MAJOR ARTICLE

Epidemiology of Multiple Respiratory Viruses in Childcare Attendees

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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.

The introduction of molecular-based detection of respiratory viruses has resulted in the detection of more viruses than ever before. As simultaneous molecular diagnostics for 15 viruses or more are increasingly available and affordable in the clinical setting, it is no surprise that there is more frequent detection of viral coinfections in the context of acute respiratory illnesses. Although earlier estimates of viral coinfection based on culture, serology, and some molecular methods were documented to be approximately 10% [1], current estimates of the prevalence of viral coinfections now range up to 44% in young children [2].

Coinfections with multiple human pathogens have been linked to more severe disease in many infections. Malaria, herpes simplex virus 2, and tuberculosis infections in patients with human immunodeficiency virus (HIV) are associated with increased HIV viral load [3] and may result in more serious morbidity or mortality. Bacterial coinfections may also contribute to the severity of influenza illness [4, 5]. Similarly, rhinoviral infections have been shown to increase the severity of invasive pneumococcal disease [6–9]. It is unclear whether infection with multiple viruses influences the severity and progression of viral respiratory illness. Contradictory conclusions on the impact of viral coinfections have been reported in studies of hospitalized children, with some studies finding an increased risk of severe disease associated with multiple viruses [1, 10–16], others finding no difference [1, 2, 17–19], and some studies finding less severe disease among children with viral coinfections [20–23].

Many viral respiratory studies rely on convenience samples obtained at hospital or emergency department admission [17, 18, 23, 24]. Little information is

Received 3 August 2012; accepted 23 October 2012; electronically published 3 January 2013.

The findings described here were presented in part at the 51st Interscience Conference on Antimicrobial Agent and Chemotherapy, 2011. Chicago, Illinois, abstract 994.

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The Journal of Infectious Diseases 2013;207:982–9

DOI: 10.1093/infdis/jis934

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 (tympanic or rectal temperature of ≥100.4, or axillary of ≥99.4), 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.

Respiratory secretions were sampled by inserting a nylon flocked swab (Copan Diagnostics, Corona, CA) into the mid to posterior nasopharynx, rotating 180°, and rinsing in 0.5 mL of lysis buffer [27]. Total nucleic acid was extracted from 200 μL of lysis buffer containing dithiothreitol and external control RNA and processed as previously described [28]. Specimens were tested for human metapneumovirus (HMPV), respiratory syncytial virus (RSV), parainfluenza types 1 through 4 (PIV1–4), influenza viruses (flu A and flu B), rhinovirus (HRV), human coronaviruses (HCoV) group 1 (229E and NL63) and group 2 (OC43 and HKU1), human bocavirus clade 1 (HBoV), and adenovirus (AdV) by separate real-time PCR and reverse transcriptase PCR (RT-PCR) assays. All 4 coronaviruses were detected in a single assay and not differentiated [21, 29, 30].

Comparisons of continuous measures of log viral load and shedding duration between viruses in multiple virus illnesses and single virus illnesses were made using nonparametric Mann-Whitney tests. Shedding events were defined as continuous virus-specific positive results interrupted by no more than one sequential negative result, and duration was calculated as number of days between the first and last positive swab of the event. Median duration and interquartile range (IQR) were calculated to describe virus-specific shedding, and shedding was compared between viruses using a binary variable indicating the occurrence of documented shedding duration exceeding 7 days. Pairwise correlations between the prevalence and quantity of specific viruses were determined by Pearson correlation coefficients displayed with a heat map (R, Vienna, Austria). Odds ratios (ORs) and 95% confidence intervals (CIs) comparing differences in patient characteristics and illness symptoms between multiple virus and single virus illnesses were calculated using generalized estimating equations (GEE) with a robust estimator to account for correlation in events measured from the same children. P values < .05 were considered statistically significant.

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

Table 1. Characteristics of 225 Children During 455 Illnesses, by Detection of Viral Coinfections at Symptom Onset

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 Viruses[a] (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)
Race[b]				
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.

