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MAJOR ARTICLE

Burden of respiratory viruses in children less than two years in a community-based longitudinal U.S. birth cohort

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Background: Respiratory viral infections are a major cause of morbidity and hospitalization in young children. Nevertheless, the population burden of respiratory viral infections, especially asymptomatic cases, is not known due to the lack of prospective community-based cohort studies with intensive monitoring.

Methods: To address this gap, we enacted the PREVAIL cohort, a CDC-sponsored birth cohort in Cincinnati, Ohio where children were followed from birth to 2 years of age. Weekly text surveys were administered to mothers to record acute respiratory illnesses (ARIs), which were defined as the presence of cough or fever (≥38^{o}C). Weekly mid-turbinate nasal swabs were collected and tested using the Luminex Respiratory Pathogen Panel, which detected 16 viral pathogens. Viral infection was defined as one or more positive tests from the same virus or viral subtype within 30 days of previous positive. Maternal report and medical chart abstractions identified health care utilization.

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Results: From 4/2017 to 7/2020, 245 mother-infant pairs were recruited and followed. From the 13,781 nasal swabs tested, a total of 2,211 viral infections were detected, of which, 821 (37%) were symptomatic. Children experienced 9.4 respiratory viral infections/child-year; half were rhinovirus/enterovirus. Viral ARI incidence was 3.3 episodes/child-year. Emergency department visits or hospitalization occurred with only 15% of respiratory syncytial virus infections, 10% of influenza infections, and only 4% of all viral infections. Regardless of pathogen, most infections were asymptomatic or mild.

Conclusions Respiratory viral infections are common in children 0-2 years. Most viral infections are asymptomatic or non-medically attended, underscoring the importance of community-based cohort studies.

INTRODUCTION

Respiratory viral infections contribute a significant burden of disease in infants and young children [1,2]. Respiratory viruses are a leading cause of acute respiratory infections (ARIs) globally and cause a wide spectrum of respiratory syndromes from asymptomatic infection to bronchiolitis and death [3-6]. Acquisition of viral infections in early life can also be associated with long-term morbidity, including wheezing, asthma, and recurrent respiratory infections [7,8].

In hospitalized children, the burden of respiratory viruses such as respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, and rhinovirus is well documented.[9-12] However, the natural history of these pathogens and their community-level burden in U.S. children is inadequately characterized. Most studies have relied on hospital-based surveillance to identify infections, leading to an over-representation of children with symptomatic disease requiring medical care or hospital admission.[13,14] Furthermore, most existing studies focus on recruiting children with specific respiratory symptoms, limiting our understanding of the full spectrum of asymptomatic and symptomatic illnesses. Community-based cohort studies, including birth cohorts, attempt to overcome these limitations through prospective, longitudinal data collection.[15] Cohort studies provide a comprehensive picture of the burden, spectrum, and natural history of respiratory viral infections, which can inform our prevention and control strategies.[14,16-18]

The Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal (PREVAIL) cohort is a Centers for Disease Control and Prevention (CDC)-funded investigation that conducted prospective, longitudinal, community-based surveillance for endemic respiratory and enteric pathogens through weekly nasal swab and stool testing of a birth cohort. The objective of this analysis is to describe the overall burden, symptomatology, and patterns of medical attendance among children less than two years of age with respiratory viral infections.

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METHODS

Healthy mother-infant pairs in the greater Cincinnati region were enrolled into the PREVAIL cohort from April 2017 to July 2018. As enrollment occurred on a rolling basis, participants were followed from birth until two years of age across multiple respiratory seasons between April 2017 and July 2020. Pregnant mothers ≥18 years of age, who were at least at 34 weeks of gestation with a singleton pregnancy, were eligible and invited to participate in the study. Mothers who elected to participate provided written consent and were provisionally enrolled, but excluded if they did not complete their first postnatal study visit at week two. Details on the study design, enrollment strategy, inclusion criteria, data collection, sample collection, and cohort methodology have been extensively described elsewhere.[19] Methods relevant to this work are described here. This study was reviewed and approved by the institutional review boards at the CDC, Cincinnati Children's Hospital Medical Center, and the enrolling birth hospitals: The Christ Hospital and University of Cincinnati Medical Center.

Sample collection and testing

Mothers collected mid-turbinate nasal swabs from infants on a weekly basis. Nasal samples were delivered to the study laboratory via courier within 48 hours of sample collection. Multiplex polymerase chain reaction (PCR) testing was performed on all samples using NxTAG Respiratory Pathogen Panel (Luminex Molecular Diagnostics, Toronto, Canada) which contains 18 viral targets including adenovirus, human bocavirus, endemic human coronaviruses (types 229E, HKU1, NL63, and OC43), influenza viruses (type A and B), human metapneumovirus, parainfluenza viruses (types 1, 2, 3, and 4), rhinovirus/enterovirus, and RSV (subtype A and B). Viral controls along with an internal control, bacteriophage MS2 (*Emesvirus zingeri*), were run to ensure adequate sample collection and assay validity.

Data collection

Automated weekly text-messaging surveys were sent to enrolled mothers via mobile phone to ascertain the presence of fever and/or cough. Reports of fever or cough triggered additional questions, including start-date of symptoms, if symptoms were ongoing or resolved, and presence of additional respiratory and gastrointestinal symptoms such as earache, runny nose, wheezing, vomiting, and diarrhea. Once resolved, end-of-illness surveys were sent to capture summary information on the episode, including duration of symptoms, all medically attended visits, and anti-microbial medication use. Abstraction of medical and immunization records further identified unreported medical office attendance, emergency department visits, and hospital admissions.

