Investigating the influence of virus-virus interactions on susceptibility to subsequent infections

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Abstract

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Background: The co-circulation of multiple respiratory pathogens can lead to competitive or cooperative forms of pathogen–pathogen interactions through broad-acting innate or adaptive immunity leading to enhanced or reduced risk of subsequent infections.

Methods: We retrospectively analyzed 6,396 unique individuals from the Seattle Flu Study to investigate viral interference among six respiratory pathogens. Using longitudinal data collected from March 2020 to July 2022, we assessed respiratory virus interference effects in the Seattle metropolitan area. Statistical analysis included investigating viral circulation proportion, Cox regression modeling to calculate the hazard risk of subsequent viral detection, and mixed effects modeling to assess associations between primary respiratory infection onset and viral load via cycle threshold (Ct) of subsequent infections.

Results: Surveillance of the Seattle metropolitan area revealed persistent circulation of human rhinovirus (13.0% of samples positive), SARS-CoV-2 (3.6%), and adenoviruses (1.23%) with a reduction in detection of other pathogens after COVID-19 mitigation efforts began in March 2020. The risk of subsequent infections varied by pathogen and time since primary detection. Significance was observed for human rhinovirus, SARS-CoV-2, adenoviruses, and respiratory syncytial virus. A significant reduction of secondary detection (HR; 0.20-0.61) within the 0-14 risk period after primary virus detection, a significantly lower risk of subsequent infection after 60 days (HR; 0.20-0.60) and a non-significant higher risk of subsequent infection within 14-30 day risk period was observed.

Conclusions: Primary virus detection was associated with a lower risk of subsequent virus detection within the first 14 and following 60 days and a higher risk within 14-30 days after primary infection. Results provide insights into viral dynamics, aiding public health strategies.

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1. Introduction

Circulating respiratory viruses contribute to a substantial portion of mortality, morbidity, and economic burden globally and in the United States.[1-4] With the emergence of the SARS-CoV-2 pandemic, the circulation of common respiratory viruses was noticeably reduced.[5,6] This reduction was shown in a lesser effect following the termination of non-pharmaceutical interventions (NPIs).[5] Rhinoviruses were the exception, which declined in case count at the initial onset of the SARS-CoV-2 pandemic; however, in contrast to the majority of other endemic respiratory viruses, their prevalence swiftly rebounded and endured despite the continuous implementation of community NPIs.[6,7]

Respiratory viruses often circulate simultaneously and can potentially infect the same host concurrently or sequentially. In this setting, there is potential for biological mechanism interactions known as viral interference.[8] This can lead to competitive or cooperative forms of pathogen–pathogen interactions through broad-acting innate or adaptive immunity.[8] An example of viral interference is shown in studies exploring early host-virus dynamics that demonstrate prior rhinovirus infection blocks SARS-CoV-2 replication,[9] as well as studies exploring the association between subsequent respiratory virus infections and their impact on transmission dynamics. [10-12] The results of these studies show varying effects, where some suggest decreased susceptibility to subsequent respiratory viral infection following infection and others suggest an increased susceptibility or risk for infection.[11-12] These varying results may be due to the respiratory viruses involved, the timing of each infection, and the interplay between the host response and each virus.[8]

Recent literature exploring the risk of subsequent respiratory virus detection supports the hypothesis for varying interference effects.[13-15] Two studies, one conducted in the Philippines and another longitudinal study in King County, WA, both demonstrate an increased risk of subsequent viral infections following an initial infection. The study in the Philippines revealed an elevated risk of subsequent viral infections after an initial infection,[13] and the longitudinal study in King County, WA, indicated that primary virus detection was associated with a higher risk of subsequent virus detection within the first 90 days.[14] Furthermore, a study exploring the association between symptomatic acute respiratory infection (ARI) and subsequent ARI in young children showed no significant associations.[15]

In the present study, we explore the epidemiology of six human respiratory pathogens; rhinovirus (HRV), SARS-CoV-2, adenovirus, respiratory syncytial virus (RSV), influenza A, and influenza B. We assessed whether prior respiratory virus detection affects the risk of subsequent viral detection in longitudinal samples from participants of the Seattle Flu Study (SFS). Furthermore, we investigated whether prior respiratory virus detection impacts subsequent infection cycle threshold (Ct) value.

2. Methods

2.1 Study Design & Data Collection

To analyze viral interference between respiratory viral infections, we retrospectively analyzed data from an unstructured longitudinal observational study, where participants were repeatedly tested. The study was designed to assess respiratory virus circulation in the Seattle, Washington metropolitan area from March 23rd, 2020 to July 27th, 2022. Samples were tested using polymerase chain reaction (PCR) to detect the presence of respiratory pathogens using OpenArray Real-Time PCR system and recorded with a unique identifier. All adult participants provided written informed consent. Child participants enrolled after proxy consent via a parent or guardian.

Volunteer participants were enrolled in the SFS and received a free testing kit for self-collection of nasal swabs.[16] Participants can enroll for the Seattle Coronavirus Assessment Network (SCAN) site with either a symptomatic or asymptomatic status. Daily limits are set on the number of symptomatic and asymptomatic enrollments to ensure representativeness. Data was collected and stored through the SFS metabase surveillance system.[16] The study is set in the Seattle and King County region and is part of the collaborative effort led by the Brotman Baty Institute, UW Medicine, the Fred Hutchinson Cancer Center, and Seattle Children's Hospital. The University of Washington Institutional Review Board (IRB) approved this study as STUDY00010432.