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, 107 of 122 (88%) AdV, 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–

Table 2. Symptoms at Initial Symptom Interview, by Detection of Viral Coinfections, from 455 Available Interviews

Symptom	None Detected (n = 73)	Single Virus at Week 0 (N = 219)	Multiple Viruses at Week 0 (n = 163)	OR Comparing Multiple to Single Viruses[a] (95% CI; *P*)
Fever[b]	26 (36)	95 (44)	49 (30)	.56 (.35, .90; .02)
Rhinorrhea	69 (95)	205 (94)	152 (93)	1.03 (.50, 2.14; .94)
Congestion	65 (89)	178 (81)	140 (86)	1.40 (.81, 2.42; .22)
Cough	65 (89)	192 (88)	142 (87)	.96 (.49, 1.85; .89)
Wheeze	15 (21)	49 (22)	42 (26)	1.33 (.84, 2.09; .22)
Myalgia	6 (8)	8 (4)	5 (3)	.86 (.22, 3.40; .83)
Malaise	5 (7)	16 (7)	7 (4)	.51 (.21, 1.26; .15)
Fatigue	28 (38)	82 (37)	54 (33)	.84 (.56, 1.25; .38)
Decreased Activity	28 (38)	90 (41)	54 (33)	.72 (.48, 1.06; .10)
Earache	8 (11)	30 (14)	20 (12)	.94 (.52, 1.71; .84)
Shortness of breath	3 (4)	11 (5)	12 (7)	1.50 (.63, 3.56; .36)
Decreased appetite	29 (40)	82 (37)	54 (33)	.86 (.57, 1.30; .48)
Vomiting	11 (15)	36 (16)	26 (16)	1.00 (.53, 1.82; .95)

[a] Confidence intervals (CI) correct for correlation between multiple illnesses collected from individual children using generalized estimating equations with a robust variance estimator.

[b] Fever was missing or incomplete in 29 illnesses (7 with no virus; 11 with single virus; 11 with multiple viruses). Sensitivity analyses assuming all missing data were positive or negative for fever, alternately, showed no effect of missing data on final conclusions.

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.

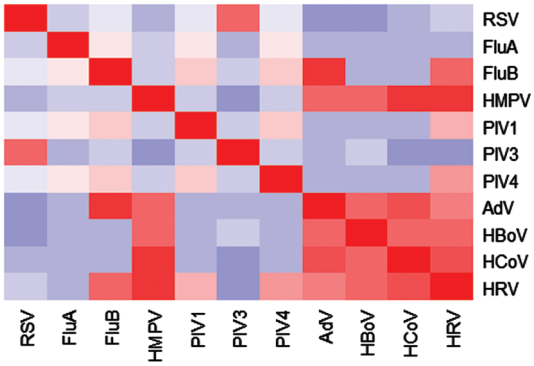

Figure 1. Correlations between the presence and quantity of 15 viruses and subtypes detected in 823 swabs from children with mild to moderate respiratory illness. Red scale: Synergistic virus combinations that occur more frequently or at higher viral loads when together. Blue scale: Antagonistic virus combinations that occur less frequently or with inversely correlated viral loads when together. Strength of color represents magnitude of positive (red) or negative (blue) correlation coefficient. Note that data are mirrored across the diagonal. Abbreviations: AdV, adenovirus; Flu, influenza; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; MVI, multiple virus illness; PIV, parainfluenza; RSV, respiratory syncytial virus; SVI, single virus illness.