Outcome definitions

An **acute respiratory infection (ARI)** was defined as the presence of maternally reported fever (temperature ≥ 38^{o}C rectally or ≥37^{o}C axillary) or cough at any time in the past week.

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A **viral infection** was defined as a positive viral detection from a nasal swab. The detection was considered part of the same viral infection if the same virus or viral subtype(when applicable) was detected in two respiratory samples ≤30 days apart, regardless of any interval negative swabs.

A **viral ARI** was defined as a viral infection associated with ARI symptoms reported within ± 7 days of the first day of the viral infection.

Statistical analysis

For analyses involving symptomatology and healthcare-seeking behavior, viral infections with detection of multiple respiratory viruses were excluded because symptoms and severity could not be attributed to a single virus.[15] The full cohort was used for most analyses except for calculating the proportion of the cohort who experienced at least one viral infection by swab collection. In this calculation, a sub-cohort of 101 highly adherent participants who submitted at least 70% of eligible weekly samples and were followed to at least 18 months of age were used, as consistency of follow-up would significantly impact these calculations.

Descriptive analysis of age at first viral infection and median duration of viral ARI was performed using median and interquartile range (IQR). Non-parametric, pairwise comparisons were made using the Kruskal-Wallis rank-sum test with Holm-Sidak corrections. The denominators (weeks at risk) used for pathogen-specific viral infection incidence rates were calculated using the number of unique epidemiological weeks in which a swab was submitted per participant, minus all weeks in which that specific pathogen was detected. Weeks at risk for pathogen-specific viral ARIs were similarly calculated, but by subtracting the weeks during which the specific viral ARI occurred. Weeks at risk for incidence of any viral infection was calculated using the total weeks at risk without subtractions as it is assumed a child may be at risk for any respiratory virus for each swab. Weeks at risk for total viral ARI incidence was calculated by subtracting the number of weeks with any viral ARI from the total number of unique weeks of submitted swabs.

The odds ratio (OR) with 95% confidence intervals (CI) of a viral infection being symptomatic and being medically attended was calculated for each respiratory virus using logistic regression with generalized estimating equations (GEE) modeling and an exchangeable correlation matrix to account for clustering among participants, comparing each respiratory virus to all other respiratory viral pathogens combined as a composite reference group. Respiratory virus-specific attributable fraction (AF) was then calculated from the odds ratio of viral infections being asymptomatic vs. symptomatic using the formula, AF= (OR-1)/OR. An AF of 0 indicates that the odds of detecting a specific virus during an asymptomatic viral infection was equal to the odds of detecting that virus during a symptomatic viral infection (OR=1). All statistical analysis was performed using STATA (StataCorp, College Station, Texas) and the R Environment for Statistical Computing.[20]

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RESULTS

Demographics and cohort characteristics

A total of 265 mothers were enrolled in the third trimester of pregnancy, of whom 245 met final postpartum eligibility criteria. Most mothers were 25-34 years of age (63%), married or partnered (67%), and publicly insured (57%) (Table 1). A plurality of households reported an annual income of ≥$50,000 (43%), while nearly a third reported incomes below $25,000 (32%). Most children were born greater than 38 gestational weeks (78%), initiated breastfeeding (87%), and received at least one dose of influenza vaccine (70%).

Of 13,781 nasal swabs tested, 4,238 (31%) were positive for at least one respiratory virus. Based on our outcome definitions, these positive detections were further categorized into 2,211 viral infections and 821 viral ARIs, of which 1,470 (66%) viral infections and 508 (62%) viral ARIs did not involve co-detections of multiple respiratory viruses.

Viral infection incidence and odds of symptomatic infection

Enrolled children contributed a total of 12,242 unique child-weeks (or 235.4 child-years) of follow-up based on weeks of nasal swab submission. The overall incidence of any respiratory viral infection was 9.4 infections per child-year and the overall incidence of viral ARI was 3.3 infections per child-year (Table 2). The incidence of any respiratory viral infection and viral ARIs were similar between the highly-adherent cohort (n=101) and the non-highly adherent cohort (n=144) with 8.5 vs 10.4 infections per child-year for any viral infection and 3.4 vs 3.8 symptomatic infections per child-year, respectively.

Rhinovirus/enterovirus accounted for nearly half of all viral infections detected in the cohort (49%, n=1,076/2,211 viral infections) and had the highest incidence of both viral infection and viral ARIs among all respiratory viruses (6.42 viral infections per child-year and 1.89 viral ARIs per child-year). The lowest incidence was seen with influenza B virus with only eight viral infections detected, accounting for an incidence of 0.03 viral infections per child-year and 0.02 viral ARIs per child-year. The seasonality of respiratory viruses across study years is shown in supplemental figure 1.

Among the 2,211 viral infections identified in our cohort, 63% (n=1,390) were reported as asymptomatic. The odds of a detected viral infection being symptomatic varied widely across different respiratory viruses and viral subtypes (Table 3). RSV B (OR=8.9, 95% CI 3.94, 20.05; p=<0.01), parainfluenza 2 (OR=6.36, 95% CI 1.96, 20.65; p=0.002), and RSV A (OR=4.97, 95% CI 2.66, 9.28; p=<0.001) viral infections had the highest odds of being symptomatic compared with all other respiratory viruses detected by PCR combined as a reference group. Endemic coronaviruses (any type), bocavirus, and rhinovirus/enterovirus viral infections, when detected, were less likely to be symptomatic, although this was only statistically significant for bocavirus and rhinovirus/enterovirus.

