Observational epidemiology for evaluating respiratory virus vaccine impact using population surveillance data

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Abstract

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Observational studies of vaccine preventable diseases (VPDs) are needed to answer questions not addressed by clinical trials and inform policy decisions. In addition, data from community, rather than clinical, settings are essential as clinical surveillance largely does not capture individuals at low risk for severe disease and may not be representative of subclinical disease. Also, mild illness can contribute to onward community transmission and result in missed work or school. Such observational data from community settings is needed both for diseases with licensed vaccines in routine use and diseases with vaccines in clinical development that are expected to be licensed. This dissertation aims to address two epidemiologic gaps for respiratory viruses: (1) estimating effectiveness of COVID-19 booster vaccination among healthy, young adult populations and (2) describing genomic diversity of respiratory syncytial virus (RSV) to inform future vaccine impact studies.

In **Chapter 1**, we provide a rationale for the use of observational epidemiology in the study of VPDs and provide a brief introduction of the specific aims of this dissertation.

In **Chapter 2**, we used data from the Husky Coronavirus Testing Study (HCT), a large SARS-CoV-2 university testing program, to estimate relative vaccine effectiveness (VE) of COVID-

19 mRNA vaccine primary series plus monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection. Data are from September 2021 to July 2022 in a community-based university population. Relative VE was estimated using the test-negative design and adjusted logistic regression implemented via generalized estimating equations (GEE). Analyses included 2,218 test-positive cases (59% received monovalent booster dose) and 9,615 test-negative controls (62%) from 9,066 individuals, with median age of 21 years. Estimated adjusted relative VE of primary series plus monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection was 40% (95% CI: 33–47%) during the overall analysis period and 46% (39–52%) during a period of Omicron circulation. In this relatively young and healthy adult population, an mRNA monovalent booster dose provided increased protection against symptomatic SARS-CoV-2 infection.

In **Chapter 3**, we used data from the Seattle Flu Study (SFS), a community-based respiratory virus surveillance study in Seattle, USA and publicly available RSV genomes to assess genomic diversity of RSV over four respiratory virus seasons (2019–2020, 2020–2021, 2021–2022, and 2022–2023). SFS nasal swabs were collected and tested for RSV by RT-qPCR, with whole genome sequencing (WGS) performed for a subset. Among SFS samples collected from children and adults, 1.8% (232/13014) and 0.2% (86/46042) respectively tested positive for RSV-A and 1.1% (139/13016) and 0.3% (119/46043) for RSV-B. In Washington, USA during 2019–2020, RSV-A and RSV-B co-circulated (majority clades A.D.1 and B.D.4.1.1). No RSV was observed in 2020–2021 following implementation of nonpharmaceutical interventions to reduce SARS-CoV-2 transmission. Subsequently, RSV re-emerged off-season in 2021–2022 and shifted to mostly RSV-B (clade B.D.E.1) and then RSV-A (clade A.D.5.2) in 2022–2023. Shifts in genomic diversity over time were similar for all ages.

We used community-based respiratory virus surveillance data from two studies to answer questions regarding two VPDs, COVID-19 and RSV. Results may inform the evidence base for policy recommendations for COVID-19 and RSV vaccines as well as provide a comparison for future monitoring of SARS-CoV-2 and RSV using similar surveillance data.

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Dedication

To my grandmother, Clara Louise Jordan Bennett, who survived malaria as a child and passed away from COVID-19 in April 2020. Both diseases are now, thankfully, vaccine preventable.

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Acronyms

Acute Respiratory Infection (ARI)

Anterior Nasal Swab (ANS)

Cycle Threshold (Ct)

Emergency Use Authorization (EUA)

Food and Drug Administration (FDA)

Generalized Estimating Equations (GEE)

Husky Coronavirus Testing Study (HCT)

Interquartile Range (IQR)

Midturbinate Swab (MTS)

Monoclonal Antibody (mAb)

Multiple Sequence Alignment (MAFFT)

Relative Cycle Threshold (Crt)

Respiratory Syncytial Virus (RSV)

Seattle Coronavirus Assessment Network (SCAN)

Seattle Flu Study (SFS)

Universal Transport Media (UTM

University of Washington (UW)

Vaccine Effectiveness (VE)

Vaccine Preventable Disease (VPD)

Variant of Concern (VOC)

Whole Genome Sequencing (WGS)

Chapter 1: Introduction

Vaccine-preventable disease epidemiology and observational studies

Robust epidemiologic methods using observational data are needed to evaluate population-level vaccine impact and guide policy decisions for (1) licensed vaccines in routine use and (2) vaccines in clinical development that are expected to be licensed in the next few years. While phase III vaccine clinical trials generate robust estimates of vaccine efficacy and safety necessary for licensure, they also require large numbers of participants and resources and are typically not possible nor ethical to conduct post-licensure. Clinical trials also fail to answer key questions regarding implementation and population-level impact of vaccines. For example, trials often do not, or are not able, to provide data on impact among populations excluded from trials, capture rare or delayed outcomes, assess indirect effects (i.e., 'herd immunity'), identify differences across epidemiologically diverse populations, quantify waning immunity, or estimate future effectiveness for evolving pathogens[1,2].

