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BRIEF REPORT

Burden of Disease and Spectrum of Illness From Enterovirus-D68 Infections in US Children 0-2 Years of Age From a Longitudinal Community-Based Cohort, 2017-2019

Zheyi Teoh[1,*]; Claire M. Midgley[2]; Shannon Conrey[3,4]; Allison Burrell[3,4]; Brendon White[3]; Claire P. Mattison[2,5]; Meredith L. McMorrow[2]; Elizabeth P. Schlaudecker[3,6]; Marissa Vawter-Lee[6,7]; Ardythe L. Morrow[3,4]; Daniel C. Payne[3,6]; Mary Allen Staat[3,6,*]

[1]Division of Infectious Diseases, Department of Pediatrics, Seattle Children's Hospital/University of Washington, Seattle, WA, United States; [2]National Center for Immunization and Respiratory Diseases, U.S. Centers for Disease Control and Prevention, Atlanta, GA, United States; [3]Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; [4]Department of Environmental and Public Health Sciences, Division of Epidemiology, University of Cincinnati College of Medicine, Cincinnati, OH, United States; [5]Cherokee Nation Operational Solutions, Tulsa, OK, United States; [6]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH, United States; [7]Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States

[*]Corresponding authors: Mary Allen Staat, MD, Division of Infectious Diseases, Cincinnati Children's Hospital, 3333 Burnet Ave, Cincinnati, OH 45229, United States (mary.staat@cchmc.org) and Zheyi Teoh, MD, Division of Infectious Diseases, Seattle Children's Hospital/University of Washington, Seattle, WA, United States (zheyi.teoh@seattlechildrens.org).

In this 2017-2019 community-based cohort, 245 healthy children were followed from birth to age 2 years. A total of 46 enterovirus-D68 infections were detected by nasal swabs, all occurring between August and November 2018, with no detections in other study periods. About 46% of infections met acute respiratory illness criteria, of which 33% were medically attended; none required hospitalization.

Key words: viral infections; enteroviruses; EV-D68; birth cohort; epidemiology.

INTRODUCTION

Enterovirus D68 (EV-D68) causes acute respiratory illness (ARI), sometimes with severe respiratory manifestations, such as asthma exacerbation and pneumonia, or non-respiratory manifestations, including acute flaccid myelitis (AFM). During 2014-2019, surveillance for EV-D68 expanded in the United States, and a short-term biennial late summer/fall pattern of detections was found.[1,2]

The burden of asymptomatic, subclinical, and non-medically attended EV-D68 cases has not been well described due to the lack of community-based studies. Describing the epidemiology and spectrum of clinical characteristics seen with EV-D68 infections in the community advances our understanding of EV-D68 transmission dynamics and seasonality. It also informs burden estimates, providing a more detailed understanding of the potential impact and benefits of future interventions aimed at interrupting EV-D68 transmission.

The Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal (PREVAIL) cohort is a prospective birth cohort of US children conducted to enhance our understanding of the epidemiology, natural history, and immune responses of respiratory and enteric infections in children. The objective of this analysis is to describe the epidemiology, incidence, virologic characteristics, symptoms, and medical care sought for EV-D68 infections in our community-based cohort of healthy children <2 years of age between April 2017 and July 2020.

METHODS

The Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal is a prospective, community-based, longitudinal birth cohort that enrolled healthy mother-infant pairs from the greater Cincinnati, Ohio region. We recruited mother-infant pairs from April 2017 to July 2018 on a rolling basis and followed children from birth to 2 years of age, spanning 3 respiratory virus seasons from April 2017 to July 2020. Details on the cohort methodology are described elsewhere.[3] Briefly, mothers collected weekly mid-turbinate nasal swabs from infants and young children and swabs were tested using NxTAG Respiratory Pathogen Panel (Luminex Molecular Diagnostics, Austin, TX), which includes targets for rhinovirus/EV as well as adenovirus, bocavirus, endemic coronaviruses, influenza virus, human metapneumovirus, parainfluenza virus, and respiratory syncytial virus (RSV). We performed additional testing for EV-D68 in children

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Table 1. Spectrum of Symptoms and Medical Attention Among EV-D68 Infections ($n = 46$) in US Children 0-2 Years of Age in a Community-Based Cohort, PREVAIL, 2018

	Did not meet ARI criteria[a] $n = 25$	Met ARI criteria[a], not medically attended $n = 14$	Met ARI criteria, primary care visit $n = 4$	Met ARI criteria, ED visit $n = 3$
Age in months, median (IQR)	6 (4-11)	8 (4-13)	8 (5-12)	6 (5-8)
Cough	-	13 (93%)	3 (75%)	2 (67%)
Congestion	N/A[b]	12 (86%)	3 (75%)	1 (33%)
Fever	-	5 (36%)	4 (100%)	3 (100%)
Wheezing	N/A[b]	2 (14%)	1 (25%)	1 (33%)
Diarrhea	N/A[b]	2 (14%)	2 (50%)	0 (0%)
Vomiting	N/A[b]	0 (0%)	1 (25%)	1 (33%)
Earache	N/A[b]	0 (0%)	1 (25%)	0 (0%)
Sore throat	N/A[b]	1 (7%)	0 (0%)	0 (0%)

[a]ARI criteria = presence of cough and/or fever (≥99 °F, rectal) occurring 7 days before or after initial detection of EV-D68.