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Viral infection acquisition by age

The median age and range of ages at which a participant acquired their first viral infection detected by PCR was variable based on the type of respiratory virus detected (Table 4). Children acquired their first rhinovirus/enterovirus infection significantly earlier (median age: 2 months, IQR 1-3 months, p <0.0001) when compared with all other respiratory viruses. For all other respiratory viruses, the median age at first viral infection occurred after six months of age.

By 12 months of age, 97% of the highly-adherent cohort had at least one rhinovirus/enterovirus infection detected, but a minority of participants at 12 months of age had experienced an infection from adenovirus (48%), RSV (40%), human metapneumovirus (24%), and influenza virus (11%). By two years of age, all participants had experienced at least one rhinovirus/enterovirus infection and most participants had experienced at least one parainfluenza virus (82%), endemic coronavirus (79%), bocavirus (77%), RSV (66%), and adenovirus (59%). A minority of participants had human metapneumovirus (47%) and influenza virus (27%) detected by two years of age.

Symptomatology of viral infections

The proportion of maternally-reported fever, earache, cough, vomiting, and diarrhea as well as the overlap between these symptoms varied widely by respiratory virus type (Figure 1 and Supplemental Figure 2). A combination of fever and cough were reported in 70% (n=14/20) of influenza ARIs and 54% (n=33/64) of RSV ARIs, while isolated fever or cough were uncommon (5% and 15% for influenza and 3% and 41% for RSV for fever and cough respectively) for both viruses (Supplement table 1). Isolated cough was seen in 61% (n=17/28) of human metapneumovirus, 54% (n=118/220) of rhinovirus/enterovirus, and 50% (n=9/18) of bocavirus ARIs, while isolated fever was seen in 35% (n=12/34) of adenovirus ARIs.

The median duration of symptoms during an ARI episode for all respiratory viruses was five days (IQR: 3-9 days; supplemental table 1). There was no significant difference in median duration of ARI between most respiratory viruses, except ARIs associated with RSV (7 days) were significantly longer than ARIs associated with adenovirus or endemic coronavirus (5 days each, p<0.05).

Healthcare utilization

Among all 1470 viral infections without co-detections, 17% (n=255) were medically attended. Among the medically attended infections, 13% (n=192) were evaluated by a primary care physician (PCP) or at an urgent care clinic, 4% (n=55) evaluated at an emergency department, and 0.5% (n=8) hospitalized (Figure 2). The proportions of viral infections that were medically attended varied, with RSV infections having the highest proportion of medical attendance (56%, n=47/84) while endemic coronavirus (12%, n=19/155), rhinovirus/enterovirus (12%, n=102/822), and bocavirus (6%, n=7/115) infections had the smallest proportion of infections

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that were medical attended. The odds of a viral infection being medically attended was highest for RSV infections (OR 7.03, 95% CI 4.48, 11.02; p=<0.001) compared with all other respiratory viruses; bocavirus (OR 0.32, 95% CI 0.15, 0.67; p=0.002) and rhinovirus/enterovirus (OR 0.45, 95% CI 0.34, 0.59; p=<0.001) infections had the lowest odds of medical attendance. Adenovirus (OR 2.49, 95% CI 1.48, 4.19; p=0.001) and influenza virus (OR 2.43, 95% CI 1.15, 5.15; p=0.02) infections had similar odds of medical attendance.

Hospitalizations were rare across all respiratory viruses. Two RSV infections, two rhinovirus/enterovirus infections as well as one human metapneumovirus, one coronavirus NL63, one bocavirus, and one adenovirus infection each resulted in hospitalization. There were no hospitalizations for viral infections due to influenza viruses or parainfluenza viruses in our cohort.

DISCUSSION

In our community-based, prospective U.S. birth cohort, respiratory viral infections were common in children during the first two years of life. We observed an average of nine respiratory viral infections per child annually, with detection of rhinovirus/enterovirus accounting for almost half (49%) of all infections. While the timing and frequency of viral acquisition was diverse across the cohort, most respiratory viral infections were asymptomatic and not medically attended. However, the odds of symptoms and medical attendance varied widely by respiratory virus. Viruses that are typically associated with severe disease, such as influenza and RSV, were also more likely to be symptomatic and medically attended in our cohort. However, the vast majority of influenza (90%) and RSV (85%) infections never required evaluation at an emergency department or hospital admission. Among non-influenza and RSV infections, parainfluenza viral infections, especially type 2 infections, had higher odds of being symptomatic than other viruses, while adenovirus infections had higher odds of medical attendance than other viruses. Bocavirus, endemic coronavirus and rhinovirus/enterovirus infections had lower odds of both developing symptoms and medical attendance, compared to all other viruses.

Our overall incidence rate estimate of 9.42 viral infections/child-year is comparable to those reported in the 'Observational Research in Childhood Infectious Disease' (ORCHID) birth cohort in Australia.[15] Similar to our study, ORCHID also utilized routine weekly nasal sampling regardless of symptoms, which better captures both symptomatic and asymptomatic childhood viral infections. Our findings supplement those of the ORCHID cohort, which was geographically distinct and performed during different respiratory seasons. In contrast, most birth cohorts rely on the ascertainment of specific respiratory symptoms in order to determine timing of sample collection, and report a lower incidence of viral infections.[21-29] The disparity in incidence rates likely reflects the under-detection of asymptomatic and subclinical infections, leading to an underestimation of the true incidence of respiratory viral infections.