We therefore rely on observational data, typically obtained through routine surveillance systems, cohorts, or case-control studies to answer questions regarding "real world" VE. However, observational VE studies are often limited by biases and confounding, as vaccinated and unvaccinated individuals often have different health and healthcare-seeking behaviors[3,4]. In addition, following successful vaccine introductions, VPDs can become increasingly rare and consequently difficult to study[5]. Therefore, well-designed epidemiologic studies are critical for demonstrating and identifying gaps in vaccine impact and optimizing policy recommendations in ever changing epidemiologic environments.

This dissertation aims to address two epidemiologic gaps for respiratory viruses: (1) estimating effectiveness of COVID-19 booster vaccination among healthy, young adult populations and (2) describing genomic diversity of RSV to inform future vaccine impact studies.

COVID-19 vaccine effectiveness within a university population

Clinical trials estimated the efficacy of two-dose primary series mRNA COVID-19 vaccines against symptomatic infection with the Wuhan-Hu-1 lineage of SARS-CoV-2 after approximately 8 weeks of follow-up[6,7]. Subsequent mRNA vaccine boosters received authorization on the basis of safety and immunogenicity data alone.[8,9] Post-authorization observational studies, conducted primarily among high-risk populations, have shown waning immunity overtime, variable VE against variants of concern (VOCs), and protection against severe COVID-19 from booster doses[10–17]. Such observational studies provide essential data to policymakers. However, understanding COVID-19 VE in community and non-hospitalized populations of generally young and healthy adults is necessary to make informed vaccine recommendations as individuals with mild and asymptomatic illness contribute to community transmission and are associated with missed work and school and risk of long COVID[18–24]. This is particularly important in an evolving VOC landscape with updated vaccine formulations annually and changing population-level immunity resulting from a hybrid of both immunization and natural infections[25–27].

HCT provided voluntary COVID-19 testing to students, faculty, and staff at University of Washington (UW) from September 2020 to June 2023. The HCT study population was highly vaccinated (>98% of individuals completed a primary COVID-19 vaccine series[28]) and on average younger and healthier than the general population. In **Chapter 2**, we used data from HCT to estimate relative VE of COVID-19 mRNA vaccine primary series plus a monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection.

Respiratory syncytial virus molecular epidemiology in community settings

RSV causes significant morbidity and mortality among children <5 years and adults ≥60 years of age globally[29–32]. Prior to 2023, no efficacious treatments or vaccines for RSV were licensed for general population use[33]. RSV vaccines targeting maternal and older adult populations and a general infant population monoclonal antibody (mAb) were licensed in 2023 and first used during the 2023–2024 respiratory virus season[34–36]. In light of multiple newly available RSV vaccines for use in adults and mAbs for use in children, analyses of pre-vaccine epidemiologic data, including on RSV genomics, are needed to establish baseline data for comparison in post-licensure studies, which will assess population-level impact, shifts in RSV epidemiology and transmission dynamics, and potential vaccine and mAb escape.

Additionally, the COVID-19 pandemic altered RSV epidemiology. RSV declined to historically low levels in 2020 due to nonpharmaceutical interventions aimed at reducing SARS-CoV-2 transmission[37]. When pandemic restrictions were lifted, RSV re-emerged and caused larger than expected interseasonal outbreaks in 2022[38]. Other shifts in RSV epidemiology were variable across countries and included a shift to more severe disease among children of a wider age range than was historically typical[39–41]. The pandemic may also have altered RSV genomic diversity. Two RSV subtypes, RSV-A and RSV-B, each with multiple clades and lineages, typically circulate in community settings and predominance of each type can vary over time and geography[42,43]. In Australia, reductions in RSV genomic diversity were observed during the COVID-19 pandemic, although this has not been widely studied elsewhere and it is unclear if these changes were sustained over time[44].

In order for future RSV vaccine impact studies to evaluate population-level changes on genomic diversity of RSV, baseline data, including data describing impact of the COVID-19 pandemic, are needed. In **Chapter 3**, we leveraged data from SFS, a community-based respiratory virus surveillance study conducted from 2018–2022, in addition to publicly available whole genome sequences, to describe RSV genomic diversity prior to, during, and following implementation of COVID-19 pandemic restrictions.