2.2 Statistical analysis

Data Preparation

The proportion of respiratory viral detections in SFS study SCAN specimens and the Seattle metropolitan area by calendar time was plotted for the six respiratory viruses of interest consisting of HRV and SARS-CoV-2, ADV, RSV, influenza A, and influenza B . Analysis was performed for data available from March 23rd, 2020 to July 27th, 2022.

To capture interference in our observational data, we restricted the sample to individuals with ≥2 tests to establish a cohort and specifically define multiple detections as ≥2 swab specimens collected at distinct time points from the same participant over the study period. For study specimens that met the definition of multiple detections, primary detection was defined as the first virus detected and secondary detection as the subsequent virus detected. According to the definition of primary infection, for each of the 6 pathogens, when a participant had >2 positive nasal swab specimens, a detection could be both secondary (relative to preceding detections) and primary (relative to subsequent detections). To further avoid misclassification of the same infection, recurrent infections of the primary infections were not included as viable subsequent infections. This was done to ensure that samples collected represent unique viral detection of unique illnesses. The frequency of primary and secondary pairs of viral detection was recorded in our study sample.

Analysis

A Cox regression model was developed to calculate the risk of subsequent respiratory viral detection after a primary respiratory viral detection. Due to the violation of Cox proportional hazard (Supplementary Figure 1), detection was modeled as a time-dependent covariate that is categorized into 5 risk periods based on the days since the last primary detection; 0-14 (including co-infections), 14-30, 30-60, 60-180, 181-365 days compared to no prior detection with the primary virus within the past year. Additionally, the model was constructed with and without adjustment for age group, sex, and presence of symptoms at the time of encounter. Hazard ratios (HRs) were calculated, and risk ratios were approximated by HRs. Calendar time was used as the time scale, and secondary detection was used as the response variable. Separate models were developed for each type of respiratory virus for primary detection. Wald tests, with robust standard errors, were used to evaluate the time-dependent covariates and Schoenfeld residuals were calculated to evaluate each variable in the time-dependent Cox regression models. We examined the smoothed incident rate for secondary detection of all respiratory pathogens with subsequent infections. Since we had no information on prior detections at the time of enrollment, it is likely that the time-dependent covariate described above would be misclassified as “no prior detection” at the start of follow-up in some participants. Therefore, we performed sensitivity analyses for SARS-CoV-2 where we discarded the first 30 days of follow-up and reran the Cox models.

We also aimed to examine the association between cycle threshold (Ct) values as a proxy for the inverse viral load in subsequent respiratory infections and primary infection. We employed a mixed-effects modeling approach while controlling for covariates such as symptoms, sex, and age group. This model accommodates the correlated nature of longitudinal data, particularly within-individual correlations and individual heterogeneity by introducing a random intercept. The model provides estimates of Ct value after primary infections induced by rhinovirus or SARS-CoV-2. All data analyses were performed using R software, version 4.1.1[17]

Results

I. Demographics of participants and infection events

Surveillance data from the Seattle metropolitan SCAN study from March 2020 to July 2022 shows the trend of circulating pathogens (Figure 1 A,B). Throughout the period, SARS-CoV-2, rhinovirus, and adenovirus were continuously circulating. With the progression of the pandemic in Seattle, a reduction in the detection of other pathogens was observed, especially rhinovirus and adenovirus, however, the proportion of rhinovirus positive samples was later sustained as SARS-CoV-2 was spreading.

Between March 2020 and July 2022, there were a total of 14,167 samples collected from 6,396 unique individuals who met the inclusion criteria of having at least two subsequent tests (Table 1). There were more females (59.4%) and individuals within the age group of 17-45 (62.0%) relative to other age groups. Furthermore, subjects from the majority of samples reported having symptoms (85.3%) including the presence of chills or shivering, cough, feeling feverish, headaches, muscle or body aches, sore throat, and runny or stuffy nose.

Overall, rhinovirus was more commonly detected in comparison to all other pathogens with a sample proportion of 13.0% relative to SARS-CoV-2 (3.6%), adenovirus (1.23%), RSV (0.54%), influenza A (0.06%) and, influenza B (0.02%). Additionally, 129 samples showed co-detection of two of the six pathogens (0.9%). Among samples with viral presence, the trend continued with most infections observed in the 17-45 age group. However, while the distribution of SARS-CoV-2 infections was fairly consistent across age groups, samples positive for rhinovirus, adenovirus, and RSV tended to be from younger participants.

The frequency of secondary detections, given a particular primary virus detection, is shown in Table 2 with a primary infection of rhinovirus having the most subsequent infections (n = 179) within 365 days. The median time between primary and secondary tests among participants was 50 days. Furthermore, There were no subsequent secondary detections within the specified risk period following influenza B due to the small sample size.

II. Risk of Subsequent Secondary Infections

The risk of secondary detection varied depending on the type and timing since the most recent primary virus detection (Table 3). The models were adjusted for sex, age group, and presence of symptoms. For the risk period from 0-14 days, there was a significant reduction in risk of subsequent infections following rhinovirus, SARS-CoV-2, and RSV (HR = 0.61 [95% CI 0.46–0.81]), (HR = 0.20 [95% CI, 0.14–0.29]), and (HR = 0.45 [95% CI, 0.29–0.70]) respectively while there was a no difference risk following adenovirus and Influenza A (HR = 1.04 [95% CI, 0.79–1.13]) and (HR = 1.12 [95% CI, 0.30–4.86]). Furthermore, a non-significant upward trend is observed within 14-30 day risk period for all pathogens that decreased following 30 days. There is a significant reduction in risk of subsequent infections observed within 60-180 risk periods for rhinovirus, adenovirus, and RSV with risk ratios within 60-180 risk period of 0.60 (95% CI; 0.41–0.89), 0.40 (95% CI; 0.25–0.51), 0.55 (95% CI; 0.33–1.00) respectively. This significantly lower risk of subsequent infection also persisted for rhinovirus, SARS-CoV-2, and Adenovirus within 180-360 day risk period with risk ratios of 0.36 (95% CI; 0.24–0.50), 0.20 (95% CI; 0.10–0.61), 0.20 (95% CI; 0.10–0.40) respectively. Results from these sensitivity analyses regarding significantly reduced risk of subsequent infection for SARS-CoV-2 following 180 days were consistent with the main analysis (Supplementary Table 1).

