Epidemiology of Multiple Respiratory Viruses in Childcare Attendees

Emily T. Martin,1 Mary P. Fairchok,2 Zach J. Stednick,3 Jane Kuypers,4 and Janet A. Englund5

1Department of Pharmacy Practice, Wayne State University, Detroit, Michigan; 2Department of Pediatrics, Madigan Army Medical Center, Tacoma, Washington; 3Vaccine and Infectious Diseases Department, Fred Hutchinson Cancer Research Center, Seattle, Washington; 4Department of Laboratory Medicine, University of Washington, Seattle, Washington; and 5Department of Pediatrics, Seattle Children’s Hospital, Seattle, Washington

Background. The identification of multiple viruses during respiratory illness is increasing with advances in rapid molecular testing; however, the epidemiology of respiratory viral coinfections is not well known.

Methods. In total, 225 childcare attendees were prospectively followed for up to 2 years. Nasal swabs were collected at respiratory illness onset and every 7–10 days until illness resolution. Swabs were tested by polymerase chain reaction for 15 respiratory viruses and subtypes.

Results. At least 1 virus was detected in 382 (84%) of 455 new-onset illnesses with multiple viruses identified in 212 (46%). The proportion of subject swabs with multiple viruses detected changed as respiratory illnesses progressed from week to week, as did the prevalence of individual viruses. Children with multiple viruses detected at the time of illness onset had less frequent fever (odds ratio [OR], 0.56; 95% confidence interval [CI], 0.35, 0.90), however, these children more often had illness symptoms lasting over 7 days (OR, 1.94; 95% CI, 1.20, 3.14).

Conclusions. A high proportion of daycare attendees had multiple viruses detected during respiratory illnesses. Delay between onset of illness and viral detection varied by virus, indicating that some viruses may be underrepresented in studies of virus epidemiology that rely on only a single test at symptom onset.

Keywords. respiratory virus; coinfection; childcare.
available regarding children with multiple viruses experiencing mild respiratory symptoms who do not require inpatient or emergency room care. Additionally, the progression of viral illnesses associated with multiple viruses over time is not well characterized, as the majority of previous studies have focused on single samples collected at admission. In this study, we analyzed the incidence and outcomes of multiple virus respiratory illnesses among healthy young children followed prospectively over 3 respiratory seasons during a study of the epidemiology of respiratory viruses in childcare [25, 26].

MATERIALS AND METHODS

Patients and Methods

Study Design

Children between 5 weeks and 30 months of age attending 3 childcare centers on a military base in Tacoma, Washington, were eligible for enrollment. Eligibility criteria included at least 20 hours per week of childcare attendance and provision of informed consent by the parents. Children expected to leave the center within 3 months were excluded from enrollment. Between 1 February 2006 and 28 April 2008 and again from 28 October 2008 to 30 June 2009, children were continuously enrolled throughout the study period and followed until 40 months of age, or until they no longer met the eligibility criteria (eg, reduced enrollment to <20 hours per week).

At the time of enrollment, baseline demographics, household characteristics, and medical history were obtained from parent interviews and participant medical records. Midturbinate nasal swabs were collected at the study enrollment visit by the study nurse whether symptoms were present or not. Children were followed throughout the study period for incident respiratory illness, defined as at least 2 of 5 symptoms including cough, rhinorrhea, wheezing, fever (typanic or rectal temperature of ≥100.4°F, or axillary of ≥99.4°F), and nasal congestion. At illness onset, the study nurse was contacted by parents and/or the childcare provider, and the nurse contacted the childcare center and/or parents weekly to identify unreported illness and follow illness progression. The study nurse reviewed the illness symptoms with the parent, and a midturbinate nasal swab was collected at illness onset. Swabs were repeated at 7–10 day intervals until resolution of symptoms or until polymerase chain reaction (PCR) results were negative, whichever came first. Parents completed a daily symptom diary for 10 days following initial illness symptoms. These data were compiled to determine the occurrence of extended respiratory symptoms, defined as at least one of the following lasting over 7 days: wheeze, cough, congestion, and/or hoarseness.