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We found that few (4%) of all viral infections in our cohort were evaluated in the emergency department or resulted in hospitalization, suggesting that reliance on hospital-based surveillance also may miss a substantial number of asymptomatic and symptomatic viral infections among infants and young children. Also, studies that rely on hospital or emergency department surveillance may over-represent viruses associated with symptomatic and severe infections, such as influenza virus and RSV. Additional community-based, cohort studies that focus on routine longitudinal multiviral testing are needed to better characterize the experiences of respiratory viral infections in different settings and patient populations. The few published birth cohort studies that performed weekly or longitudinal viral sampling typically focused on the epidemiology of a single viral pathogen.[30-34]

There are several limitations in the interpretation of results related to our cohort methodology. The definition of a viral infection and parameters used to attribute symptoms to a viral infection were similar to comparable cohort studies,[1,15,16] but no standardized or universally agreed definition exists. Given the intermittency of viral detection, the cutoff utilized for defining a viral infection may imprecisely capture true episodes. This may be especially problematic for viruses such as human rhinovirus and bocavirus that are known to intermittently shed over a prolonged period.[32,33,35] Another important limitation is our inability to differentiate between rhinovirus and enterovirus, which is a common challenge found with many commercial multiplex PCR platforms. Utilizing the full cohort of 245 mother-infant pairs allows us to better describe the full diversity of respiratory viral infections, but adherence to weekly nasal sample submissions varied; 41% (n=101) of mother-infant pairs submitted at least 70% of eligible swabs and are demographically different from our full cohort (supplemental table 2).[19] However, in our sensitivity analysis, we found that incidence of viral infections and viral ARIs as well as proportion of medically attended infections were similar between the highly adherent and non-highly adherent cohort, a finding that is likely due to using weeks of viral sample testing as the person-time denominator. The adjunctive use of serology and molecular tests has been shown to better capture the full extent of viral infections experienced by children.[36] While the use of weekly longitudinal nasal specimen collection allows us to better capture the spectrum of asymptomatic and symptomatic viral infections, the addition of respiratory viral serological assays would supplement future studies from our cohort. Finally, our ARI symptom ascertainment focused on fever and/or cough which may miss symptomatic ARIs that presented with other isolated respiratory symptoms. However, given our study design and reliance on self-reported symptom surveys, fever and/or cough were targeted as these are specific and verifiable, while indicating a degree of clinical concern for children less than two years.

Overall, there was high adherence to our study protocol, including high rates of median weekly survey responses and nasal sample submission.[19] The collection of respiratory samples on a weekly basis across four respiratory seasons provided a more complete overview of the respiratory viral experience among young children in the community. In addition, Cincinnati Children's Hospital Medical Center is the major inpatient and outpatient pediatric provider for

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the region and serves >97% of children living in Hamilton County. The combination of data from medical chart abstraction and self-reported medical visits allowed us to confidentially capture most medically attended visits for participating children. As nearly all samples (98%) were collected prior to the first reported case of COVID-19 in the U.S, our study also provides a unique opportunity to characterize childhood respiratory viral infections in the pre-pandemic era.[37]

In conclusion, our community-based, prospective birth cohort provides a comprehensive overview on the incidence, symptomatology, and severity of viral infections in children 0-2 years of age and how these varied by respiratory virus. Viral infections were commonly detected during the first two years of life but the majority of infections, irrespective of virus or viral subtype, were asymptomatic and not medically attended. The high proportions of asymptomatic and non-medically attended infections has implications on our understanding of the dynamics of respiratory viral transmission in the community, and future research studies and public health policies must account for these infections.

NOTES

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Author Contributions: ZT, SC, DCP, ALM, and MAS conceptualized this study. ZT, SC, AB, RB, CM, and ALM assisted with data curation. ZT, SC, and ALM performed formal analysis. ALM, DCP, SC, AB, and MAS acquired financial support for the project. MML, AB, ALM, and MAS were responsible for conducting the research and investigation process. DCP, ALM, and MAS were responsible for development and design of methodology. AB, and MAS were responsible for project administration. MML, and MAS were responsible for provision of resources. ZT, SC, and ALM were responsible for software programming. DCP, MMW, ALM, and MAS provided oversight and supervision. ZT, SC, and ALM were responsible for validation. ZT conducted data visualization. ZT, SC, ALM, and MAS wrote the original draft. All authors reviewed and edited the manuscript

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. CDC. ZT, SC, ALM, MAS had full access to all the data used in this study and all authors had final responsibility for the decision to submit for publication.

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Abbreviations

ARI - Acute respiratory infection

CDC- Centers for Disease Control and Prevention

CI- Confidence interval

GEE- Generalizing estimating equations

IQR- Interquartile range

OR- Odds Ratio

ORCHID- Observational Research in Childhood Infectious Disease

PCR- Polymerase chain reaction

PCP- Primary care physician

PREVAIL- Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis Longitudinal

RSV- Respiratory syncytial virus

References

1. Sarna M, Ware RS, Sloots TP, Nissen MD, Grimwood K, Lambert SB. The burden of community-managed acute respiratory infections in the first 2-years of life. *Pediatr Pulmonol.* 2016;51(12):1336-1346.
2. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. *Pediatrics.* 2004;113(6):1758-1764.