Sex was not significantly associated with the risk of subsequent infections with HR ranging from 0.96 to 1.09 (95% CI; 0.82, 1.26). Among all primary pathogens, age was significantly associated with the risk of subsequent infection. Age group <5 was significantly associated with an increase of subsequent infections relative to the referent group (17-45). This pattern was also observed for age group 6-16. Furthermore, there was a significantly reduced risk of subsequent infections for older age groups 45-65 and 66+ compared to the referent group. Among all pathogens, there was an increased risk of subsequent infection for individuals presenting with symptoms.

The adjusted Cox regression models for subsequent respiratory virus infection showed a significant reduction of secondary detection within the 0-14 risk period after primary virus detection and a significantly lower risk of subsequent infection after 60 days. Due to the COVID-19 pandemic and inability to adjust for non-pharmaceutical interventions (NPIs), the number of respiratory viruses detected likely decreased with calendar time. The incidence of respiratory viruses and incidence of secondary detection are visualized (Figure 2 A, B). Overall, there was a pattern of decreased risk of infections past the primary virus detection. This pattern increased for the 14-30 day period and then decreased thereafter. Furthermore, the proportional Schoenfield residual hazard test was not significant for time-dependent covariates for both models and adjusted covariates representing valid proportional hazard assumption ($P > 0.1$, $df = 4$).

III. Cycle Threshold Analysis

The coefficients estimated for subsequent infection cycle threshold (Ct) values are summarized in Table 4. Positive coefficients denote an increase in Ct values, suggesting a decline in viral load, while negative coefficients indicate a reduction in Ct values, signifying a higher viral load. The distribution of cycle threshold values based on the presence of symptoms for SARS-CoV-2 and rhinovirus, adenovirus, and RSV demonstrate lower Ct values for symptomatic individuals relative to asymptomatic individuals infected with these pathogens (Figure 3).

Controlling for sex, age group, symptoms, and seasonality, primary HRV infection displays no difference estimate in SARS-CoV-2 Ct within the risk period of 0 to 14 days [estimate -0.88 (95% CI: -4.47, 2.72)], and a significant trend of increasing Ct within risk periods 30-60 days [estimate 4.79 (95% CI: 0.61, 8.96)]. Conversely, primary SARS-CoV-2 infection shows a significant association with changes in rhinovirus Ct with an estimate of 2.52 (95% CI: 0.61, 4.43) for the risk period of 0 to 14 days. Additionally, younger age groups (<5 and 6-16 years) are significantly associated with Ct reduction following SARS-CoV-2 primary infection for HRV. The presence of symptoms significantly correlates with a reduction in Ct for both primary infections.

Discussion

In this longitudinal study, we aimed to examine the respiratory viral-viral interference of six different pathogens including rhinovirus, SARS-CoV-2, adenovirus, respiratory syncytial virus (RSV), influenza A, and influenza B with samples collected over 2 year period in Seattle, Washington. Infection with rhinovirus, SARS-CoV-2, and adenovirus was significantly associated with a reduction in risk of subsequent infection within the 0-14 day period. Across all respiratory pathogens for which subsequent infections were observed, there was a non-significant increase in the risk of subsequent infections within the 14-30 day period following the primary infection. A significant reduction in risk was also observed after 60 days since the initial infection, in comparison to individuals without prior detection of respiratory viruses during follow-up. Notably, younger individuals exhibited a significantly increased risk, while older age groups displayed a decreased risk relative to the 17-45 age group. However, the presence of symptoms was found to be significantly associated with an increased risk of subsequent infections.

During the initial study period in spring and summer 2020, there was a substantial decrease in seasonal respiratory virus detection in Seattle metropolitan community samples due to COVID-19 mitigation efforts with viral detection increasing steadily into 2021 and 2022 (Figure 1 B). This has also may have contributed to a decrease in the number of viral detections available to study specifically for influenza viruses.[4,18-19] This sample size limitations restricted our ability to comprehensively examine specific viral pairings, some of which have been hypothesized to result in viral interference.[20-24] Therefore, this model of any subsequent viral detection was used to maximize the analysis sample size.

Prior literature investigating respiratory viral interference suggests different hypotheses on how an interference effect may take place. These findings highlight the heterogeneity involved in multiple viral infections and emphasize the significance of investigating viral interference on both a population-wide and individual basis especially since they continue to be a significant cause of morbidity and mortality. A number of studies highlight the ability of several respiratory viruses including Rhinovirus, RSV, and Influenza to block or suppress the growth of other viruses when simultaneously present in the same host,[22-26] through mechanisms of innate immunity, we find a significant decrease in subsequent infections in risk period 0-14 which includes co-infection which may reflect these mechanisms. Furthermore, we find a significant reduction in subsequent infection risk after 60 days since the primary infection. The mechanism by which a reduction in risk may occur is suggested by evidence of persistent adaptive immunity following primary infection.[27,28] An example of this is a recent study by Sieber et al, showing that SARS-CoV-2 infection induced a robust and lasting neutralizing antibody response for up to 12 months post-infection and long-term immunity elicited by Measles presents another classic example.[27, 29] Nonetheless, it is crucial to highlight that studies suggest that decay in antibody production after infection or vaccination is not linear and varies by pathogen from several months to years.[30]

