HHS Public Access

Author manuscript

Pediatr Infect Dis J. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Pediatr Infect Dis J. 2015 October ; 34(10): 1074–1080. doi:10.1097/INF.0000000000000812.

Respiratory Viral Detections During Symptomatic and Asymptomatic Periods in Young Andean Children

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Abstract

Background—Viruses are commonly detected in children with acute respiratory illnesses (ARI) and in asymptomatic children. Longitudinal studies of viral detections during asymptomatic periods surrounding ARI could facilitate interpretation of viral detections but are currently scant.

Methods—We used reverse transcription-polymerase chain reaction (RT-PCR) to analyze respiratory samples from young Andean children for viruses during asymptomatic periods within 8-120 days of index ARI (cough or fever). We compared viral detections over time within children and explored RT-PCR cycle thresholds (CT) as surrogates for viral loads.

Results—At least one respiratory virus was detected in 367 (43%) of 859 samples collected during asymptomatic periods, with more frequent detections in periods with rhinorrhea (49%) than those without (34%, p<0.001). Relative to index ARI with human rhinovirus (HRV), adenovirus (AdV), respiratory syncytial virus (RSV), and parainfluenza virus (PIV) detected, the same virus was also detected during 32%, 22%, 10%, and 3% of asymptomatic periods, respectively. RSV was only detected 8-30 days after index RSV ARI, whereas HRV and AdV were detected throughout asymptomatic periods. Human metapneumovirus (MPV) and influenza were rarely detected during asymptomatic periods (<3%). No significant differences were observed in the CT for HRV or AdV during asymptomatic periods relative to ARI. For RSV, CT were significantly lower during ARI relative to the asymptomatic period (p=0.03).

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The authors have no conflicts of interest to disclose.

Conclusions—These findings indicate that influenza, MPV, PIV, and RSV detections in children with ARI usually indicate a causal relationship. When HRV or AdV is detected during ARI, the causal relationship is less certain.

Keywords

acute respiratory illness; children; Peru; respiratory virus

BACKGROUND

Increasing availability of molecular diagnostic methods has enhanced detection of viruses in the upper respiratory tracts of patients with acute respiratory illnesses (ARI). However, viral detection in a patient with an ARI does not confirm an etiologic role for the virus. Viral detection may, alternatively, represent coincidental asymptomatic infection or persistent shedding after a prior infection.(1, 2)

Understanding the role of viral detection in ARI is essential to inform clinical management decisions and research priorities. Many published studies, including some of our own, have evaluated patients presenting or admitted to healthcare centers with respiratory infections in developed countries and compared them with different groups of healthy patients without respiratory symptoms. However, potential underlying differences between these groups could impact the validity of these comparisons.

Longitudinal assessments of the same individual could address some of these concerns by collecting repeated respiratory samples over time within a given individual during both symptomatic and asymptomatic periods. Nevertheless, most available longitudinal studies have evaluated only a limited number of viruses, were conducted in urban, high population-density areas in developed countries, and surveillance was performed in health care facilities, not in the patient homes, which potentially impacts the generalizability of those data. (3-13)

Our prospective household-based cohort study of young children from communities in the Peruvian Andes(14) is uniquely suited to provide information about the detection of common respiratory viruses during symptomatic ARI and from sequential respiratory samples obtained from the same child during prospective follow-up in a rural, high-altitude setting. The objective of the current study was to evaluate the presence and persistence of respiratory viral detections in individual children during asymptomatic periods relative to periods of symptomatic ARI.

MATERIALS AND METHODS

Study Design

This study utilizes data from the study of Respiratory Infections in Andean Peruvian children (RESPIRA-PERU), a prospective cohort study designed to evaluate the epidemiology of common respiratory viruses, the distribution of colonizing bacteria, the role of respiratory viruses in pneumococcal colonization, the impact of environmental factors on bacterial colonization, and the incidence and severity of ARI among young Andean children.

(15-20) This study was approved by the Vanderbilt Institutional Review Board and by the Ethics Committee of the Instituto de Investigacion Nutricional.