Contributions of this dissertation

Results from this dissertation address gaps in data on COVID-19 VE among healthy, young adults and changes in RSV genomic diversity following the COVID-19 pandemic. We estimated relative VE of COVID-19 monovalent booster doses and established baseline, pre-vaccine RSV genomic data that described impact of the COVID-19 pandemic.

Chapter 2. Utilizing a university testing program to estimate relative effectiveness of monovalent COVID-19 mRNA booster vaccine versus two-dose primary series against symptomatic SARS-CoV-2 infection

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Abstract

VE studies utilizing the test-negative design are typically conducted in clinical settings, rather than community populations, leading to bias in VE estimates against mild disease and limited information on VE in healthy young adults. In a community-based university population, we utilized data from a large SARS-CoV-2 testing program to estimate relative VE of COVID-19 mRNA vaccine primary series and monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection from September 2021 to July 2022. We used the test-negative design and logistic regression implemented via GEE adjusted for age, calendar time, prior SARS-CoV-2 infection, and testing frequency (proxy for test-seeking behavior) to estimate relative VE. Analyses included 2,218 test-positive cases (59% received monovalent booster dose) and 9,615 test-negative controls (62%) from 9,066 individuals, with median age of 21 years, mostly students (71%), White (56%) or Asian (28%), and with few comorbidities (3%). More cases (23%) than controls (6%) had COVID-19-like illness. Estimated adjusted relative VE of primary series and monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection was 40% (95% CI: 33–47%) during the overall analysis period and 46% (39–52%) during the period of Omicron circulation. Relative VE was greater for those without versus those with prior SARS-CoV-2 infection (41%, 34–48% versus 33%, 9%–52%, P<0.001). Relative VE was also greater in the six months after receiving a booster dose (41%, 33–47%) compared to more than six months (27%, 8–42%), but this difference was not statistically significant (P=0.06). In this relatively young and healthy adult population, an mRNA monovalent booster dose provided increased protection against symptomatic SARS-CoV-2 infection, overall and with the Omicron variant. University testing programs may be utilized for estimating VE in healthy young adults, a population that is not well-represented by routine VE studies.

Introduction

Clinical trials for two-dose primary series mRNA COVID-19 vaccines estimated efficacy against symptomatic infection with the Wuhan-Hu-1 lineage of SARS-CoV-2 after approximately 8 weeks of follow-up and received emergency use authorizations (EUAs) from the US Food and Drug Administration (FDA) in December 2020.[6,7,45,46] In the first year of COVID-19 vaccination programs, an estimated 14 million deaths from COVID-19 were prevented worldwide.[47] Subsequent monovalent (third dose) and bivalent (fourth dose) mRNA vaccine boosters received FDA EUAs on the basis of safety and immunogenicity data alone.[8,9] Post-EUA observational studies have shown that immunity from COVID-19 vaccines wanes over time, VE varies across VOCs, and booster doses provide increased protection against severe COVID-19[10–17]. These 'real-world' COVID-19 VE studies provide critical data, but typically do not capture outcomes in healthy young adults who are at low risk for severe disease.[48]

University populations are generally at low risk for severe COVID-19 but can experience greater SARS-CoV-2 transmission due to congregate living, frequent social gatherings, and travel to and from home locales multiple times a year.[18,49–53] Understanding COVID-19 VE in this population is necessary to inform vaccine recommendations, university policies, and public health actions as mild and asymptomatic illness have an associated burden of missed work and school, can still cause long COVID, and may contribute to onward community transmission.[19–24,54,55] Only two prior studies have estimated COVID-19 VE among university populations, one in the US and one in Japan. Both were conducted during a period of Omicron circulation and evaluated VE of the primary series and a monovalent booster dose compared to primary series only and unvaccinated individuals against any SARS-CoV-2 infection.[56,57] The Japan study included only approximately 500 individuals and did not capture data on waning immunity or prior SARS-CoV-2 infections. While the US study evaluated prior infection history and waning immunity among a large population, there was only one month of testing data included and evidence was insufficient to detect any difference in VE by prior infection history or time since vaccine receipt.

Using data from HCT, which provided voluntary COVID-19 testing to students, faculty, and staff at a large public university in Seattle, WA, USA, we estimated relative VE of the COVID-19 mRNA vaccine primary series and a monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection among a university population over time and by prior SARS-CoV-2 infection status.