Several studies have discussed the relationship between concurrent and subsequent respiratory infections, cycle threshold, and symptom severity. Lower Ct values, indicating higher viral loads, have been hypothesized to be linked to more severe symptoms in patients with influenza, human metapneumovirus, respiratory syncytial virus, coronavirus, and rhinovirus.[31-33] Furthermore, the duration of infectiousness in COVID-19 cases has been found to correlate with Ct values, with a decline in the probability of culturing the virus in samples with higher Ct values.[34] In contrast, several studies have shown evidence that Ct values do not correlate either with the severity of clinical outcomes or with the manifestation of symptoms.[35,36] Taking into account that the seasonality of respiratory viral infections, influenced by environmental factors such as temperature and humidity, may also play a role in the severity of these infections.[37]

In the present analysis, we find that the presence of symptoms in primary infection was significantly associated with a reduction in the cycle threshold of subsequent infections (Figure 3). Furthermore, we find that prior infection of SARS-CoV-2 was significantly associated with an increase in subsequent Rhinovirus cycle threshold for risk periods 14-30 days since the primary infection. We also find that there’s no significant association between primary Rhinovirus infection and subsequent SARS-CoV-2 infection cycle threshold for that risk period. These results are consistent with previous findings reported by Burstein et al. where they explored the effect of pathogen-pathogen interaction of SARS-CoV-2 and Rhinovirus on Ct.[5] In contrast, Rhinovirus infection was associated with an increase in Ct of SARS-CoV-2 for risk periods 14-30 day and 30-60 days since the primary infection.

Limitations

The present study has several limitations which should be considered when interpreting our findings. It was primarily conducted during the COVID-19 pandemic, which may have reduced viral cocirculation, limiting the sample size. Furthermore, the pandemic likely affected participants' behavior, including COVID-19 vaccination, which was not included in this analysis due to >70% missing data in reporting, use of nonpharmaceutical interventions, and other factors that may affect exposure and infection and were not accounted for in this analysis. This may affect the generalizability of our findings, limiting them to the pandemic period and location of study. Nonetheless, the estimates generally presented a persistent pattern across the different pathogens studied and were supported by probable biological mechanisms.

The construction of the models adjusts for relevant covariates including demographic characteristics and the presence of symptoms, nonetheless, there may be unmeasured confounders associated with the risk of primary and subsequent detections impacting conclusions regarding causality.

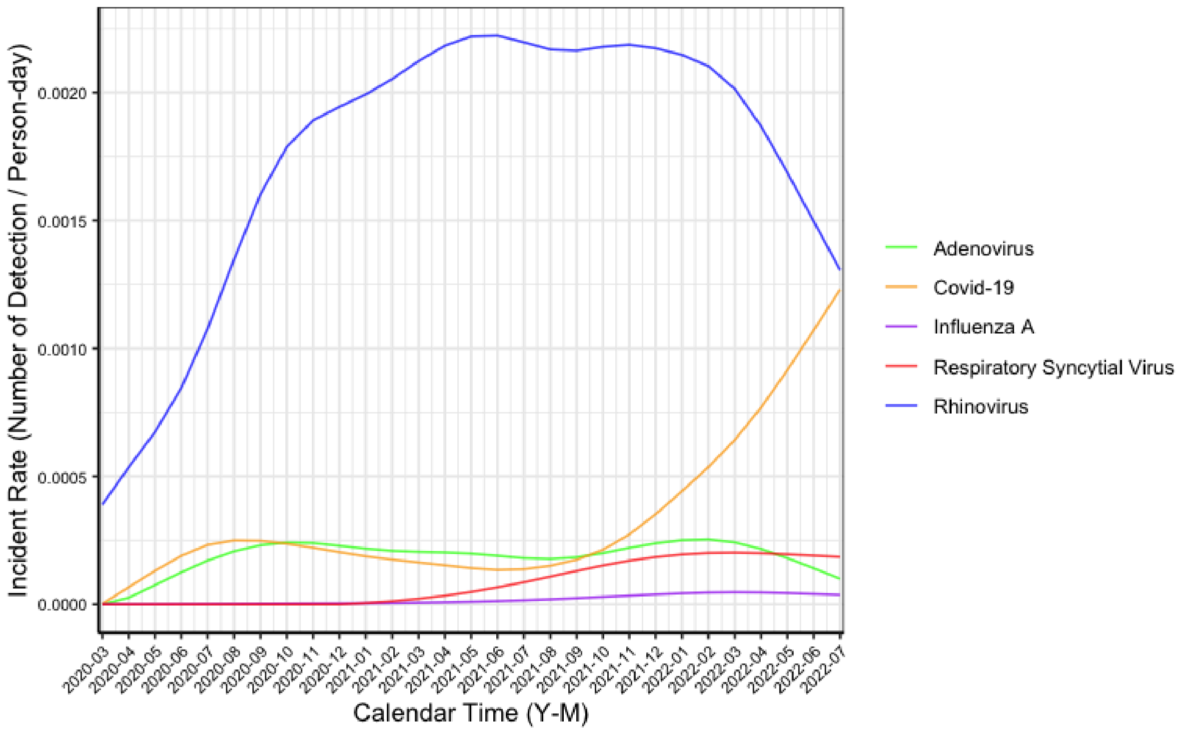
Conclusion

Our study examined the temporal dynamics of respiratory virus interactions, highlighting patterns in subsequent infection risk. The results suggest a significant reduction in risk with another respiratory virus, within 14 days of infection and following two months of primary infection suggesting a potential period of heightened immune response efficacy and pathogen-pathogen interference. The statistical significance of the risk of subsequent virus detection varied by primary virus etiology. These findings contribute to our understanding of the complex interplay between respiratory viruses and underscore the imperative for further research aimed at enhancing protective measures against these pathogens.