Study Setting

RESPIRA PERU was conducted in the Province of San Marcos, Department of Cajamarca, located in the northern highlands of Peru. San Marcos includes areas ranging from approximately 1500-4000 meters above sea level. The population is primarily rural, with low income, low educational level, and limited access to health-care services, as previously described.(20)

Enrollment and Follow-up

After a local census, broad selection criteria were used for enrollment, consisting of: 1) families with children aged <3 years; and 2) intention to remain in the study area for the next year. Children born during the study period in the study communities were enrolled to replace children who attained 3 years of age, with the aim of maintaining a dynamic cohort with approximately 500 children <3 years under observation at any time. Weekly active household-based ARI surveillance was conducted from May 1, 2009 through September 30, 2011 by trained field workers. ARI was defined as the presence of either cough or fever.(21) An ARI episode encompassed the period from the date of symptom onset to the last day of ARI symptoms that was followed by 7 or more days free of ARI symptoms. Children were followed until their third birthday, loss to follow-up, withdrawal of consent, death, or the end of the study, whichever came first.

Household Visits

Trained field workers visited the home of each enrolled child weekly to collect information on respiratory signs and symptoms through the use of a standardized questionnaire. At each visit, field workers assessed from the caretaker whether the children were experiencing ARI symptoms on the day of or up to 7 days prior to the household visit. In children with ARI symptoms on the day of or the day before the visit, the presence of World Health Organization (WHO) danger signs or lower respiratory tract infection (LRTI) was assessed by physical examination. If these signs were present, the field workers notified a study physician or nurse by cell phone to arrange a household visit or to coordinate referral to a healthcare center.

Respiratory Sample Collection

Nasal swab collection—During weekly household visits, field workers collected a nasal swab (NS) from any child with an ARI, following procedures previously reported.(22) In brief, one non-flocked polyester-tipped swab was placed into each nostril sequentially and rotated beneath the turbinates to collect epithelial cells and absorb secretions. After each nostril was swabbed, the swab was inserted into a tube with Remel M4RT® viral transport medium and transported in ice packages to the local research laboratory within 8 hours of sample collection. Samples were preserved at −70°C prior to viral diagnostic testing. Nasal swabs were collected for each new episode of ARI, but were not collected in consecutive weeks unless a new ARI developed.

Nasopharyngeal swab collection—Nasopharyngeal (NP) swabs were collected monthly from each child under observation, whether or not respiratory symptoms were present, and during complicated ARI, defined by the presence of WHO danger or LRTI signs (subcostal retractions, nasal flaring, etc.).(23) NP samples were processed according to WHO recommendations for identifying pneumococcal colonization.(24, 25) Briefly, samples were collected with a non-flocked deep nasopharyngeal Rayon swab and then immediately placed in 1 mL of Skim Milk-Tryptone-Glucose-Glycerol (STGG) transport medium. Specimens were transported in cold packs to the laboratory within 8 hours of collection and then preserved at −70°C.

Selection of Nasopharyngeal Samples

Because NP samples were collected monthly from all subjects under observation, multiple asymptomatic NP specimens were available from most subjects. We restricted sample selection for this study to observation periods after a first ARI so that the temporal relationship with an ARI was always known. For analysis of viral detections during asymptomatic periods, we selected a random sample of NP specimens collected during asymptomatic periods 8-30 or 31-120 days before or after an ARI (hereafter referred to as index ARI) (**Figure 1**).

Viral Detection Techniques

Both NS and NP samples were analyzed for the identification of influenza viruses (A and B), respiratory syncytial virus (RSV), human metapneumovirus (MPV), rhinovirus (HRV), adenovirus (AdV), and parainfluenza viruses 1-3 (PIV) using nucleic acid extraction and real-time reverse transcription-polymerase chain reaction (RT-PCR) as previously described.(22, 26-29) Despite different collection methods, prior studies by our group and others have demonstrated a very high agreement (89%-99%) in the detection of common respiratory viruses between NS and NP samples collected in this manner.(16, 30) For samples with virus detections, RT-PCR cycle thresholds (CT) from asymptomatic and ARI periods were considered as surrogates for viral loads, with higher CT indicating lower viral load. CT values of ≤40 were used to define sample positivity. Each RT-PCR was capable of detecting less than 50 RNA copies based on RNA runoff transcripts.

Statistical Analysis

The prevalence of detection of each respiratory virus during four asymptomatic periods relative to an index ARI within a given child was calculated (**Figure 1**). We also compared viral detections during asymptomatic periods between children with specific virus-positive and virus-negative ARI using the Fisher's exact test. Viral detection during asymptomatic periods was also stratified by the presence or absence of rhinorrhea and compared using the Fisher's exact test. CT for viral detections from asymptomatic and ARI periods were compared using the Wilcoxon sign-rank test.