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

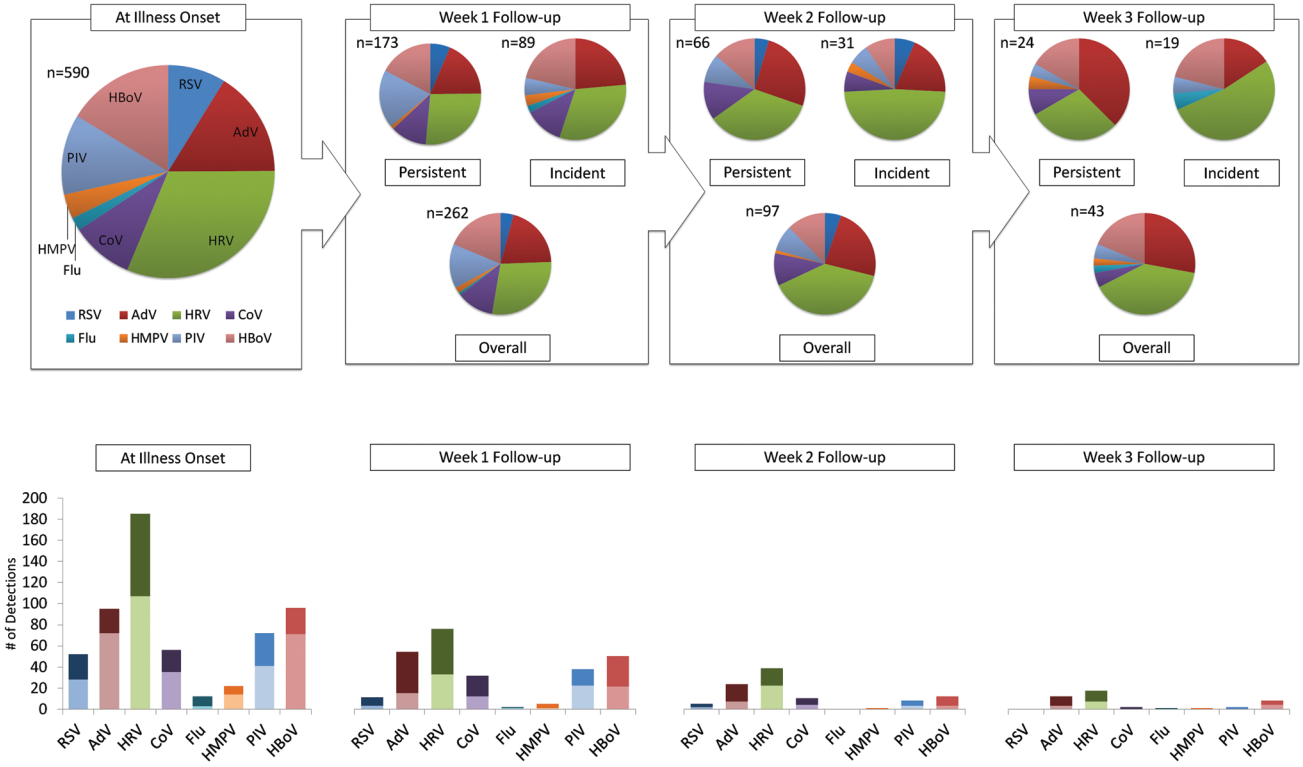

Figure 2. Distribution of virus detections at illness onset and 3 subsequent weekly swabs (weeks 1–3). Pie charts indicate the no. of viruses detected at illness onset and at 3 subsequent weekly follow-up swabs. Distributions are represented overall and by incident detection (first detection of the virus during the illness) and by persistent detection (repeated detection of that virus during the illness). Bar graphs indicate overall no. of detections, by swab time point. Dark bars indicate no. of detections with another virus, and light bars indicate no. of single detections for that virus. Abbreviations: AdV, adenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; MVI, multiple virus illness; PIV, parainfluenza; RSV, respiratory syncytial virus; SVI, single virus illness.

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

Table 3. Median Duration of Detection During Respiratory Illness of Extended Shedding Events (Detection Period ≥7 Days), by Multiple vs Single Virus Illness

Virus		Median days (IQR; max) during respiratory illness
RSV	SVI (n = 2)	8 (7,9; 9)
	MVI (n = 10)	12 (10, 15; 47)
HMPV	SVI (n = 0)	…
	MVI (n = 3)	8 (7, 8; 8)
HBoV	SVI (n = 0)	…
	MVI (n = 35)	11 (9, 20; 44)
AdV	SVI (n = 4)	8 (8,12.5; 17)
	MVI (n = 36)	12.5 (9, 21; 44)
HCoV	SVI (n = 1)	9 (…)
	MVI (n = 23)	11 (9, 16; 18)
HRV	SVI (n = 2)	8.5 (8, 9; 9)
	MVI (n = 60)	11.5 (9, 20; 41)
PIV	SVI (n = 5)	9 (8,9; 11)
	MVI (n = 29)	9 (8, 15; 26)
Flu	SVI (n = 0)	…
	MVI (n = 0)	…

Abbreviations: AdV, adenovirus; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HRV, rhinovirus; MVI, multiple virus illness: at least one other virus detected during illness; PIV, parainfluenza; RSV, respiratory syncytial virus; SVI, single virus illness: no other viruses detected during illness.

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 coinfections 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 cosuppression 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 coinfections. 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. Detections 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.

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