If the child’s illness required medical care, one of 3 study physicians documented medical visit information related to the illness(es) using a standardized form.

Results

Altogether, 225 children enrolled in center-based daycare in Fort Lewis, Washington, were followed for an average of 264 days, with a range of 12–811 days. The mean age at enrollment was 10 months (range, 5 weeks to 25 months of age). Participants were 49% male (n = 110), and 99 (44%) identified as white, 60 (27%) as black, 8 (4%) as Asian/Pacific Islander, 32 (14%) as Hispanic, and 39 (7%) as multiple races/ethnicities (Table 1). Only 4 (1.8%) children had a smoker in the home. One hundred and thirteen (50%) children had at least one sibling (median siblings, 1; range, 0–5). All participants were dependents of military personnel or civilians working on base, and most children had at least one active-duty parent (n = 201; 89%). We captured 455 incident illnesses in 163 children, and 109 (67%) children had more than one incident
respiratory illness (median, 2 illnesses; IQR, 1–4). Mean age at illness was 12 months (range, 1.7–39 months of age). At study enrollment, 127 children had no respiratory symptoms present and had a nasal swab collected at that time. Thirty percent (n = 38) of these asymptomatic samples had no respiratory virus detected, one respiratory virus was detected in 57 (45%) of samples, and multiple viruses were detected in 32 samples (25%; 2 viruses in 27 [21%] samples; 3 viruses in 5 [4%]).

Weekly respiratory samples collected during incident illness events detected at least one respiratory virus at onset or during the follow-up of 382 (84%) illnesses. A single respiratory virus was detected throughout the duration of 170 (37%) illnesses (single virus illness [SVI]), and multiple respiratory viruses were detected throughout the duration of 212 (46%) illnesses (multiple virus illness [MVI]). Two viruses were detected during 130 (61%) MVIs, 3 viruses during 54 (25%) MVIs, 4 viruses during 24 (11%) MVIs, and 5 viruses during 4 (2%) MVIs. No patient characteristics were identified to be risk factors for the presence of multiple viruses at illness onset (Table 1). Among children with a single virus detected at illness onset (week 0; n = 219), detection of additional viruses later in illness increased the duration of extended respiratory symptoms, although this was not statistically significant (OR, 1.37; 95% CI, .68, 2.74; P = .38). Other markers of illness severity did not differ between children with multiple viruses detected throughout the course of illness compared with children with only a single virus, based on visits to a healthcare provider (OR, 1.09; 95% CI, 0.73, 1.63; P = .66) and antibiotic prescriptions (OR, 1.03; 95% CI, 0.60, 1.77; P = .91). Only one child was hospitalized—a 9–month-old infant with RSV, AdV, HRV, and HCoV detected during the course of illness.

Multiple viruses were detected right at the time of illness onset (week 0) in 163 (36%) of illnesses. Interviews at illness onset, and daily symptom diaries were completed for 152 of these multiple virus illnesses and for 360 illnesses overall. When compared to single virus illnesses, the detection of multiple viruses at illness onset was significantly associated with a lower prevalence of fever at the onset of symptoms (OR, 0.56; 95% CI, .35, .90; P = .02; Table 2), but these children more frequently had extended respiratory symptoms (defined as at least one of the following lasting over 7 days: wheeze, cough, congestion, and/or hoarseness; OR, 1.94; 95% CI, 1.20, 3.14; P = .007).

The proportions of illnesses with multiple viruses detected throughout the illness, by virus, were as follows: 38 of 53 (72%) RSV illnesses, 20 of 26 (77%) HMPV, 147 of 223 (66%) HRV, 97 of 121 (80%) HBoV, 59 of 70 (84%) HCoV, 60 of 78 (77%) PIV (all types), 10 of 15 (67%) influenza (all types). Possible synergistic and antagonistic patterns (red and blue, respectively) in the presence and quantity of virus combinations are detailed in Figure 1. AdV, HBoV, HCoV, HMPV, and HRV frequently occurred together, as indicated by red boxes. RSV and HRV were detected in the same specimen less often than expected by chance (Pearson correlation coefficient: −0.12; P < .005). No other specific virus patterns were statistically significant.