Comparison of viral detections between symptomatic and asymptomatic periods—To assess the association between viral detections and symptomatic periods, we compared the proportion of specific viral detections between symptomatic and asymptomatic periods. For this, we used univariate logistic regression models and calculated

odds ratios (ORs) for the detections of each virus. The dependent variable in each model was symptomatic (i.e. ARI) or asymptomatic period (all combined) at the time of sample collection, and the independent variable was detection of each individual virus (positive or negative RT-PCR). Given the within-subject nature of our assessments, all regression models accounted for the clustering of observations at the individual level using the Huber-White sandwich variance estimator.(31) In addition, we estimated attributable fractions for each virus and expressed those as percentages using the equation: AF% = (OR – 1)*100 / OR.(32-34) Thus, for each group of symptomatic periods with positive detections for a specific virus, the AF% represented the proportion that could be attributed to the virus detected (i.e. considering that viruses could also be detected during asymptomatic periods). For example, if the odds of a viral detection were the same during symptomatic and asymptomatic periods, the odds ratio would be 1.0; accordingly, the corresponding AF would be 0% suggesting that the symptomatic periods might not be attributed to viral detections. In contrast, an OR of 5.0 yields an AF% of 80 indicating that 80% of symptomatic periods with virus detected might be attributed to the detected virus.

All analyses were done in Stata 13 (StataCorp, College Station, TX).

RESULTS

Characteristics of the Study Population

In total, 892 children representing 810 households in 58 communities were enrolled in the RESPIRA-PERU study. A detailed description of enrolled households and children has been published previously.(20) A total of 4475 ARI episodes were observed, with at least one virus detected in 67% of ARI. HRV was the most common virus identified, detected during 32% of ARI, followed by AdV (5%), PIV (5%), influenza (5%), RSV (3%), and MPV (2%). (15) Detection of two or more viruses was observed in approximately 13% of ARI.(15)

Viral Detection During Asymptomatic Periods

Nine hundred nine (909) NP samples collected during asymptomatic periods were randomly selected from a total of 3470 samples to undergo viral testing. Of those, 859 had enough residual material for comprehensive viral testing. At least one virus was detected in 42.7% of these 859 NP samples, while co-detection of more than one virus was observed in 48 samples (5.7%). HRV and AdV were detected most commonly, in 270 (31.4%) and 95 (11.0%) asymptomatic periods, respectively, followed by RSV in 29 (3.4%), MPV in 13 (1.5%), PIV in 11 (1.3 %), and influenza in 6 (0.7%) asymptomatic periods (**Table 1**).

Viruses were detected more frequently in children with rhinorrhea than those without ($p<0.001$; **Table 2**). The association of viral detection with rhinorrhea was primarily driven by HRV and AdV. This association was not observed for MPV, RSV, PIV, and influenza; however, these viruses were rarely detected during asymptomatic periods and thus, those comparisons had limited power.

Viral Detections During Asymptomatic Periods Relative to Index ARI

Seven hundred seventy-four (774) observations in which NP samples were available from an asymptomatic period and within 120 days of an index ARI were identified, representing 774 unique ARI-asymptomatic sample pairs (**Figure 1**). **Figure 2** displays the proportions of viral detection during each asymptomatic period relative to an index ARI. Black bars indicate observations in which a given virus was detected, both during the index ARI and asymptomatic periods. Gray bars represent detection of a given virus during an asymptomatic time period relative to an index ARI in which the same virus was not detected. For ARI in which HRV, AdV, RSV, and PIV were detected, the overall proportions of detection of the same virus during asymptomatic periods within 120 days of the ARI in a given subject were 32%, 22%, 10%, and 3%, respectively (**Table 3**). No subject who had a virus-positive ARI for influenza (n=25) or MPV (n=32) had the same virus detected during an asymptomatic time period.

Viral detections varied during asymptomatic periods. Asymptomatic detections of RSV were restricted to the 8-30 day period after RSV-positive ARI. Although detections of HRV and AdV were observed throughout the asymptomatic periods, HRV was significantly more likely to occur in the 8-30 day asymptomatic period after HRV-positive ARI than after ARI in which HRV was not detected. Detection of AdV was significantly more likely to occur in the 8-30 day periods both before and after an index ARI in which AdV was detected. Detection of PIV was rare during asymptomatic periods relative to ARI in which PIV was detected.

