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Upsurge of Enterovirus D68 Infection in the Lower Hudson Valley, New York, 2016

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BACKGROUND

In 2014, a nationwide outbreak of severe respiratory illness associated with *Enterovirus* D68 was reported in the US. There were no EV-D68 cases during the 2015 enterovirus season per CDC's National Enterovirus Surveillance System (NESS).

Upsurge of EV-D68 infection were reported in Europe in 2016 [1-2], but there were only sporadically cases in the US per CDC [3].

The aims of this study are to determine if EV-D68 was circulating in patients in the Lower Hudson Valley, New York in 2016, and if so, whether there were any significant variations in the virus genome or the severity of clinical diseases.

MATERILAS & METHODS

Study site. Lower Hudson Valley, New York, USA.

Clinical samples. Nasopharyngeal (NP) specimens from patients with respiratory illness and/or neurologic symptoms.

Respiratory multiplex PCR (RP) assay. NP specimens were examined for *Rhinovirus/ Enterovirus* (RhV/EV) and other respiratory pathogens using the FilmArray™ RP assay.

EV-D68 rRT-PCR and Next-Generation Sequencing. NP specimens positive for RhV/EV were analyzed with an EV-D68-specific rRT-PCR assay [4] and next-generation sequencing (NGS) on the Illumina MiSeq [5].

Phylogenetic analysis was performed using the BioNumerics v7.6. (Applied Maths, Belgium).

RESULTS

Detection of RhV/EV by FilmArray RP assay, 2014-2016. A total of 11,715 NP specimens were analyzed. The overall positivity was 49.4% by RP, with ~25% positive for RhV/EV (**Fig.1 & Table 1**).

EV-D68 detection, 2014-2016. EV-D68 was confirmed in 94 cases in 2014 and none in 2015 (**Fig. 2**).

EV-D68 was detected in 1/108 (0.9%) RhV/EV-positive NP specimens from January through May 2016, and 159/442 (36.0%) NP specimens from June through October 2016 by rRT-PCR (**Table 2**).

EV-D68 genomes and phylogenetic analysis of 2016 strains. Complete EV-D68 genomes from 22 patient specimens in 2016 were obtained. A new subclade B3 strain, which was ~4.5% divergent in nucleotides from the B1 causing outbreak in 2014, was identified (**Table 3, Fig. 3 & Fig. 4**).

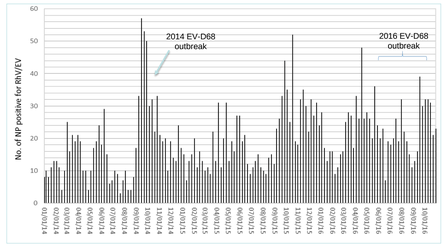

Figure 1. Weekly distribution of RhV/EV-positive NP specimens by the FilmArray RP assay, 2014 through October 2016.

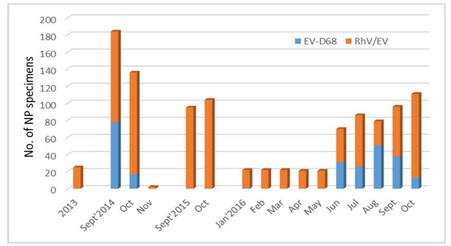

Figure 2. Enterovirus D68 detected by rRT-PCR in NP specimens collected from 2013 through October 2016.

Table 1. Number of nasopharyngeal specimens examined by FilmArray RP during the period from 2014 to 2016
[a] *one or more target(s) detected by the FilmArray RP assay.*

Month & year	Total no. by RP	No. of positive[a]	(%)	No. of RhV/EV positive	RhV/EV positivity (%)
Jan-Dec 2014	3,762	1,769	47.0	917	24.4
Jan-Dec 2015	4,310	2,131	49.4	1,034	24.0
Jan-Oct 2016	3,643	1,882	51.7	985	27.0
Total	11,715	5,782	49.4	2,936	25.1

Table 2. Number of nasopharyngeal specimens examined by the FilmArray Respiratory Panel and EV-D68 rRT-PCR from January to October 2016

Month in 2016	No. of RhV/EV-positive	No. tested by rRT-PCR (%)	EV-D68 rRT-PCR		
			No. of negative	No. of positive	Positivity (%)
Jan-May	416	108 (26.0)	107	1	0.9
June	71	70 (98.6)	39	31	44.3
July	86	86 (100)	60	26	30.2
August	80	79 (98.8)	28	51	64.6
September	96	96 (100)	58	38	39.6
October	112	111 (99.1)	98	13	11.7
Total	861	550 (63.9)	390	160	29.1

Identification of a new subclade B3 circulating in the US in 2016 (Figure 3)