AVAILABILITY

All code can be accessed through https://github.com/Eabousam/respiratory-interference-SFS

Figure 1. The Proportion of Respiratory Pathogens Over Time from Seattle SCAN SFS data, 2020-2022. (A) and Proportion of Positive Samples (B).



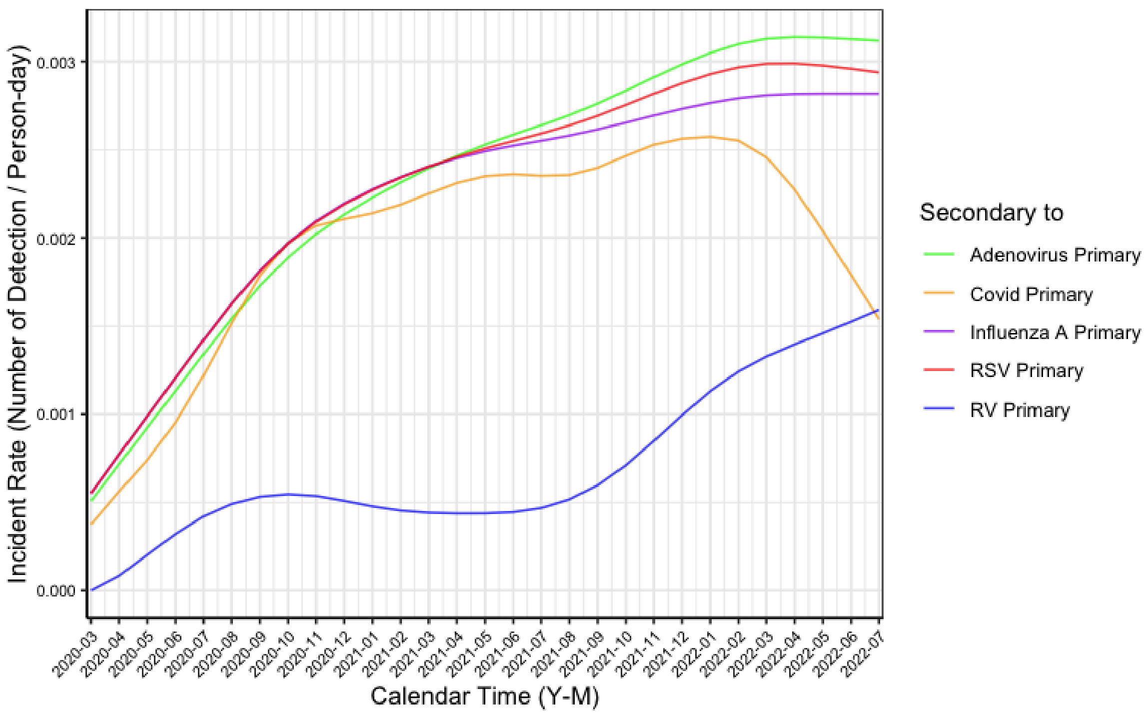


Figure 2. Smoothed incidence rate of respiratory viruses detected (A). Smoothed Incidence rate of a subsequent detection of a respiratory virus, by type of primary virus detected over calendar time of follow-up using SCAN data, Seattle, WA (Mar 2020- July 2022) (B).