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3. Troeger C, Blacker B, Khalil IA, et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases.* 2018;18(11):1191-1210.
4. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *The Lancet.* 2010;375(9725):1545-1555.
5. Jansen RR, Wieringa J, Koekkoek SM, et al. Frequent detection of respiratory viruses without symptoms: toward defining clinically relevant cutoff values. *J Clin Microbiol.* 2011;49(7):2631-2636.
6. Galanti M, Birger R, Ud-Dean M, et al. Rates of asymptomatic respiratory virus infection across age groups. *Epidemiol Infect.* 2019;147:e176.
7. de Steenhuijsen Piters WAA, Watson RL, de Koff EM, et al. Early-life viral infections are associated with disadvantageous immune and microbiota profiles and recurrent respiratory infections. *Nat Microbiol.* 2022;7(2):224-237.
8. Fauroux B, Simoes EAF, Checchia PA, et al. The Burden and Long-term Respiratory Morbidity Associated with Respiratory Syncytial Virus Infection in Early Childhood. *Infect Dis Ther.* 2017;6(2):173-197.
9. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. *J Pediatr.* 2009;154(5):694-699.
10. Poehling KA, Edwards KM, Griffin MR, et al. The burden of influenza in young children, 2004-2009. *Pediatrics.* 2013;131(2):207-216.
11. Hall CB, Weinberg GA, Blumkin AK, et al. Respiratory syncytial virus-associated hospitalizations among children less than 24 months of age. *Pediatrics.* 2013;132(2):e341-348.
12. Iwane MK, Prill MM, Lu X, et al. Human rhinovirus species associated with hospitalizations for acute respiratory illness in young US children. *J Infect Dis.* 2011;204(11):1702-1710.
13. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med.* 2009;360(6):588-598.
14. Taylor S, Lopez P, Weckx L, et al. Respiratory viruses and influenza-like illness: Epidemiology and outcomes in children aged 6 months to 10 years in a multi-country population sample. *J Infect.* 2017;74(1):29-41.
15. Sarna M, Lambert SB, Sloots TP, et al. Viruses causing lower respiratory symptoms in young children: findings from the ORChID birth cohort. *Thorax.* 2018;73(10):969-979.
16. Sarna M, Ware RS, Lambert SB, Sloots TP, Nissen MD, Grimwood K. Timing of First Respiratory Virus Detections in Infants: A Community-Based Birth Cohort Study. *J Infect Dis.* 2018;217(3):418-427.
17. Turner S. Childhood respiratory cohort studies: do they generate useful outcomes? *Breathe.* 2012;8(3):194.
18. Byington CL, Ampofo K, Stockmann C, et al. Community Surveillance of Respiratory Viruses Among Families in the Utah Better Identification of Germs-Longitudinal Viral Epidemiology (BIG-LoVE) Study. *Clin Infect Dis.* 2015;61(8):1217-1224.
19. Morrow AL, Staat MA, DeFranco EA, et al. Pediatric Respiratory and Enteric Virus Acquisition and Immunogenesis in US Mothers and Children Aged 0-2: PREVAIL Cohort Study. *JMIR Res Protoc.* 2021;10(2):e22222.

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20. Core Team R. R: A Language and Environment for Statistical Computing. 2014.
21. van Benten I, Koopman L, Niesters B, et al. Predominance of rhinovirus in the nose of symptomatic and asymptomatic infants. *Pediatr Allergy Immunol.* 2003;14(5):363-370.
22. Kusel MM, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J.* 2006;25(8):680-686.
23. von Linstow ML, Holst KK, Larsen K, Koch A, Andersen PK, Hogh B. Acute respiratory symptoms and general illness during the first year of life: a population-based birth cohort study. *Pediatr Pulmonol.* 2008;43(6):584-593.
24. van der Zalm MM, Uiterwaal CS, Wilbrink B, et al. Respiratory pathogens in respiratory tract illnesses during the first year of life: a birth cohort study. *Pediatr Infect Dis J.* 2009;28(6):472-476.
25. Toivonen L, Karppinen S, Schuez-Havupalo L, et al. Burden of Recurrent Respiratory Tract Infections in Children: A Prospective Cohort Study. *Pediatr Infect Dis J.* 2016;35(12):e362-e369.
26. Regamey N, Kaiser L, Roiha HL, et al. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J.* 2008;27(2):100-105.
27. Kumar P, Mukherjee A, Randev S, et al. Effect of acute respiratory infections in infancy on pulmonary function test at 3 years of age: a prospective birth cohort study. *BMJ Open Respir Res.* 2020;7(1).
28. Emanuels A, Hawes SE, Newman KL, et al. Respiratory viral coinfection in a birth cohort of infants in rural Nepal. *Influenza Other Respir Viruses.* 2020;14(6):739-746.
29. Anders KL, Nguyen HL, Nguyen NM, et al. Epidemiology and virology of acute respiratory infections during the first year of life: a birth cohort study in Vietnam. *Pediatr Infect Dis J.* 2015;34(4):361-370.
30. Galanti M, Shaman J. Direct Observation of Repeated Infections With Endemic Coronaviruses. *J Infect Dis.* 2021;223(3):409-415.
31. Frank AL, Taber LH, Wells CR, Wells JM, Glezen WP, Paredes A. Patterns of shedding of myxoviruses and paramyxoviruses in children. *J Infect Dis.* 1981;144(5):433-441.
32. Martin ET, Kuypers J, McRoberts JP, Englund JA, Zerr DM. Human Bocavirus 1 Primary Infection and Shedding in Infants. *J Infect Dis.* 2015;212(4):516-524.
33. Loeffelholz MJ, Trujillo R, Pyles RB, et al. Duration of rhinovirus shedding in the upper respiratory tract in the first year of life. *Pediatrics.* 2014;134(6):1144-1150.
34. Munywoki PK, Koech DC, Agoti CN, et al. The source of respiratory syncytial virus infection in infants: a household cohort study in rural Kenya. *J Infect Dis.* 2014;209(11):1685-1692.
35. Howard LM, Johnson M, Gil AI, et al. Molecular Epidemiology of Rhinovirus Detections in Young Children. *Open Forum Infect Dis.* 2016;3(1):ofw001.
36. Zhang Y, Sakthivel SK, Bramley A, et al. Serology Enhances Molecular Diagnosis of Respiratory Virus Infections Other than Influenza in Children and Adults Hospitalized with Community-Acquired Pneumonia. *J Clin Microbiol.* 2017;55(1):79-89.
37. Holshue ML, DeBolt C, Lindquist S, et al. First Case of 2019 Novel Coronavirus in the United States. *N Engl J Med.* 2020;382(10):929-936.