[b]Additional symptoms besides cough and fever were only ascertained if caregivers reported the presence of cough and/or fever.

Abbreviations: AGE, acute gastroenteritis; ARI, acute respiratory illness; ED, emergency department; EV-D68, enterovirus-D68; IQR, interquartile range; N/A, not applicable; PREVAIL, Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal.

with a swab positive for rhinovirus/EV in the months of July to November, the months EV-D68 is typically detected.[1,2] Molecular testing for EV-D68 was performed using a Centers for Disease Control and Prevention (CDC)-developed real-time reverse transcription polymerase chain reaction (RT-PCR) assay.[4] Only samples with a cycle threshold value of ≤40.00 were considered positive and included in this analysis.

We sent mothers automated, weekly text surveys to ascertain respiratory and gastrointestinal symptoms. Text surveys and medical chart abstraction identified medically attended visits. This study was reviewed and approved by CDC, Cincinnati Children's Hospital Medical Center, Christ Hospital, and the University of Cincinnati Medical Center institutional review boards (§See 45 C.F.R. part 46; 21 C.F.R. part 56). Mothers provided informed, written consent prior to enrollment into the study.

Acute respiratory illness was defined as the presence of cough and/or fever (≥99 °F, rectal); acute gastroenteritis (AGE) was defined as ≥3 loose/watery stools or ≥1 vomiting episode in a 24-h period. Children met ARI or AGE definitions if symptoms occurred 7 days before or after initial virus detection. The detection of EV-D68 from respiratory samples 30 days or less apart, regardless of any interval negative swabs, was counted as the same episode, and the duration of shedding in weeks was determined from each episode.

Findings were summarized with descriptive statistics: median and interquartile range (IQR) for continuous variables and proportions for categorical variables. As EV-D68 infections were only detected in 2018, 2 different incidence rates for EV-D68 infections were calculated. Incidence rates for the epidemiological year 2018 (January 1, 2018 to December 31, 2018) were calculated using all weeks in which a nasal swab was submitted as the denominator, excluding any weeks in which EV-D68 continued to be detected after the initial detection because they were not at risk for a new infection during this time. Incidence rates for the EV-D68 2018 season were calculated using weeks in which a nasal swab was submitted during our testing period (July 1, 2018 to November 30, 2018) as the denominator, excluding any weeks in which EV-D68 continued to be detected. Statistical analyses were performed using STATA 17 (StataCorp, College Station, TX).

RESULTS

Overall, 245 children were enrolled in the cohort, submitting a total of 5812 nasal swabs between the months of July and November across all study years (median of 26 swabs/child). Of these, 1246 (21%) were positive for rhinovirus/EV and all were further tested for EV-D68; 46 EV-D68 episodes were detected.

All EV-D68 infections detected in this cohort were between August 6, 2018 and November 27, 2018; no infections occurred between July and November of other study years. No nasal swabs in July 2020 were positive for rhinovirus/EV, so none were tested for EV-D68. The incidence of EV-D68 for epidemiological year 2018 was 0.78 infections per 100 child-weeks (95% CI 0.57, 1.04 per 100 child-weeks), while the incidence of EV-D68 for the EV-D68 2018 season was 1.64 infections per 100 child-weeks (95% CI 1.20, 2.20 per 100 child-weeks).

During July to November 2018, 204 children submitted at least 1 nasal swab, and 23% of children had an EV-D68 infection. Among the 2961 swabs submitted between July and November 2018, 21% ($n = 630$) were positive for rhinovirus/EV; among these rhinovirus/EV-positive swabs, 9% ($n = 59$) were positive for EV-D68. The median age among children in which EV-D68 was detected was 7.5 months (IQR 4-11 months). No repeat infections were found for any participants during their 2 years of follow-up.

Molecular Characteristics

Median duration of shedding was 1 week, occurring in 83% of EV-D68 infections. Five (11%) EV-D68 infections were detectable for 2 weeks, 2 (4%) for 3 weeks; a single 2-month-old child, who did not meet ARI criteria and was otherwise healthy, had detectable virus for 5 weeks. The median cycle threshold for initial swabs of each EV-D68 infection was 27.4 (IQR 24.7-30.6, range of 17.8-34.3).

Viral co-detections in EV-D68-positive swabs were infrequent (10.8%), occurring in only 5 children. Co-detections with rhinoviruses or EVs of different types could not be determined due to the lack of typing. Co-detections included 1 adenovirus, 3 bocavirus, and 1 parainfluenza virus 4 detection. Twenty-four (52%) EV-D68 infections had a preceding viral detection within the prior 30 days, of which rhinovirus/EV ($n = 19$) was most commonly found, followed by bocavirus ($n = 4$) and RSV B ($n = 1$).