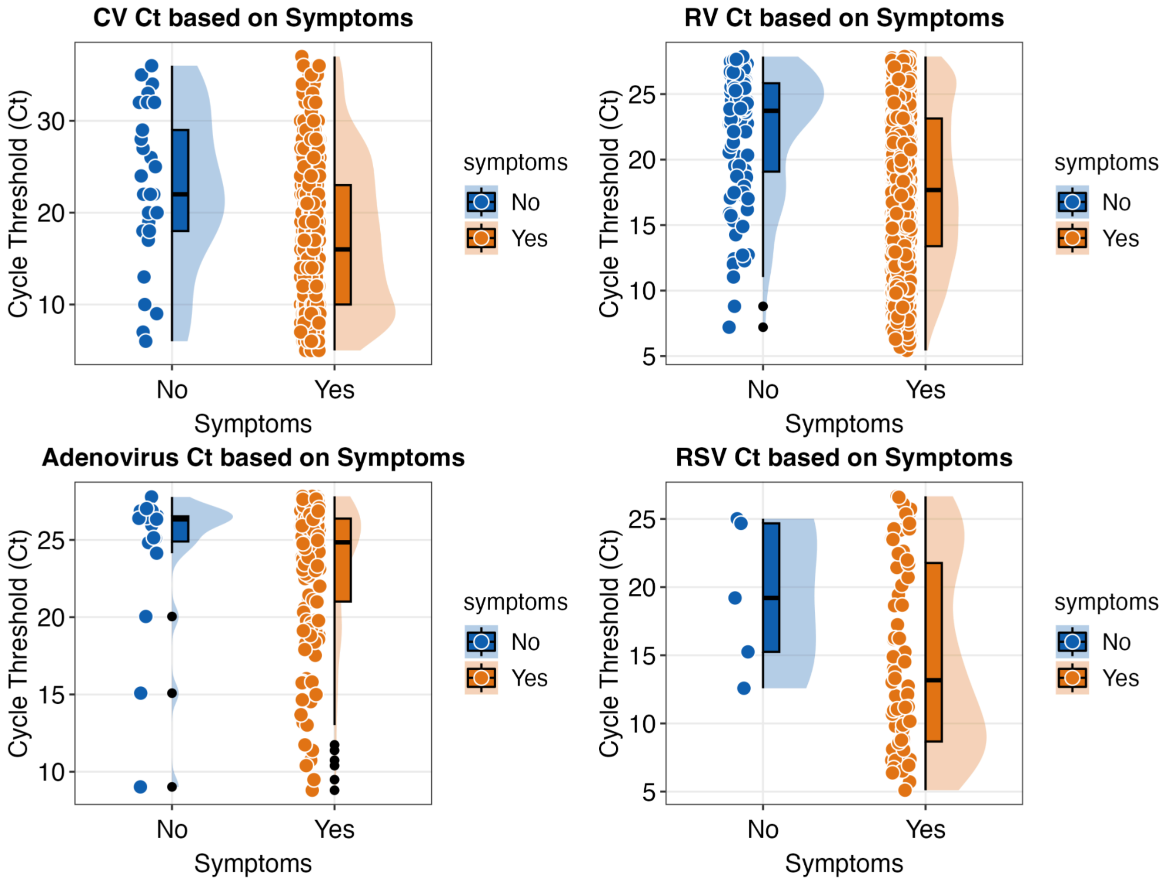


Figure 3. Distribution of cycle threshold values based on the presence of symptoms for SARS-CoV-2 (CV), Human Rhinovirus (RV), Adenovirus, and Respiratory Syncytial Virus (RSV) infected individuals.

Table 1. Demographic and Clinical Characteristics of SCAN Study Samples (n = 14,167) by Type of Respiratory Virus Detected Collected from n = 6,396 Unique Individuals (2020-2022)

		Type Of Respiratory Virus Detected N (%)[2]							
Covariate	Total Samples Tested 14,167 (6,396)[1]	None Detected 11,691 (82.5)	Rhinovirus 1,842 (13.0)	SARS-CoV-2 503 (3.6)	Adenovirus 175 (1.23)	Respiratory syncytial virus 77 (0.54)	Influenza A 8 (0.06)	Influenza B 3 (0.02)	Any Co-detection[3] 129 (0.9%)
Sex									
Male	5,751 (40.6)	4,688 (40.1)	803 (43.5)	213 (42.3)	68 (38.9)	34 (44.2)	4 (50.0)	0 (0.0)	56 (43.4)
Female	8,416 (59.4)	7,003 (59.9)	1,039 (56.5)	290 (57.7)	107 (61.1)	43 (55.8)	4 (50.0)	3 (100)	73 (56.6)
Age group									
< 5	1,289 (9.1)	562 (4.8)	647 (35.1)	37 (7.4)	90 (51.4)	34 (44.2)	0 (0.0)	0 (0.0)	78 (60.5)
6 - 16	1,188 (8.4)	839 (7.2)	282 (15.3)	44 (8.7)	19 (10.9)	10 (13.0)	3 (37.5)	2 (66.7)	11 (8.5)
17 - 45	8,786 (62.0)	7,663 (65.6)	763 (37.2)	307 (61.0)	56 (32.0)	26 (33.8)	5 (62.5)	1 (33.0)	35 (27.1)
46 - 65	2,335 (16.5)	2,104 (18.0)	127 (6.9)	96 (19.1)	8 (4.6)	4 (5.2)	0 (0.0)	0 (0.0)	4 (3.1)
66 +	569 (4.0)	523 (4.5)	23 (1.3)	19 (3.8)	2 (1.1)	3 (3.9)	0 (0.0)	0 (0.0)	1 (0.8)
Self-Reported Symptoms[4]									
Yes	12,092 (85.3)	9,771 (83.6)	1,738 (94.3)	473 (94.1)	157 (89.7)	72 (93.5)	6 (75.0)	2 (66.7)	125 (96.9)
No	2,075 (14.7)	1,920 (16.4)	104 (5.7)	30 (6.0)	18 (10.3)	5 (6.5)	2 (25.0)	1 (33.3)	5 (3.9)

[1]Unique individuals
[2]Data represent no. or no. (%); percentages are column percentages.
[3] Viral codetection was defined as a nasal swab specimen collected from a unique participant in which >1 respiratory pathogen was concurrently detected
[4]Reported symptoms include any presence of chills or Shivering, cough, feeling Feverish, headaches, muscle or Body Aches, sore Throat, and runny or Stuffy Nose

Table 2. Frequency of primary and secondary viral detection pairs given a specific primary virus detection

	Secondary Viral Detection[1]					
Primary Viral Detection	**Day < 14**[2]	**14 < Day < 30**	**30 < Day <= 60**	**90 < Day <= 180**	**180 < Day <= 365**	**Total Secondary Detections**
Rhinovirus	72 (1,556)[3]	5 (250)	12 (331)	41 (776)	49 (501)	179
SARS-CoV-2	31 (548)	2 (44)	3 (46)	11 (79)	3 (32)	50
Adenovirus	75 (216)	7 (65)	14 (92)	22 (119)	20 (86)	138
Respiratory Syncytial Virus	21 (90)	5 (30)	8 (35)	17 (58)	0[e] (32)	51
Influenza A	2 (12)	0[e] (3)	1 (7)	1 (12)	0[e] (2)	4
Influenza B	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]

[1]Includes Human Rhinovirus (HRV), SARS-CoV-2, Adenovirus, Respiratory Syncytial virus (RSV), Influenza A, and Influenza B excluding recurrent primary infections
[2]Includes co-detection with any specified respiratory pathogen (Risk period = 0)
[3] Total secondary groups tested within risk period
[e]No subsequent detection within the specified risk period