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Table 1. Demographic characteristics of mother-infant pairs enrolled in the PREVAIL cohort (n=245), Cincinnati, Ohio, April 2017-July 2020

Characteristics	n (%)
Maternal age	
18-24 years	50 (20.4%)
25-34 years	155 (63.3%)
≥ 35 years	40 (16.3%)
Maternal race	
White	127 (51.8%)
Black	106 (43.3%)
Other	12 (4.9%)
Insurance	
Public	139 (56.7%)
Private	106 (43.3%)
Marital status	
Married/partnered	163 (66.5%)
Single	82 (33.5%)
Maternal education	
Less than high school	22 (9.0%)
High school graduate	93 (38.0%)
Associate/trade	35 (14.3%)
College graduate	95 (38.8%)
Annual household income	
<US $25,000	78 (31.8%)
US $25,000-US $49,999	49 (20.0%)
≥US $50,000	106 (43.3%)
Unknown	12 (4.9%)
Infant sex	
Female	127 (51.8%)
Male	118 (48.2%)
Infant gestational age	
35-36 weeks	11 (4.5%)
37 weeks	44 (18.0%)
38-42 weeks	190 (77.6%)
Breastfeeding	
Initiated	212 (86.5%)
Never breastfed	33 (13.5%)
Infant influenza vaccine status	
Never received influenza vaccine	74 (30.2%)
Received at least one dose of influenza vaccine	171 (69.8%)

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Table 2. Swab positivity and incidence of viral infections and viral ARIs by respiratory virus in the PREVAIL cohort (n=245), by respiratory virus.

Pathogen	Positive weeks, n (%)	Viral infections		Viral ARIs	
		n	Incidence per child-year[a] (95% CI)	n	Incidence per child-year[a] (95% CI)
Adenovirus	302 (7.8%)	196	0.86 (0.74, 0.99)	81	0.35 (0.28, 0.44)
Bocavirus	764 (19.7%)	257	1.19 (1.05, 1.34)	70	0.32 (0.25, 0.41)
Coronavirus 229E	19 (0.5%)	13	0.06 (0.03, 0.09)	1	0.00 (0.00, 0.02)
Coronavirus HKU1	78 (2.0%)	62	0.27 (0.20, 0.34)	20	0.09 (0.05, 0.13)
Coronavirus NL63	138 (3.6%)	74	0.32 (0.25, 0.40)	26	0.11 (0.07, 0.16)
Coronavirus OC43	134 (3.5%)	88	0.38 (0.30, 0.47)	37	0.16 (0.11, 0.22)
Influenza A	50 (1.3%)	37	0.16 (0.11, 0.22)	25	0.11 (0.07, 0.16)
Influenza B	10 (0.3%)	8	0.03 (0.01, 0.07)	5	0.02 (0.01, 0.05)
Human Metapneumovirus	108 (2.8%)	75	0.32 (0.25, 0.40)	38	0.16 (0.12, 0.22)
Parainfluenza 1	30 (0.8%)	25	0.11 (0.07, 0.16)	15	0.06 (0.04, 0.11)
Parainfluenza 2	25 (0.6%)	23	0.10 (0.06, 0.15)	16	0.07 (0.04, 0.11)
Parainfluenza 3	129 (3.3%)	100	0.43 (0.35, 0.52)	55	0.24 (0.18, 0.31)
Parainfluenza 4	62 (1.6%)	48	0.21 (0.15, 0.27)	26	0.11 (0.07, 0.16)
Rhinovirus/Enterovirus	2462 (63.4%)	1,076	6.42 (6.04, 6.81)	317	1.89 (1.69, 2.11)
RSV A	112 (2.9%)	69	0.30 (0.23, 0.38)	45	0.19 (0.14, 0.26)
RSV B	79 (2.0%)	60	0.26 (0.20, 0.33)	44	0.19 (0.14, 0.25)
Any virus detected		2,211	9.39 (9.00, 9.78)[b]	691[c]	3.30 (3.01, 3.50)[d]

[a]Incidence rate and 95% CI provided in child-years

[b]Total duration of follow-up (235.40 years) used as denominator as it is assumed a child may be at risk for any virus for each swab

[c]Excluded viral infections associated with same ARI episode

[d]Years-at-risk calculated as total duration of follow-up (235.40 years) minus years of viral ARI (23.22 years)=212.17 years as it is assumed a child with a current viral ARI is not at risk for a new viral ARI

