Clinical and Virologic Characterization of Children Presenting with Enterovirus-D68 Infection in Seattle, WA

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Abstract (modified)

Background: In mid-2014, severe respiratory disease associated with enterovirus D68 (EV-D68) was reported in pediatric patients. We studied virologic characteristics and clinical disease due to EV-D68 in patients at Seattle Children's Hospital (SCH).

Methods: Mid-nasal swabs from children with respiratory symptoms were evaluated. A single specimen from each patient with rhinovirus/enterovirus (HRV/EV) detected via commercial multiplex polymerase chain reaction (PCR) platform underwent real-time reverse-transcriptase PCR for EV-D68 using an assay developed in our laboratory. Cycle threshold (CT) was used as a proxy for semi-quantitative viral load. Patient characteristics were compared between EV-D68 positive and EV-D68 negative patients.

Results: From Aug-Dec 2014, 878 patient swabs tested positive for HRV/EV, and 611 had samples available for further testing. 186 (30%) of HRV/EV+ samples tested had EV-D68 detected, an unusually high fraction for a single strain of HRV/EV+ when compared to previous years (7% in 2012, P<0.01). EV-D68 was only detected from Aug. 22-Dec. 18, 2014. Among patients evaluated in the ED or outpatient setting, 144/179 (80%) of EV-D68 positive patients were admitted vs. 259/380 (68%) of patients positive for HRV/non-D68 EV (p = 0.03). Of those hospitalized, 2 (1%) of patients with EV-D68 required mechanical ventilation (MV) compared to 13 (3%) of patients with HRV/non-D68 EV (P=0.16). Median CT was lower in EV-D68 positive patients requiring MV than those who did not (17.4 v. 25.3, P = 0.04) and between those requiring any supplemental oxygen and those remaining on room air (24.2 v. 26.2, P < 0.01). Median CT did not significantly differ between those with EV-D68 who were and were not admitted to the hospital (25.3 v. 24.7, P=0.31).

Conclusion: Over 30% of pediatric patients positive for HRV/ENT with samples available were infected with EV-D68. Compared to patients with confirmed HRV/non-D68 EV, patients with EV-D68 had a higher rate of hospitalization but no difference in rate of MV. Patients with EV-D68 who required oxygen had lower CT than those that did not, suggesting that viral load may be a marker of clinical severity. Rapid and quantitative testing may help stratify risk; further research is required to understand the pathogenesis of EV-D68.

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Background

- **EV-D68 first identified in 1962, with few reported cases until 2008 (1)**
- **Sporadic, small outbreaks emerged in 2008 in Asia, Europe, and United States (2)**
- **EV-D68 outbreak in summer 2014 first identified at Children's Mercy Hospital in Kansas City, later identified across North America and subsequently in Europe and South America (3)**
- **Outbreak led to significant increase in ED and Urgent Care visits (4)**

Methods

- **Mid-nasal swabs from children with respiratory symptoms were evaluated.**
- **A single specimen from each patient with rhinovirus/enterovirus (HRV/EV) detected via commercial multiplex polymerase chain reaction (PCR) platform underwent real-time reverse-transcriptase PCR for EV-D68 using an assay developed in our laboratory. (5)**
- **Cycle threshold (CT) was used as a proxy for semi-quantitative viral load.**
- **Patient characteristics were compared between EV-D68 positive and EV-D68 negative HRV/EV positive patients.**
- **Lower respiratory tract infection (LRTI) was defined requiring respiratory support OR radiographic findings suggestive of infection (confirmed) or with symptoms or exam findings consistent with LRTI (probable).**
- **Respiratory score was used as a proxy for disease severity, with higher score indicating worse clinical illness. This score has been shown to be predictive of admission among asthmatic patients and to have good inter-observer agreement (6,7)**

