is one difference in L121I, which has not been addressed in other case series. That might represent amino acid polymorphism and the association with disease severity was unknown. Whether if different EV-D68 subclade strains have any impact on its pathogenesis and the likelihood in association with neurological complications remains unclear and needs further investigation.

In September 2017, there was another case of rhombencephalitis attributed to EV-D68 infection in Taiwan, reported by the EVSC surveillance system. The surveillances of EV-D68 are important for disease prevention and control. The routine laboratory-based surveillance networks are key elements for our enterovirus identification. To strengthen the surveillance of EV-D68, TCDC would constantly enhance case-based surveillance either by risk communications with clinicians to report suspected cases and keep screening EV-D68 infection among AFP cases.

### Conflict of interest

The authors declare that there are no conflicts of interest.

### Acknowledgments

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