Table 3. Estimated Hazard Ratio of Secondary Detection by Time Since Primary Detection in Time-Dependent Covariate Cox Model

	HR (95% CI) of Secondary Detection by Primary											
	Timing of Secondary Virus Detection[d,s]							Age Group[c]				
Primary Virus Detection	None	0-14 d	14-30 d	30-60 d	60-180 d	180-360 d	Sex[c]	< 5	6-16	46-65	66 +	Presence of Symptoms[c]
Rhinovirus	1.0	**0.61**[p] (0.46, 0.81)	1.34 (0.56, 3.14)	1.01 (0.57, 1.79)	**0.60**[p] (0.41, 0.89)	**0.36**[p] (0.24, 0.50)	0.96 (0.82, 1.13)	**3.35**[p] (2.69, 4.18)	**1.27**[p] (0.78, 0.97)	0.83 (0.67, 1.00)	0.75 (0.49,1.15)	**1.37**[p] (1.02, 1.86)
SARS-CoV-2	1.0	**0.20**[p] (0.14, 0.29)	1.11 (0.28, 4.03)	0.91 (0.27, 2.28)	0.63 (0.34, 1.15)	**0.20**[p] (0.10, 0.61)	0.96 (0.86, 1.06)	**6.05**[p] (5.50, 6.90)	**2.29**[p] (1.97, 2.64)	**0.54**[p] (0.44, 0.68)	**0.46**[p] (0.24, 0.81)	**2.07**[p] (1.67, 2.56)
Adenovirus	1.0	1.04 (0.79, 1.13)	1.24 (0.60, 2.45)	0.98 (0.60, 1.65)	**0.40**[p] (0.25, 0.51)	**0.20**[p] (0.10, 0.40)	0.99 (0.91, 1.09)	**4.74**[p] (4.20, 5.36)	**1.92**[p] (1.70, 2.19)	**0.70**[p] (0.52, 0.79)	**0.53**[p] (0.40, 0.72)	**2.11**[p] (1.71, 2.58)
Respiratory syncytial virus	1.0	**0.45**[p] (0.29, 0.70)	1.44 (0.60, 3.48)	0.99 (0.58, 2.92)	**0.55**[p] (0.33, 1.00)	0[e]	1.09 (0.94, 1.26)	**4.85**[p] (4.25, 5.35)	**1.92**[p] (1.68, 2.20	**0.70**[p] (0.57, 0.78)	**0.52**[p] (0.38, 0.70)	**2.00**[p] (1.63, 2.44)
Influenza A	1.0	1.20 (0.30, 4.86)	0[e]	2.25 (0.27, 3.07)	0.58 (0.12, 2.55)	0[e]	0.98 (0.89, 1.07)	0[e]	**1.93**[p] (1.71, 2.19)	0[e]	0[e]	**1.99**[p] (1.68, 2.34)
Influenza B	1.0	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]	0[e]

[d]Includes Human Rhinovirus, SARS-CoV-2, Adenovirus, Respiratory Syncytial virus (RSV), Influenza A, and Influenza B.
[s]Schofield residual proportional hazards test > 0.05 representing for valid proportional hazard assumption
[c]Age is a categorical variable, referent age group 17-45; Sex is a binary variable, female is the referent group; symptoms is a binary variable, No presence of symptoms is referent group.
[e]Zero events; therefore, P values could not be calculated.
[p]P < .05.

Table 4. Mixed-Effects Model: Association Between Primary Respiratory Infection and Cycle Thresholds of Secondary Infection