Median and range log viral load overall was as follows: RSV: 7.0 (3.1–9.4); HMPV: 5.9 (4.0, 9.4); HBoV1: 4.3 (2.9–

| Characteristic | No Viruses Detected (n = 73) | Single Virus at Week 0 (N = 219) | Multiple Viruses at Week 0 (n = 163) | OR Comparing Multiple to Single Virusesa (95% CI; P) |
|----------------|-------------------------------|-----------------------------------|-------------------------------------|--------------------------------------------------|
| Male           | 36 (49)                       | 109 (50)                          | 97 (60)                             | 1.49 (.98, 2.27; .06)                             |
| Age at illness, months |                               |                                   |                                     |                                                  |
| <6             | 5 (7)                         | 37 (17)                           | 20 (12)                             | Ref                                              |
| 6–23           | 64 (88)                       | 171 (78)                          | 135 (83)                            | 1.48 (.88, 2.47; .14)                            |
| ≥24            | 4 (5)                         | 11 (5)                            | 8 (5)                               | 1.34 (.70, 2.57; .38)                            |
| Raceb          |                               |                                   |                                     |                                                  |
| White          | 36 (49)                       | 102 (47)                          | 69 (42)                             | ref                                              |
| Black          | 23 (32)                       | 52 (24)                           | 46 (28)                             | 1.31 (.77, 2.24; .32)                            |
| Asian/Pacific Is. | 1 (1)                        | 9 (4)                             | 3 (2)                               | .50 (.12, 2.10; .34)                             |
| Other/Multiple | 12 (16)                       | 53 (25)                           | 41 (25)                             | 1.16 (.69, 1.95; .58)                            |
| Tobacco use in home | 1 (1)                    | 3 (1)                             | 2 (1)                               | .89 (.14, 5.79; .90)                             |
| Any siblings   | 44 (60)                       | 105 (48)                          | 91 (56)                             | 1.37 (.91, 2.07; .13)                            |
| Hours/week of childcare |                 |                                   |                                     |                                                  |
| 20–39          | 11 (15)                       | 33 (15)                           | 33 (20)                             | ref                                              |
| ≥40            | 62 (85)                       | 186 (85)                          | 130 (80)                            | .70 (.38, 1.30; .26)                             |

a Confidence intervals (CI) correct for correlation between multiple illnesses collected from individual children using generalized estimating equations with a robust variance estimator.
b Race not reported for 8.
11.1); AdV: 4.1 (2.0–9.1); CoV: 6.4 (2.8–10.1); PIV1: 6.8 (5.9–7.7); PIV3: 5.8 (2.2–9.0); PIV4: 5.6 (3.9–9.8); FluA: 6.7 (3.6–8.6); FluB: 6.7 (5.1, 8.2). Semiquantitative results were obtained for rhinovirus, which had a median cycle threshold (Ct) of 28.2 (range, 16.6–40). HRV viral load, approximated by Ct was significantly lower when detected along with HMPV (median 33 with HMPV vs median 28 in all other HRV detections). No other significant associations in prevalence or quantity of specific virus combinations were found, and no overall association was found between viral load and the presence of multiple viruses.