Comparison of viral detections between symptomatic and asymptomatic periods—In regression models evaluating symptomatic ARI and asymptomatic sample pairs, detections of PIV, MPV, and influenza were most highly associated with ARI, resulting in AFs of 91% (95% confidence interval (CI): 80, 86), 79% (CI: 46, 92), and 73% (CI: 21, 90), respectively (**Table 4**). RSV and HRV detections were also significantly associated with ARI, but with lower AF% of 67% (CI: 43, 81) and 41% (CI: 22, 54), respectively. Only AdV was not significantly associated with ARI in this cohort, with an AF % of 5% (CI: −37, 34), meaning its detection was not significantly increased during symptomatic periods relative to asymptomatic periods.

RT-PCR CT for Viral Detections during ARI and Asymptomatic Periods

No significant differences were observed in CT values during asymptomatic periods [median 29.7, (IQR 24.4-34.7)] relative to index ARI [27.2 (23.2-32.1), p=0.49] in which HRV was detected (**Table 3**). Similarly, no differences were observed in CT values for AdV during asymptomatic periods [median 35.6, (IQR 31.6-37.1)] relative to index ARI's [36.1 (32.7-37.3), p=0.78]. However, in the 7/25 (28%) samples in which RSV was detected during the asymptomatic period 8-30 days after RSV-positive index ARI, the median CT values were significantly higher during asymptomatic periods than ARI, indicating lower viral loads during these asymptomatic periods following ARI [CT 28.8 (25.0-38.5)] than during ARI [CT 24.4 (18.9-29.3); p=0.028]. The median duration of RSV detection after a symptomatic ARI was 15 days (IQR 14-21).

DISCUSSION

We compared the prevalence of detection of specific respiratory viruses during asymptomatic periods compared to ARI within individual children <3 years of age. We found that detection of respiratory viruses during asymptomatic periods was common, occurring in over 40% of samples, and each one of the viruses evaluated was detected in asymptomatic periods. The majority of detections that occurred in asymptomatic periods were HRV and AdV, consistent with findings from other studies.(1, 35, 36) All asymptomatic RSV detections occurred shortly following index ARI in which RSV was detected. Detections of PIV, MPV, and influenza were rare during asymptomatic periods.

The prevalence of asymptomatic detection for both HRV and AdV varied significantly relative to the timing of index ARI. HRV was detected more frequently in the 8-30 days after ARI in which HRV was detected than following an ARI in which HRV was not detected, consistent with findings from other studies that have demonstrated that rhinovirus RNA may persist for up to 30 days or even longer after HRV symptomatic infection in young infants.(12, 37-40) There was also variation in the prevalence of asymptomatic AdV detection relative to the timing of index AdV-positive ARI, with AdV detected in 33% and 40%, respectively, of asymptomatic periods 8-30 days before and 8-30 days after AdV-positive ARI but only in 13% or less of asymptomatic samples obtained greater than 30 days apart from ARI. Additionally, detection of AdV during the 8-30 days asymptomatic periods before and after ARI was significantly more frequent surrounding AdV-positive ARI compared to AdV-negative ARI, suggesting a relationship between asymptomatic detection and ARI. While the prolonged presence of AdV following ARI has not been thoroughly evaluated, prolonged shedding has been described,(3) and AdV has been found in tonsil and adenoid tissue of asymptomatic children undergoing routine tonsillectomy,(41) suggesting that this mucosal lymphoid tissue may harbor AdV and allow intermittent shedding before or after symptomatic disease.

However, the relative viral loads of HRV and AdV, as estimated by CT values, were not higher during index ARI compared to asymptomatic periods before or after ARI, arguing against the possibility that these detections represented persistence of infection due to the same HRV or AdV strain.(42-45) Recently, Loeffelholz et al. reported that prolonged presence of the same HRV strain for >30 days was uncommon in young children, occurring in less than 5% of HRV detections.(12) Another study by the same group evaluating repeated AdV detections within individual children demonstrated that repeat detection of the same strain of AdV following AdV-associated symptomatic disease occurred, either continuously or intermittently, for more than four months in 10 of 16 (62.5%) instances, while other repeated detections represented acquisition of new AdV strains.(46) Additional genotyping of HRV and AdV detections in our cohort would be necessary to conclusively clarify these observations.