Respiratory score criteria

Score Range	Element of assessment
1-3	Respiratory rate (assessed over 60 seconds)
0-3	Retractions (1,2, or 3 areas)
0-3	Dyspnea (age-based assessments)
0-3	Auscultation (degree of wheezing)
1-12	Total

Results

Cohort Description

878 nasal swabs positive for HRV/EV

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623 available for further testing/tested by CDC

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611 patient encounters after excluding duplicates (69.6% of total swabs performed)

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Real Time-PCR for EV-D68

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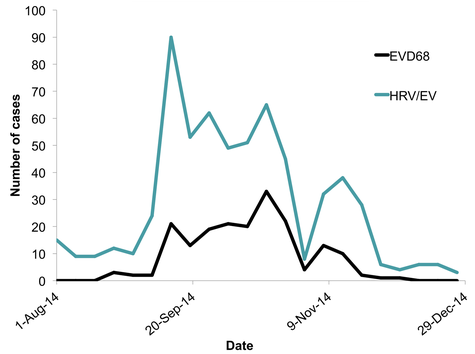
186 EV-D68 positive samples | **425 EV-D68 negative HRV/EV positive samples**

Temporal Distribution



Frequency of HRV/EV and EV-D68 infections at Seattle Children's Hospital in 2014. 186 (30%) of HRV/EV+ samples tested had EV-D68 detected . By contrast, the highest percentage of a single RhV strain in our ED was 7% for strain HRV-A10 in 2012, P<0.01 (8). EV-D68 was only detected from Aug. 22-Dec. 18, 2014.

Demographics

Characteristic	EV-D68+ N = 186 (%)	Non-EV-D68 Rhino/Entero N=425 (%)	P-value
Male Gender	112 (60)	248 (58)	0.64
Age			
<1	16 (9)	101 (24)	<0.001
1-5	87 (47)	191 (45)	0.65
6-11	70 (38)	79 (19)	<0.001
≥12	13 (7)	54 (13)	0.03
Race			
American Indian and Alaska Native	2 (1)	7 (2)	0.38
Asian	20 (11)	35 (8)	0.23
Black or African American	18 (10)	45 (11)	0.71
Native Hawaiian and other Pacific Islander	3 (2)	12 (3)	0.48
Other	39 (21)	75 (18)	0.38
White or Caucasian	93 (50)	233 (55)	0.26
Unknown	11 (6)	18 (5)	0.61

Results (continued)

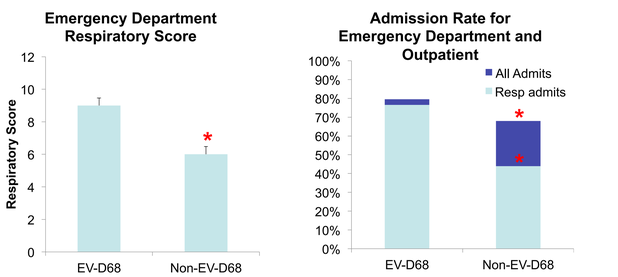
Co-morbidities

Co-morbidities	EV-D68+ N = 186(%)	Non-EV-D68 Rhino/ Entero N=425 (%)	P-value
Immunosuppressed	9 (5)	61 (14)	0.001
Immunodeficiency	2 (1)	23 (5)	0.017
Cardiac disease (unrepaired)	6 (3)	32 (8)	0.02
Pulmonary disease	132 (71)	189 (45)	<0.001
Asthma/Reactive Airway Disease	121 (65)	145 (34)	<0.001