The proportion of subject swabs with multiple viruses detected changed as respiratory illnesses progressed from week to week, as did the prevalence of individual viruses (Figure 2). The viruses detected at illness onset (week 0) did not fully represent all viruses detected throughout the course of illness, as additional incident viruses appeared at weeks 1 through 4 of follow-up. The frequency of additional virus detection during follow-up varied by specific virus: only 1 of 53 (2%) RSV detections occurred for the first time after one week of illness, followed by 6 of 78 (8%) for PIV types 1–4, 4 of 26 (15%) for HMPV, 14 of 70 for HCoV (20%), 3 of 15 (20%) for influenza, 25 of 121 (21%) for HBoV, 27 of 122 (22%) for AdV, and 38 of 223 (17%) for HRV. We also found that the duration of documented shedding varied by specific virus. Extended shedding (defined as sequential detections of the same virus at least 7 days apart with no more than a single interim negative) was documented in 0 of 15 (0%) cases of Flu A and B, 3 of 26 cases (12%) of HMPV, 12 of 53 cases (23%) of RSV, 35 of 121 cases (29%) of HBoV, 40 of 122 cases (33%) of AdV, 24 of 70 cases (34%) of HCoV, 62 of 223 cases (28%) for HRV, and 34 of 78 cases (44%) for PIV1–4. The occurrence of extended shedding was associated with MVIs for HBoV (0 of 24 SVI vs 35 of 97 MVIs, P < .001, Fisher exact test), and HRV (2 of 76 SVIs vs 60 of 147 MVIs, P < .001, Fisher exact test).
The median duration of shedding (defined as days from the first to last positive swab in an illness with no more than one interim negative) was frequently longer among MVIs (up to a median of 11.5 days for HRV, 12 days for RSV, and 12.5 days for AdV); however, the presence of multiple viruses overall was not significantly associated with shedding duration (Table 3).

**DISCUSSION**

The detection of multiple coincident viruses in clinical settings is more common with the introduction of molecular-based, multiplex point-of-care tests into hospitals and clinics [31], but the clinical significance of the detection of multiple viruses has been unclear. In this prospectively followed child-care cohort, we detected multiple viruses at a high rate among children with respiratory infections of mild to moderate severity. This percentage of illnesses with multiple viruses (47%) was higher than other recent studies of viral coinfections that have found multiple viruses in up to 40% of patients [2]. Our high rate of MVIs appeared to be a function of the high incidence of new viruses detected not only at illness onset but throughout the respiratory illness as well, in addition to the long durations of shedding seen with almost all viruses except influenza.

Other factors in our study design likely impacted the high percentage of MVIs. Our design allowed us to collect study samples very close to the onset of illness symptoms, rather than waiting until the children presented for medical care. As we demonstrate here, the timing of sample collection impacts the number and types of viruses detected. The large number of MVIs might also be influenced by our study population of healthy children with mild respiratory illness, in contrast to most studies in hospitalized children. Recent work by our group [32] and others [20, 22] have found that the prevalence of MVIs is higher in nonhospitalized children. No demographic or household characteristics, including age of child, were found to be associated with MVIs.

This study contributes new information on the potential severity of MVIs in children with respiratory infections. Children with MVIs did not have more severe illness initially than children with single virus illnesses. Children with multiple
viruses detected at illness onset had significantly lower rates of fever, however children with MVIs at onset did have longer duration of illness symptoms. We have previously reported decreased severity associated with MVI in hospitalized children, with an increased risk of oxygen requirement, extended hospital stay, and inpatient and intensive care unit admission among children with SVI [32]. Our finding of decreased severity among children with MVI has been [20, 22, 23] contrasts with reports of greater severity of illness in children with MVI [1, 10–16]. These latter studies only collected samples at the time children were admitted to the inpatient ward or emergency department; only one study included outpatients [12].

Also, many studies addressed the severity of coinfections specifically with RSV [10–12, 14, 15] while we focused on MVI patterns among 15 different viruses and subtypes of viruses.

Our analysis is strengthened by the identification of illnesses during regular, prospective follow-up by a study nurse, using parental and daycare staff input. We collected samples at the onset of a new illness, rather than waiting until children presented for medical care. The regular, repeated sampling also allowed us to document the presence and persistence of multiple viruses during a single illness. The pool of viruses detected during a respiratory illness changed over time. A surprising number of virus detections (19% overall) occurred subsequent to the onset of illness. Our results demonstrate that a single test for respiratory viruses at illness onset does not capture all viruses contributing to the severity and course of illness in children.