Table 3. Asymptomatic and symptomatic[a] viral infections, odds ratio of symptomatic infection, and attributable fraction in the PREVAIL cohort (n=245), by respiratory virus

Pathogen[b]	Viral infections[c]	Asymptomatic infections	Symptomatic infections	Odds of symptomatic infection, compared to all other pathogens (95% CI)	p value	Attributable fraction in exposed (95% CI)
RSV B	37	6	31	8.89 (3.94, 20.05)	**<0.001**	89% (75%, 95%)
Parainfluenza 2	16	4	12	6.36 (1.96, 20.65)	**0.002**	84% (49%, 95%)
RSV A	47	14	33	4.97 (2.66, 9.28)	**<0.001**	80% (62%, 89%)
Influenza A	26	9	17	3.85 (1.60, 9.22)	**0.003**	74% (38%, 89%)
Influenza B	5	2	3	3.69 (0.71, 19.11)	0.119	73% (-41%, 95%)
Parainfluenza 1	19	8	11	2.91 (1.15, 7.40)	**0.025**	66% (13%, 86%)
Parainfluenza 4	37	17	20	2.52 (1.29, 4.93)	**0.007**	60% (23%, 80%)

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Parainfluenza 3	67	33	34	2.09 (1.32, 3.30)	**0.002**	52% (24%, 70%)
Human Metapneumovirus	56	28	28	2.05 (1.20, 3.49)	**0.009**	52% (17%, 71%)
Adenovirus	68	34	34	1.81 (1.04, 3.15)	**0.036**	45% (4%, 68%)
Coronavirus OC43	61	41	20	0.91 (0.52, 1.59)	0.736	-10% (-92%, 37%)
Coronavirus NL63	45	31	14	0.86 (0.49, 1.51)	0.596	-16% (-104%, 34%)
Coronavirus HKU1	40	28	12	0.75 (0.38, 1.50)	0.417	-33% (-163%, 33%)
Rhinovirus/Enterovirus	822	602	220	0.45 (0.36, 0.56)	**<0.001**	-122% (-178%, -79%)
Bocavirus	115	97	18	0.36 (0.22, 0.60)	**<0.001**	-178% (-355%, -67%)
Coronavirus 229E	9	8	1	0.22 (0.02, 3.02)	0.258	-355% (-4900%, 67%)

a Symptomatic is defined as the presence of fever and/or cough with or without the presence of other respiratory or gastrointestinal symptoms

b Ordered by OR of symptomatic infection, highest to lowest

c Viral infections without co-detection of multiple respiratory viruses (n=1470)

Table 4. Age at first viral infection and proportion of cohort who experienced at least one respiratory viral infection detected by PCR of nasal swab in the highly-adherent PREVAIL cohort (n=101), by respiratory virus.

Pathogen	Age in months at first viral infection[a]	Proportion of cohort with at least one viral infection detected by nasal swab	
		By 12 months	By 24months
Adenovirus	8 (6-11) [1]	48%	59%
Bocavirus	10 (6-15) [2]	50%	77%
Endemic Coronavirus	11 (6-17)	55%	79%
229E	18 (11-19)	3%	9%
HKU1	13 (7-18)	20%	45%
NL63	11 (5-15.5)	23%	44%
OC43	11 (6-13)	31%	50%
Influenza virus	17 (10-20)	11%	27%
Influenza A	17 (10-20)	9%	25%
Influenza B	11.5 (6-20)	2%	4%
Human Metapneumovirus	11 (8-18)	24%	47%
Parainfluenza virus	12 (7-17)	50%	82%
PIV1	18.5 (15-21.5)	3%	20%
PIV2	9 (7-13)	9%	17%
PIV3	10 (6-14)	38%	57%
PIV4	15 (9-19)	10%	31%
Rhino/Enterovirus	2 (1-3) [3]	97%	100%
Respiratory Syncytial Virus	12 (7-18)	40%	66%
RSVA	15.5 (9-19)	16%	42%
RSVB	9 (6-17)	25%	41%

a Presented as median (interquartile range)

1 Significantly differs from median age at first viral infection of influenza virus (p=0.005), parainfluenza virus (p=0.013), and RSV (p=0.018)

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2 Significantly differs from median age at first viral infection of influenza virus (p=0.005)
3 Significantly differs from median age at first viral infection of all other respiratory virus using Kruskal-Wallis, p=<0.0001

FIGURE LEGENDS:

Figure 1. Proportional Venn diagram of symptom overlap seen during viral AR is without co-detection (n=508) in the PREVAIL cohort (n=245), by respiratory viruses (subtypes combined).