Presenting characteristics

Presenting characteristics	EV-D68+ N = 186 (%)	Non-EV-D68 Rhino/ Entero N=425 (%)	P-value
Location of HRV swab test, n (%)			
Ambulatory	10 (6)	42 (10)	0.11
ED	162 (87)	329 (77)	0.005
ICU	3 (2)	9 (2)	1
Inpatient	2 (1)	33 (8)	<0.001
Urgent Care	7 (4)	9 (2)	0.15
Unknown	2 (1)	3 (1)	1
Fever, n (%)			
No	105 (56)	241 (57)	0.82
Yes	50 (27)	135 (32)	0.22
Tactile	29 (16)	43 (10)	0.035
Unknown	2 (1)	6 (1)	1
Days of URI symptoms, median (range)	2 (0-14)	2 (0-21)	0.635
Co-infection			
Viral, n (%)	4 (2)	22 (5)	0.085
Bacterial, n (%)	23 (12)	41 (10)	0.46
Fungal, n (%)	0 (0)	1 (0)	1
Patient transferred, n (%)	64 (34)	112 (26)	0.044
If transferred, for respiratory symptoms, n (%)	60 (94)	74 (66)	<0.001

Disease severity at presentation



ED median respiratory score. Respiratory score is utilized as indicator of disease severity. * P<0.0001 (Student's T-test). Error bars represent 95% confidence interval.

Overall and respiratory admission rates among patients who were tested in ED or clinic. P-value (all admits) = 0.0033, p-value (respiratory admits) < 0.0001 via Chi-Squared. ID of primary reason for admission as respiratory based on admission assessment and plans.

Disease severity in patients admitted for respiratory illness

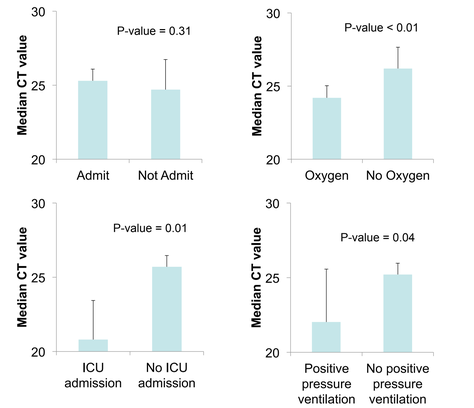
Admissions from ED and outpatient	EV-D68+ N = 137	Non-EV-D68 Rhino/ Entero N=167	P-value
Duration of admission, median days (Interquartile range)	1 (1-2)	1 (1-3)	0.10
Max respiratory score, median (Interquartile range)	10 (8-11)	7 (1-10)	0.0001
ICU admission, n (%)	16 (12)	26 (16)	0.33
Duration of ICU admission, median days (Interquartile range)	3 (1-5.5)	3.5 (2-7.5)	0.42

Results (continued)

Clinical outcomes and EV-D68 (bivariate associations)

Outcome	OR (95% CI)	P-value
Admitted to hospital	1.68 (1.11-2.54)	0.01
Admitted to ICU	0.85 (0.49-1.49)	0.58
Any respiratory support	2.91 (2.03-4.15)	<0.01
Mechanical ventilation	0.34 (0.08-1.54)	0.16
Confirmed LRTI	3.38 (2.31-4.93)	<0.01
Confirmed or probable LRTI	5.59 (3.52-8.88)	<0.01
Any continuous albuterol	5.39 (3.68-7.89)	<0.01

Viral load in relation to disease severity in EV-D68 positive patients



Conclusions

We describe here one of the first cohorts of children presenting for acute care in the ambulatory setting during the summer-fall of 2014 who had EV-D68 and other HRV strains detected and clinical disease described. Samples were available in ~70% of patients presenting for care over a 5 month period. We determined that:

- **Over 30% of pediatric patients positive for HRV/ENT from 2014 were infected with EV-D68.**
- **Compared to patients with confirmed HRV/non-D68 EV, patients with EV-D68 had a higher rate of hospitalization, requirement for respiratory support, and LRTI.**
- **Patients with EV-D68 had pulmonary disease or asthma more often than patients with HRV/non-D68 EV.**
- **Viral load was higher in EV-D68 patients requiring oxygen, positive pressure ventilation, and those admitted to ICU**
- **Rapid and quantitative testing may help stratify risk in the future, particularly as it relates to rate of admission.**

Acknowledgements

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