Rhinovirus was the most common virus detected in our study. The clinical importance of HRV detection has now clearly been associated with severe disease leading to hospitalization in children <5 years of age [33, 34]. Rhinovirus has also been identified as an important coinfection in invasive pneumococcal disease, where it is associated with increased severity of disease [7–9] and with invasive pneumococcal disease due to typically noninvasive pneumococcal serotypes [6]. The role of HRV in virus-virus infections has been unclear. HRV has been found by some investigators to be frequently codetected with other viruses [24], while others have suggested that HRV may have a competitive relationship with other viruses. In a study of 1742 specimens, Brunstein and colleagues reported a number of instances of suspected pathogen co-suppression between specific viral combinations, particularly between single-stranded RNA viruses [35]. Greer et al reported that, among 1247 specimens, HRV was negatively associated with coinfection with AdV, CoV, HBoV, HMPV, RSV, PIV, influenza A, and polyomaviruses [36].

We found an inverse correlation between the detection of RSV and HRV, indicating these viruses occur together less frequently than if the virus combinations were distributed equally throughout the population. This finding provides supportive evidence for the possibility of co-suppression. Potentially, the immune response to a first infection decreases the risk of infection by a second virus due to the induction of cytokines or other factors known to prevent viral infection. Jartti et al reported that children with atopy have a higher risk of HRV but a lower risk of RSV [37], suggesting that the risks of acquisition of these two viruses are differentially affected by a child’s immunologic state. This would make coinfections with both viruses in the same child simultaneously less likely. Other authors have similarly found nonrandom distribution of specific virus combinations [38].

Several groups have suggested that specific virus pairings may be characterized by one dominant virus paired with one nondominant virus, defined either by viral load [39] or specific virus groupings [21, 40]. While we did not have the sample size necessary to fully explore this, we did find that only 39% of MVIs included a virus from RSV, influenza, or PIV, whereas all of our multiple virus illnesses included at least one virus of HCoV, AdV, HBoV, HRV, or HMPV.

Longitudinal sampling allowed us to examine in detail the shedding patterns of each virus, both as a sole viral pathogen and when detected during a MVI. Given the high prevalence and frequent extended shedding of HBoV and HRV, it is not surprising that these viruses were frequently detected in co-infections. This may simply be a result of persistent infection extending over a prolonged time period, making it more likely
for an infection with a second virus to occur. The impact of persistent shedding on the severity of incident illness remains unknown. Our study found prolonged shedding of HRV for up to 41 days by PCR and of HBoV for up to 44 days following the onset of initial symptoms. We did not detect any extended shedding of HBoV when it was detected alone, yet extended shedding of HBoV was detected in 36% of multiple virus illnesses, similar to other studies evaluating HBoV time. This suggests that 2 potential pathways of HBoV infection may exist: one with a short, acute, and more symptomatic, single virus infection (perhaps the primary infection) and the other pathway with a long-shedding, perhaps more indolent, infection during which other viruses are frequently detected.

Long shedding patterns add another perspective to the ongoing challenge to determine when a detected virus is actually the cause of clinical symptoms [41, 42]. Reports of high rates of viral detection among asymptomatic children [25, 37, 43, 44] emphasize that not all viruses detected from a patient are necessarily causal agents of disease in that individual at the time they are detected. Our data showing extended detection of respiratory viruses emphasize the need for caution when studying associations between respiratory viruses and illness symptoms, particularly when using cross-sectional sampling to evaluate the clinical correlates of newly discovered viruses.

We did not have the benefit of daily sample collection, which would have allowed us to pinpoint the exact duration of shedding of each of the viruses; instead we collected samples only at 7–10 day intervals, making it likely that we have underestimated duration of shedding overall (Table 3). It is possible that daily sampling would have documented shedding across a shorter time frame, especially for cases of influenza which have been shown to shed for approximately 5–7 days among pediatric and adult outpatients [45]. Nonetheless, we were still able to document prolonged shedding in all of the noninfluenza viruses tested.