RSV was only detected in the 8-30 day asymptomatic period after an RSV-associated ARI. These RSV detections in asymptomatic periods shortly after an RSV-associated ARI were associated with significantly higher CT, suggesting declining viral shedding. Our observed median duration of detection 15 days after symptomatic ARI is consistent with previous

studies that have demonstrated that the duration of RSV shedding after infection in healthy young children is usually 4-10 days but may persist up to 3-4 weeks.(47-50)

We also found that viruses were detected more frequently during non-ARI periods in which rhinorrhea was present. This observation was primarily driven by HRV and AdV. Although a similar trend was observed for MPV and PIV, the small sample sizes for asymptomatic detection of these viruses may have limited our power to detect significant differences. While infection with some of these viruses may be associated with rhinorrhea alone,(51, 52) or rhinorrhea may enhance viral detection from the nasopharynx, the interpretation of this finding should take into account that our definition of ARI only included cough and fever, but not rhinorrhea.

We also examined the association of specific viral detections and symptomatic respiratory illness. Most viruses including MPV, PIV, and influenza were detected commonly during symptomatic periods but uncommonly during asymptomatic periods, suggesting a direct etiological role when detected in children with ARI. In contrast, HRV and especially AdV were frequently detected during aggregated asymptomatic periods in addition to ARI periods, yielding much lower odds ratios and lower AFs. An important caveat for the interpretation of these estimates is the variable detection of these viruses during asymptomatic periods over time. In particular, the lack of a significant association between AdV detections and symptomatic illness as determined by OR and AF calculations must be interpreted with caution, as the prevalence of asymptomatic AdV detection varied significantly in different asymptomatic periods. The AF measurement is based on a simple model of causation. For example, it did not take into account data on CT values for RSV, thus likely underestimating the true fraction of disease due to RSV. In addition, previous experimental and observational studies have demonstrated a clear pathogenic role of specific strains of HRV and AdV.(46, 53-58) Whether some specific viral strains may be more pathogenic than others remains unclear and requires further scrutiny.

Our study took place in a rural high altitude setting, with approximately 75% of our cohort residing at altitudes higher than 2314 meters above sea level. The physiologic effects of altitude are multiple, including lower baseline oxygen saturations,(59, 60) impaired respiratory ciliary function,(61) and hypoxia-driven pulmonary vasoconstriction, and may impact susceptibility to respiratory illness or disease severity. In our cohort, residence at higher altitude (≥2315-2865 meters compared to <2314 meters) was an independent risk factor for RSV infection, but not for MPV infection.(62) Choudhuri et al.(63) similarly demonstrated increased RSV-associated hospitalization rates of 25% and 53% for every 1000-meter increase in altitude among children less than one and children aged 1 to 4 years, respectively. The highest risk for RSV-associated hospitalization occurred at elevations >2500 meters. However, there were no data for other viruess. Whether altitude and other factors that could influence susceptibility to specific viral infections also impact respiratory viral disease severity requires further exploration.

The current study has several important strengths. First, the RESPIRA-PERU study is one of few large prospective intensive evaluations of mild-to-moderate respiratory illness in a rural, high-altitude setting. Additionally, rather than utilizing an external control cohort, the study

collected prospective data from individual subjects over a period of longitudinal follow-up wherein each subject served as his own control, directly accounting for inter-subject variability. Rigorous active household surveillance combined with sensitive viral detection techniques enabled a highly sensitive assessment of viruses associated with both asymptomatic and ARI conditions.

Our study also has several limitations. First, limited information regarding host immunological factors and viral-bacterial interactions in individuals over time is available, which may influence patterns of respiratory viral detection.(64-68) Additionally, no detailed molecular characterizations of each viral strain were conducted to inform interpretation of whether repeated detections in an individual are due to prolonged shedding with a single viral strain or frequent acquisition of new strains. Our highly sensitive ARI definition (fever or cough) may have resulted in noninfectious causes of fever or cough to be counted as ARI. Finally, although we tested for a large panel of respiratory viruses, we did not attempt to identify other viruses (such as coronaviruses and bocaviruses) that may have been detectable in this cohort.

Taken together, our findings established from within-person comparisons indicate that detections of HRV and AdV were common during asymptomatic periods and varied over time, occurring closely before and after index viral ARI, with similar CTs across these periods, and with lower attributable fractions for symptomatic diseases than the other viruses. RSV was infrequently detected during asymptomatic periods except in the 8-30 days following an index RSV-positive ARI. In contrast, detection of influenza, MPV and PIV during asymptomatic periods was very uncommon, indicating that detection of influenza, MPV, PIV, and RSV in a child with an ARI usually indicates a causal relationship. The causal relationship between HRV or AdV detections and ARI is less certain, and the patterns of the detection of different strains of these viruses over time requires further investigation.