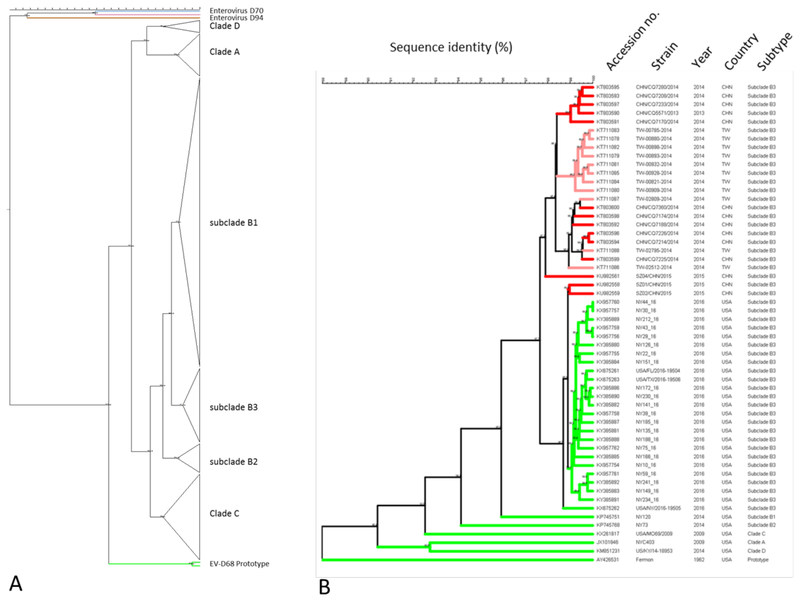

Figure 3. (A) Collapsed phylogenetic tree of enterovirus D68 based on nucleotide sequences of 341 complete or nearly complete genomes. Strains of EV-D70 and EV-D90 were used as outgroup; (B) An enlarged phylogenetic tree of EV-D68 subclade B3 strains (n = 50). Strains from China (CHN) and Taiwan (TW) were shown in red and pink, respectively, whereas strains from the US were shown in green. One strain representing each of other clades (A, C, D, B1, B2 and prototype) was included for comparison.

Table 3. Nucleotide and amino acid sequence identity between subclade B3 and other subtypes of EV-D68 strains

EV-D68 clade	Sequence identity range (%)			
	Nucleotide, genome	Nucleotide, VP1	Amino acid, polyprotein	Amino acid, VP1
B3 vs. A	90.7-91.0	87.8-88.9	98.1-98.2	95.9-97.1
B3 vs. B1	95.5-95.8	95.4-96.5	99.0-99.3	98.6-99.4
B3 vs. B2	93.8-94.2	93.2-94.5	99.2-99.5	99.1-99.8
B3 vs. C	92.0-92.4	90.8-92.2	98.8-99.1	96.6-97.8
B3 vs. D	89.7-89.9	97.3-88.8	98.2-98.3	96.4-97.4
B3 vs. Fermon	87.7-88.1	85.3-86.7	97.6-97.7	94.0-95.0

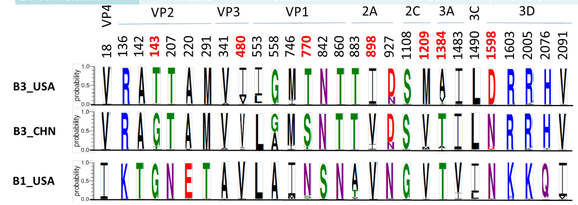

Figure 4. Amino acid (aa) polymorphisms of EV-D68 subclade B3 based on the entire polypeptide sequences of approximate 2,188 aa. Twenty-eight amino acid polymorphisms were identified in subclade B3 strains from the US in 2016, as compared to those of subclade B1 from US in 2014.

Clinical characteristics of patients with EV-D68 in 2016. The median age of 160 patients was 3.2 years (3 wks to 91 years) and 62.5% were male. 145 (90.6%) were pediatric patients. The common clinical presentations included fever (70.2%), cough (72.1%), wheezing (51%), and increased work of breathing (58.6%). Thirty-one (29.8%) pediatric patients required intensive care unit admission in 2016, comparable to 23 of 80 (28.8%) patients in 2014. Acute flaccid myelitis (AFM) was confirmed in two pediatric patients with 5 and 21 months of age, respectively.

CONCLUSIONS

A new EV-D68 subclade B3 circulating in the US caused an outbreak in the Lower Hudson Valley, New York with 160 laboratory-confirmed cases in 2016.

Multiple mutations in the viral genomes of B3 strains and variations in the spectrum and severity of clinical diseases were observed.

Enhance surveillance and more accurate lab diagnostic testing for detection of EV-D68 in clinical specimens are warranted.

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* Contributed equally to this work.