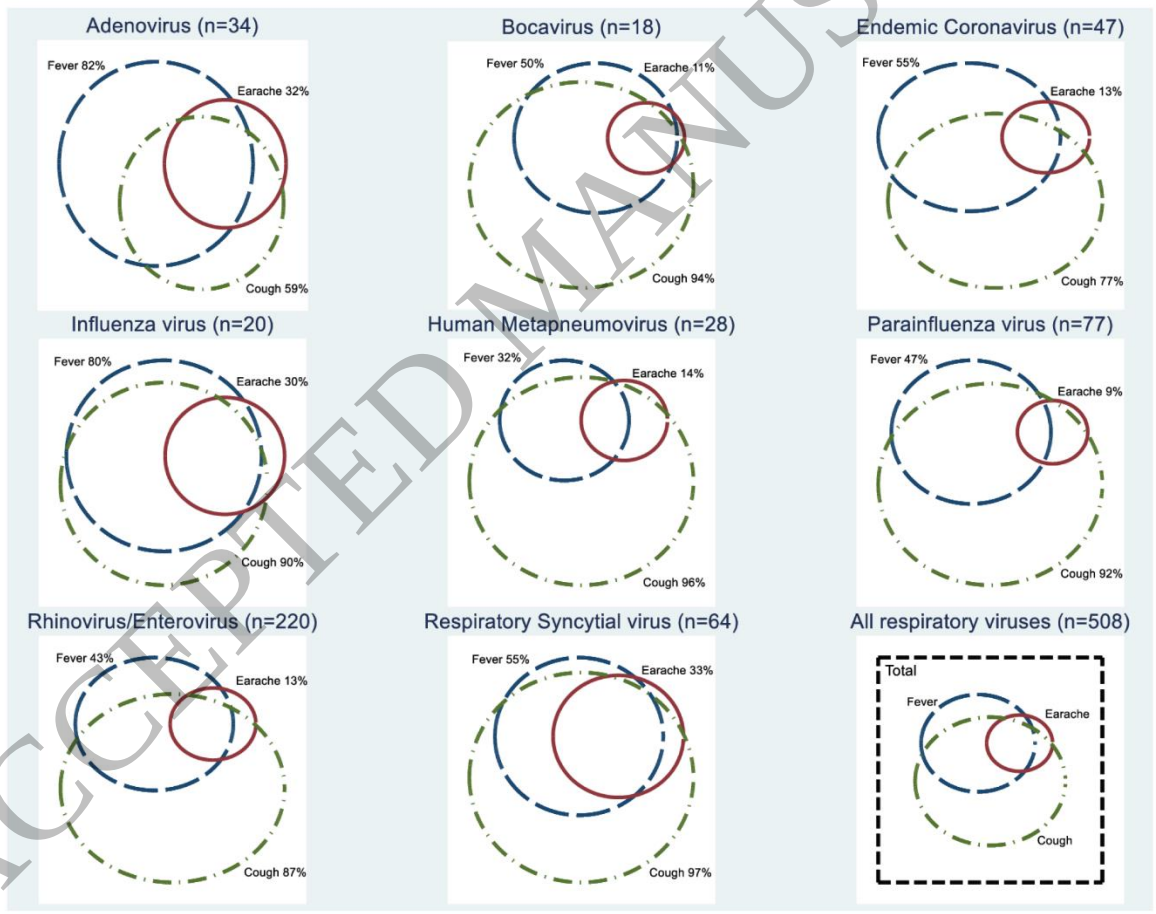

Figure 2. Medical utilization patterns of viral infections without co-detection of other viruses (n=1470) and odds ratio of a viral infection being medically attended in the PREVAILcohort (n=245), by respiratory virus (subtype combined).

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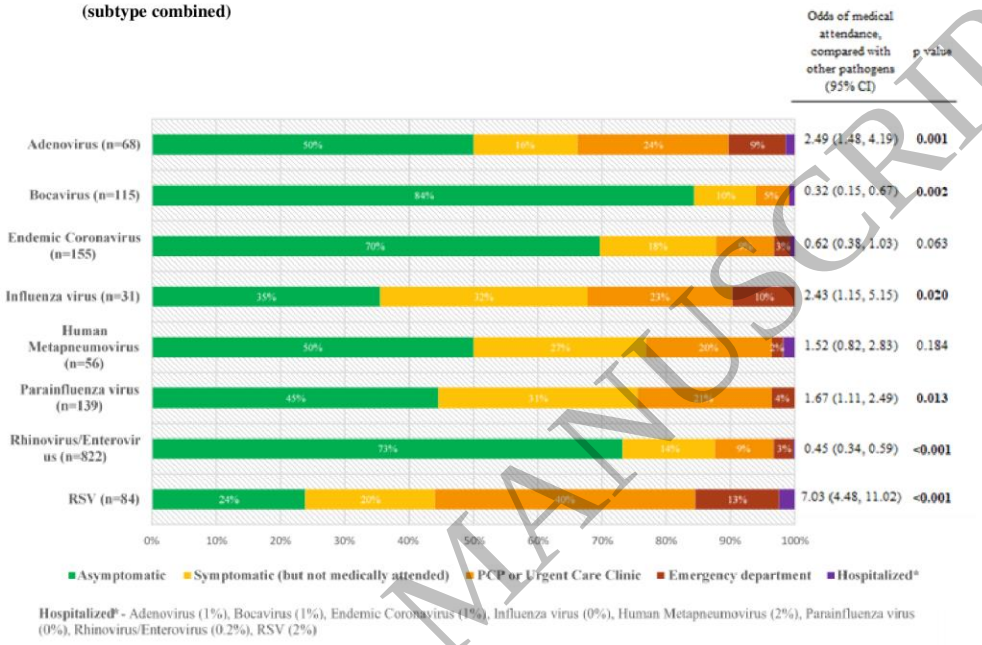
Figure 2. Medical utilization patterns of viral infections without co-detection of other viruses (n=1470) and odds ratio of a viral infection being medically attended in the PREVAIL cohort (n=245), by respiratory virus (subtype combined)



Hospitalized* - Adenovirus (1%), Bocavirus (1%), Endemic Coronavirus (1%), Influenza virus (0%), Human Metapneumovirus (2%), Parainfluenza virus (0%), Rhinovirus/Enterovirus (0.2%), RSV (2%)

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