ACKNOWLEDGMENTS

On behalf of the study of Respiratory Infections in Andean Peruvian children (RESPIRA-PERU): Vanderbilt University: Marie R. Griffin, John V. Williams, Kathryn M. Edwards, Philip J. Budge, Yuwei Zhu, Monika Johnson, Carlos G. Grijalva; Emory University: Jorge E. Vidal, Keith P. Klugman; Instituto de Investigacion Nutricional: Hector Verastegui, Stella M. Hartinger, Ana I. Gil, Claudio F. Lanata. We are indebted to the communities of San Marcos, Cajamarca Peru for their participation in this study. We also acknowledge the approval and continuous support of the Cajamarca Health Region authorities. We are also indebted to the field workers and field supervisors whose efforts in difficult geographical areas and harsh weather conditions allowed the conduct of this study.

This work was supported by the Vanderbilt University CTSA grant UL1 RR024975-01 from NIH, an investigator initiated grant from Pfizer (IIR WS1898786(0887X1-4492)) and a research grant from the Thrasher Research Fund (02832-9). L.M.H was supported by Fogarty International Center Grant Number R25TW009337 from National Institutes of Health through Vanderbilt University.

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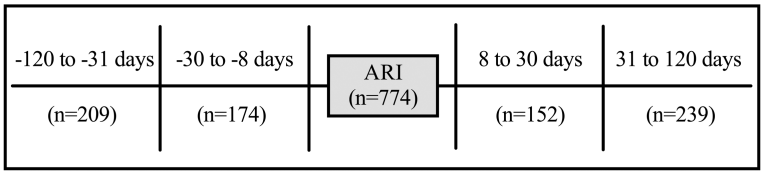

Figure 1.

Selection of nasopharyngeal samples from four asymptomatic time periods within 120 days of an index acute respiratory illness (ARI).

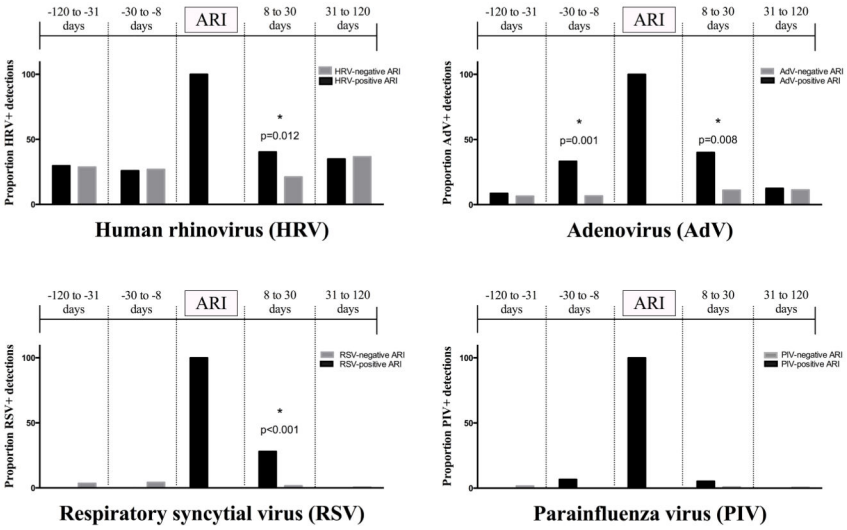

Figure 2.

Detection of virus during asymptomatic periods relative to virus-positive and virus-negative index ARI.

TABLE 1

Viral detections in asymptomatic time periods stratified by age.

Virus	Number of detections (%)*			
	All ages (n=859)	0-5 months (n=64)	6-11 months (n=178)	12-35 months (n=605)
HRV	270 (31.4)	19 (29.7)	57 (32.0)	194 (32.1)
AdV	95 (11.0)	1 (1.5)	25 (14.0)	69 (11.4)
RSV	29 (3.4)	2 (3.1)	7 (3.9)	20 (3.3)
MPV	13 (1.5)	1 (1.5)	2 (1.1)	10 (1.7)
PIV	11 (1.3)	1 (1.5)	4 (2.2)	6 (1.0)
Influenza	6 (0.7)	1 (1.5)	1 (0.6)	4 (0.7)
Any Virus	367 (42.7)	23 (35.9)	82 (46.1)	263 (43.5)
≥ 2 Viruses	48 (5.6)	2 (3.1)	14 (7.9)	32 (5.3)

TABLE 2

Viral detections in asymptomatic time periods stratified by rhinorrhea.