Symptomatology and Medical Attendance

Less than half ($n = 21$, 46%) of identified EV-D68 infections met criteria for ARI; 5 (11%) of these children additionally met criteria for AGE, but none met criteria for AGE alone. Fever (26%) and respiratory tract symptoms such as cough (39%) and nasal congestion (35%) were the most prevalent symptoms; diarrhea (9%) and vomiting (4%) were less frequent. Children meeting ARI criteria versus those who did not meet ARI criteria were similar in age (median: 6 vs 8 months), duration of shedding (median: 1 vs 1 week), cycle threshold (median: 27.3 vs 27.5) and co-detection of another virus during their EV-D68 infection (2 vs 3 children).

Of the 21 children meeting ARI criteria, 7 (33%) had an EV-D68 infection that was medically attended. Four children were seen by their primary care physician, and 3 children were evaluated in the emergency department (ED); 2 children presented with fever and wheezing and were diagnosed with bronchiolitis, while 1 child presented with fever and cough and was diagnosed with an upper respiratory tract infection. No hospitalizations occurred, and no children were diagnosed with pneumonia or AFM.

DISCUSSION

In our community-based longitudinal birth cohort of 245 healthy children 0-2 years of age from April 2017 to July 2020, we detected 46 EV-D68 infections, all occurring in late summer/fall of 2018. The incidence of EV-D68 for the full 2018 year was 0.78 infections per 100 child-weeks, comparable to the incidence of parainfluenza virus 3 (0.83 infections per 100 child-weeks) but lower than the incidence of RSV A or B (5.8 and 5.0 infections per 100 child-weeks, respectively) described in the same cohort.[5] Most of these infections did not meet ARI criteria, and among these, only one-third were medically attended. No hospitalizations or severe disease (including AFM) occurred, highlighting that most EV-D68 infections in infants and young children were mild or asymptomatic. Our study provides insight into the community burden of EV-D68 in infants and young children, as most US-based surveillance of EV-D68 is focused on medically attended infections. We also provide unique information on the molecular and clinical characteristics of EV-D68 infections in healthy infants and young children, including both severe and non-severe infections, agnostic of medical attention.

Enterovirus D68 infections in our community cohort matched the short-term biennial circulation patterns seen with medically attended ARI and AFM in the pre-coronavirusdisease 2019 (COVID-19) pandemic period.[1,6,7] Although non-biennial detection of EV-D68 has been reported to a lesser degree elsewhere,[1,8] we did not find molecular evidence of EV-D68 in our cohort, including mild or asymptomatic community-level EV-D68 infections, during non-epidemic years. The lack of detection with our longitudinal and prospective collection of weekly nasal swabs irrespective of symptoms suggests that infections in healthy young children and infants may be uncommon during the summer months of non-epidemic years. Nevertheless, larger cohorts with a wider geographical distribution would be needed to better define the circulation patterns of this virus. In addition, serological studies coupled with molecular detections are needed to enhance the detection of EV-D68 infections, as reliance on molecular detection alone can miss viral infections.[9]

There are several limitations to consider for our study. While we had 3 years of follow-up with >5812 nasal swab submissions during our study period, our findings may not be generalizable to EV-D68 infections occurring during other respiratory seasons, especially those that have occurred in the post-COVID-19 pandemic era, where the number of susceptible children may have increased after periods of low circulation.[10] As we only performed EV-D68 molecular testing during months (July to November) where EV-D68 were typically detected in prior studies, we may have missed infections during other months, including those in December when the EV-D68 season offset is delayed. There could also be false negative rhinovirus/EV detections from our multiplex assay, leading to missed EV-D68 infections. Subsequently, our incidence rates may be an underestimation of the true burden of EV-D68, including the burden across the entire epidemiological year. In addition, our study findings may not reflect the symptomatology and burden of EV-D68 infections in older children, including those who are experiencing repeat infections, as well as children with symptoms consisting primarily of nasal congestion and rhinorrhea, since these were not part of our primary ARI ascertainment. Finally, although weekly swabbing is a strength of this study for detecting EV-D68, it limited our ability to capture shedding duration more precisely at the daily level.

In conclusion, we detected 46 EV-D68 infections among healthy infants and young children in our community cohort, all between July and November 2018; most did not meet ARI criteria or require medical attention. Our findings underscore the concept that severe manifestations of EV-D68 may only represent the minority of symptomatic community-acquired EV-D68 infections in infants and young children.

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Author contributions

Z.T., C.M., D.P., and M.A.S. conceptualized this study. Z.T., S.C., and A.B. assisted with data curation. Z.T. performed formal analysis. S.C., A.B., and M.A.S. acquired financial support for the project. A.B., B.W., A.L.M., and M.A.S. were responsible for conducting the research and investigation process. D.C.P., A.L.M., and M.A.S. were responsible for development and design of methodology. A.B., C.M., and M.A.S. were responsible for project administration. D.C.P., M.M., A.L.M., and M.A.S. provided supervision. Z.T. and M.A.S. wrote the original draft. All authors reviewed and edited the manuscript.

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Conflicts of interest

All authors declare that they have no financial or personal conflicts of interest relevant to this study.

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