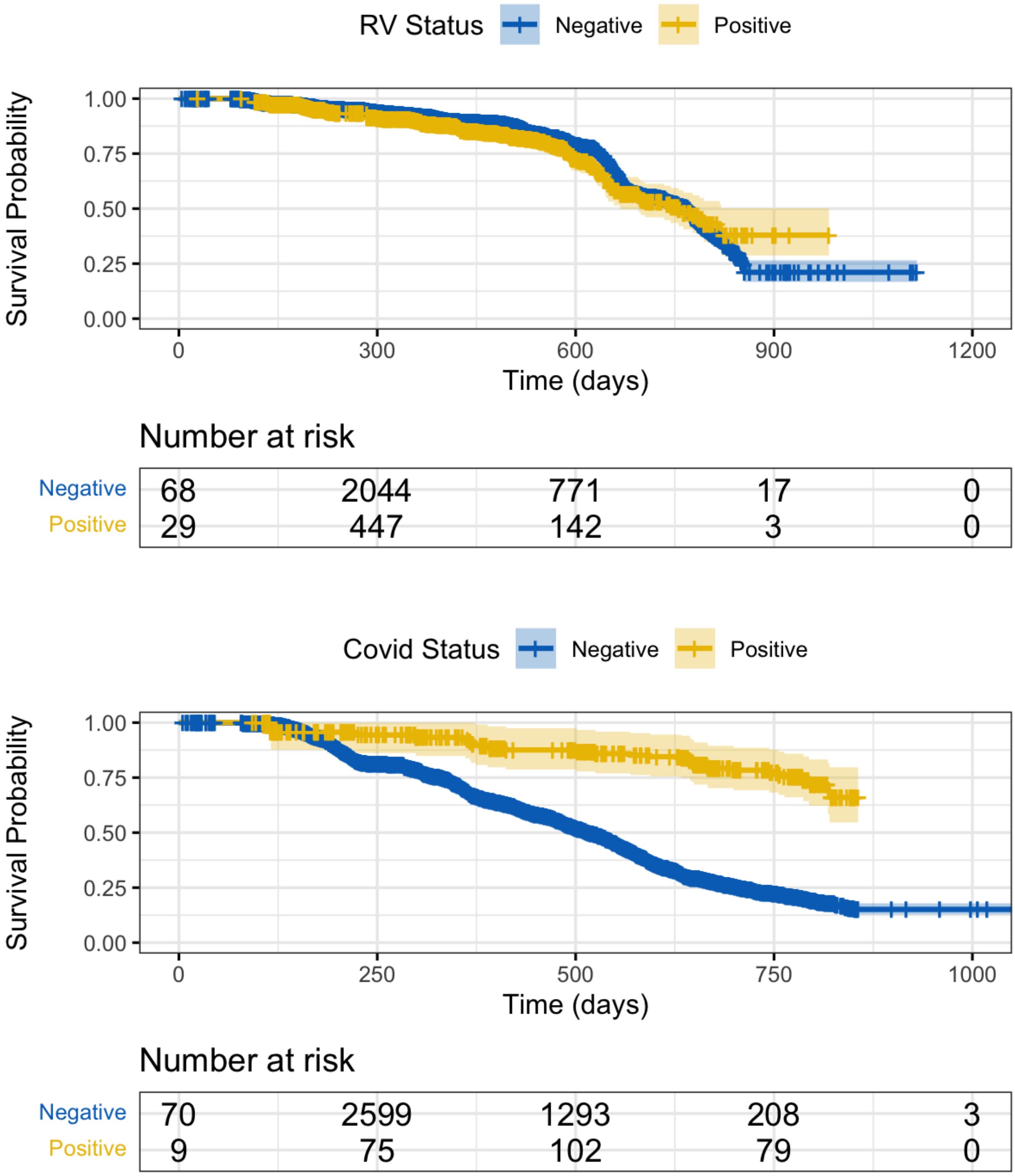
Primary infection	Ct Coefficient estimates (95% CI) of Secondary Detection by Primary											
	Estimates[c]							Age Group				
	None	0-14 d	14-30 d	30-60 d	60-180 d	180-360 d	Sex	< 5	6-16	46-65	66 +	Presence of Symptoms
Human Rhinovirus	1.0	-0.88 (-4.47, 2.72)	4.99 (-0.46, 10.44)	**4.79[p]** (0.61, 8.96)	1.40 (-1.61, 4.41)	0.92 (-1.56, 3.40)	0.16 (-1.31,1.55)	1.00 (-1.27,4.28)	-1.68 (-4.22,0.87)	0.22 (-1.46, 1.90)	0.10 (-3.13, 3.31)	**-2.99[p]** (-5.48, -0.53)
SARS-CoV -2	1.0	**2.52[p]** (0.61, 4.43)	0.95 (-2.69, 4.60)	-0.30 (-3.95, 3.36)	2.34 (-0.23, 4.91)	2.67 (-0.28, 5.60)	-0.06 (-0.59,0.48)	**-2.43[p]** (-2.80,-1.58)	**-2.08[p]** (-2.80,-1.81)	0.04 (-1.02,1.10)	2.15 (-0.10, 4.88)	**-4.23[p]** (-5.07,-3.18)

[c]SARS-CoV-2 Secondary to HRV and HRV Secondary to SARS-CoV-2
[e]Zero events; therefore, P values could not be calculated.
[p]Significant Association; P < .05.

Supplementary Table 1. Sensitivity analysis of subsequent infections to SARS-CoV-2 discarding the first 14 and 30 days of follow-up time in the study; Hazard Ratio of Secondary Detection by Time Since Primary Detection in Time-Dependent Covariate Cox Model

Primary Virus Detection	HR (95% CI) of Secondary Detection by Primary											
	Secondary Virus Detection[d,s]							Age Group[c]				
	None	0-14 d	14-30 d	30-60 d	60-180 d	180-360 d	Sex[c]	< 5	6-16	46-65	66 +	Presence of Symptoms[c]
SARS-CoV-2 (14 days removed)	1.0	**0.21**[p] (0.15, 0.31)	1.40 (0.43, 4.48)	0.70 (0.20, 2.67)	0.69 (0.40, 1.32)	**0.62**[p] (0.10, 0.70)	0.93 (0.83, 1.00)	**6.33**[p] (5.60, 7.13)	**2.39**[p] (2.07, 2.75)	**0.52**[p] (0.41, 0.62)	**0.46**[p] (0.31, 0.68)	**2.59**[p] (2.05, 3.27)
SARS-CoV-2 (30 days removed)	1.0	**0.24**[p] (0.10, 5.8)	0.98 (0.20, 5.99)	1.47 (0.40, 5.43)	0.82 (0.44, 1.53)	**0.26**[p] (0.10, 0.85)	**0.90**[p] (0.79, 0.99)	**5.90**[p] (5.21, 6.71)	**2.46**[p] (2.12, 2.86)	**0.54**[p] (0.41, 0.64)	**0.51**[p] (0.35,0.76)	**2.81**[p] (2.17, 3.65)

[d]Includes Human Rhinovirus, SARS-CoV-2, Adenovirus, Respiratory Syncytial virus (RSV), Influenza A, and Influenza B.
[s]Schofield residual proportional hazards test > 0.05 representing for valid proportional hazard assumption
[c]Age is a categorical variable, referent age group 17-45; Sex is a binary variable, female is the referent group; symptoms is a binary variable, No presence of symptoms is referent group.
[e]Zero events; therefore, P values could not be calculated.
[p]P < .05.



Supplementary Figure 1. Survival Curves of Secondary Respiratory Infection by rhinovirus (A) and SARS-CoV-2 status (B) demonstrating non-proportional hazard.

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