In this study, multiple viruses were frequently detected during the same illness among young children attending childcare. We did not find increased illness severity among children with multiple viruses detected. Detection of multiple viruses changed frequently throughout the course of symptomatic disease in young children attending daycare, a dynamic and interactive location where children share frequent and close contact with one another. Incident viruses detected later in illness progression include a combination of persistent and new incident viruses. Our findings emphasize the importance of longitudinal, repeated sampling when studying the epidemiology and viral etiologies of respiratory illness in young children.

Notes

Financial support. This study was supported by an investigator-initiated grant from MedImmune, Inc. (J. A. E.).

Potential conflicts of interest. J. A. E. has received research support from MedImmune, Inc., and Novartis, and serves as a consultant for GlaxoSmithKline and Novavax. All other authors declare no conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Drews AL, Atmar RL, Glezen WP, Baxter BD, Piedra PA, Greenberg SB. Dual respiratory virus infections. Clin Infect Dis 1997; 25:1421–9.
2. Nascimento MS, Souza AV, Ferreira AV, Rodrigues JC, Abramovici S, Silva Filho LV. High rate of viral identification and coinfections in infants with acute bronchiolitis. Clinics (Sao Paulo) 2010; 65:1133–7.
3. Barnabas RV, Webb EL, Weiss HA, Wasserheit JN. The role of coinfections in HIV epidemic trajectory and positive prevention: a systematic review and meta-analysis. AIDS 2011; 25:1559–73.
4. MacDonald KL, Osterholm MT, Hedberg CW, et al. Toxic shock syndrome. A newly recognized complication of influenza and influenza-like illness. JAMA 1987; 257:1053–8.
5. Severe coinfection with seasonal influenza A (H3N2) virus and Staphylococcus aureus–Maryland, February-March 2012. MMWR Morb Mortal Wkly Rep 2012; 61:289–91.
6. Launes C, de-SEvilla MF, Selva L, Garcia-Garcia JJ, Pallares R, Munoz-Almagro C. Viral coinfection in children less than five years old with invasive pneumococcal disease. Pediatr Infect Dis J 2012; 31:650–3.
7. Techaasensiri B, Techaasensiri C, Mejias A, Craddock GH Jr, Ramilo O. Viral coinfections in children with invasive pneumococcal disease. Pediatr Infect Dis J 2010; 29:519–23.
8. Vu HT, Yoshida LM, Suzuki M, et al. Association between nasopharyngeal load of Streptococcus pneumoniae, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. Pediatr Infect Dis J 2011; 30:11–8.
9. Zhou H, Haber M, Ray S, Farley MM, Panozzo CA, Klugman KP. Invasive pneumococcal pneumonia and respiratory virus co-infections. Emerg Infect Dis 2012; 18:294–7.
10. Semple MG, Cowell A, Dove W, et al. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. J Infect Dis 2003; 191:382–6.
11. Paraninos-Baccala G, Komurian-Pradel F, Richard N, Vernet G, Lima BFloret D. Mixed respiratory virus infections. J Clin Virol 2008; 43:407–10.
12. Konig B, Konig W, Arnold R, Werchau H, Ihorst G, Forster J. Prospective study of human metapneumovirus infection in children less than 3 years of age. J Clin Microbiol 2004; 42:4632–5.
13. Cilla G, Onate E, Perez-Yarza EG, Montes M, Vicente D, Perez-Trallero E. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. J Med Virol 2008; 80:1843–9.
14. Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T. Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response. Pediatr Infect Dis J 2005; 24:605–10.
15. Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis 2003; 9:372–5.
16. Richard N, Komurian-Pradel F, Javouhey E, et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J 2008; 27:213–7.
17. Zhang RF, Jin Y, Xie ZP, et al. Human respiratory syncytial virus in children with acute respiratory tract infections in China. J Clin Microbiol 2010; 48:4193–9.
18. Peng D, Zhao D, Liu J, et al. Multipathogen infections in hospitalized children with acute respiratory infections. Virol J 2009; 6:155.