	Any Virus	HRV	AdV	RSV	MPV	PIV	Influenza
Rhinorrhea (n=446)	219 (49.1)	163 (37.1)	60 (13.5)	12 (2.7)	7 (1.6)	9 (2.0)	3 (0.7)
No rhinorrhea (n=403)	138 (34.2)	102 (25.4)	35 (8.7)	14 (3.5)	3 (0.7)	2 (0.5)	3 (0.7)
	p<0.001	**p<0.001**	**p=0.03**	p=0.55	p=0.35	p=0.07	p=1.0

TABLE 3

Proportion of viral detections relative to virus-positive index ARI and CT differences according to virus and time period.

	Days −120 to −31	Days −30 to −8	Days 8 to 30	Days 31-120	All time points
HRV+ ARI (n=330)	30/101 (29.7)	21/81 (25.9)	25/62 (40.3)	30/86 (34.9)	106/330 (32.1)
Median CT Asymptomatic (IQR)	29.4 (25.5-36.8)	29.6 (25.4-34.9)	28.4 (23.8-31.5)	30.3 (24.1-34.7)	29.7 (24.4-34.7)
Median CT ARI (IQR)	25.6 (21.9-30.0)	28.2 (24.4-32.6)	28.0 (25.2-34.2)	27.3 (23.4-31.1)	27.2 (23.2-32.1)
	p=0.29	p=0.52	p=0.17	p=0.38	p=0.49
RSV+ ARI (n=69)	No detection	No detection	7/25 (28.0)	No detection	7/69 (10.1)
Median CT Asymptomatic (IQR)			28.8 (25.0-38.5)		28.8 (25.0-38.5)
Median CT ARI (IQR)			23.4 (18.9-27.0)		24.4 (18.9-29.3)
			p=0.028		**p=0.028**
PIV+ ARI (n=75)	No detection	1/15 (6.7)	1/19 (5.3)	No detection	2/75 (2.6)
Median CT Asymptomatic (IQR)		20.7	38.9		29.8 (20.7-38.9)
Median CT ARI (IQR)		25.6 (23.8-35.1)	21.5 (19.5-28.5)		24.2 (21.0-30.8)
		p=0.32	p=0.32		p=0.65
AdV+ ARI (n=83)	2/23 (8.7)	7/21 (33.3)	6/15 (40.0)	3/24 (12.5)	18/83 (21.7)
Median CT Asymptomatic (IQR)	33.0 (28.5-37.5)	36.0 (35.2-37.1)	33.3 (31.6-35.3)	36.8 (23.2-38.7)	35.6 (31.6-37.1)
Median CT ARI (IQR)	35.9 (33.1-37.6)	36.1 (31.4-37.0)	32.8 (30.0-36.5)	36.7 (34.8-37.8)	36.1 (32.7-37.3)
	p=0.65	p=0.40	p=0.75	p=0.59	p=0.78
MPV+ ARI (n=32)	No detection	No detection	No detection	No detection	No detection
Flu+ ARI (n=25)	No detection	No detection	No detection	No detection	No detection

TABLE 4

Prevalence of respiratory virus detection by RT-samples and samples from ARI, unadjusted odds ratios (OR (AF). PCR in paired asymptomatic), and attributable fractions

	Asymptomatic period, n (%)	ARI, n (%)	p	OR (95% CI)	AF (95% CI)
HRV	237 (30.6)	330 (42.6)	<0.001	1.68 (1.30, 2.18)	0.41 (0.22, 0.54)
AdV	80 (10.3)	83 (10.8)	0.773	1.06 (0.73, 1.53)	0.05 (−0.37, 0.34)
RSV	24 (3.1)	69 (8.9)	<0.001	3.06 (1.75, 5.35)	0.67 (0.43, 0.81)
MPV	7 (0.9)	32 (4.1)	0.001	4.73 (1.85, 12.0)	0.79 (0.46, 0.92)
PIV	7 (0.9)	75 (9.7)	<0.001	11.76 (4.96, 27.89)	0.91 (0.80, 0.96)
Influenza	7 (0.9)	25 (3.2)	0.017	3.66 (1.26, 10.62)	0.73 (0.21, 0.90)