Methods

Study Design, Data Collection, and Laboratory Methods

HCT was a prospective cohort study that provided voluntary SARS-CoV-2 testing to UW students, faculty, and staff from September 2020 to June 2023. The study design, data collection, and laboratory methods have been previously described.[18,49] In brief, eligible participants lived on or near (~100-mile radius) the main campus or one of two satellite UW campuses, had a valid university identification number, were English speaking, and were ≥13 years of age.

Participants self-reported demographics, health behaviors, symptoms, prior SARS-CoV-2 positive tests from RT-PCR tests received outside of the study or at-home rapid antigen tests, and COVID-19 vaccination status, including vaccination doses, dates, and products, through online questionnaires received via e-mail or text message. Vaccination history, health behavior, and positive SARS-CoV-2 test results from outside of the study data were updated by participants on monthly and quarterly online questionnaires. During the study period primary series COVID-19 vaccination was mandated by the university whereas booster vaccination was strongly recommended but not mandated (with the exception of health care workers, for whom both the primary series and monovalent booster dose were mandated). Participants received invitations to test for SARS-CoV-2 within the study and reasons for receiving a testing invitation evolved with changing university guidance and COVID-19 prevention goals. From September 2021 to July 2022, participants were invited to test at baseline (enrollment) and if they reported new symptoms, had a known exposure to a SARS-CoV-2 case, returned from out of state travel, or were members of a university-affiliated group experiencing an outbreak. Walk-in testing without an invitation was also available for any reason. Testing was conducted through supervised self-swabbing at staffed kiosks, unsupervised self-swabbing returned to on-campus drop boxes, and unsupervised self-swabbing returned to the laboratory via mail.[58] All data were collected and managed using Project REDCap.[59,60] All participants completed electronic informed consent (or assent and parental permission for those <18 years of age) and the UW IRB approved this study.

For all unsupervised swabs delivered to the laboratory by mail and for some supervised swabs during supply shortages, US Cotton #3 swabs (SteriPack Polyester Spun Swab) in a 10 mL tube and eluted with 1 mL Tris-EDTA were used. For all other supervised swabs and unsupervised swabs delivered to campus drop boxes, RHINOsticTM Automated Nasal Swabs (Rhinostics RH-S000001) in a MatrixTM 1.0 mL ScrewTop Tube (Thermo Fisher 3741) and eluted with 300 μL Tris-EDTA were used.[61] Specimens were tested at the UW Northwest Genomics Center for SARS-CoV-2 using RT-qPCR, a validated method previously described.[62] For all positive specimens with a high quantity of SARS-CoV-2 RNA (typically cycle threshold (Ct) values ≤30), viral genome sequencing was attempted (Illumina COVID-seq), raw sequencing reads were processed using the SFS Assembly Pipeline[63], and consensus genomes were assembled against the SARS-CoV-2 Wuhan/Hu-1/2019 reference genome using a modified iVar pipeline.[64]

Data Analysis

Data for this analysis include HCT swabs collected from September 2021 to July 2022. During this time, invitations to test did not change and were aligned with university testing guidance. Although participants were invited to test within the study, testing following an invitation was voluntary, and walk-in testing was available at any time for any reason. Therefore, in order to reduce confounding by test-seeking behavior, we restricted the primary analyses to individuals who were symptomatic at the time of testing (and excluded those who were not symptomatic and tested for other reasons, such as pre- and post-travel testing).[65]

We defined a probable prior SARS-CoV-2 infection for SARS-CoV-2 negative controls to be any previous positive SARS-CoV-2 RT-PCR or rapid antigen test result either within the study or outside of the study and self-reported on questionnaires. For SARS-CoV-2 positive cases without genomic sequencing data available, we considered a positive SARS-CoV-2 RT-PCR or rapid antigen test result >90 days prior to testing to be a probable prior infection. We also considered positive SARS-CoV-2 RT-PCR or rapid antigen test results >30 days and ≤90 days prior to a positive case to be a probable prior infection if one or more of the following were true: (1) new or worsening symptoms compatible with acute SARS-CoV-2 were reported (with a negative SARS-CoV-2 RT-PCR test result in between positive tests for prior symptomatic infections only), (2) a change in local circulation of SARS-CoV-2 showing predominance (>80%) of a new variant of concern, or (3) the positive case was asymptomatic, there was a negative SARS-CoV-2 RT-PCR test result in between positive tests, and a known exposure to SARS-CoV-2 was reported. Prior infection with genomic confirmation was defined as a previous positive SARS-CoV-2 test result for a different SARS-CoV-2 variant of concern.

Data were excluded from individuals who were unvaccinated or partially vaccinated (i.e., received one of a two-dose primary series or a dose within two weeks of testing), finished the primary vaccine series <6 months prior (i.e., not eligible for a booster dose), received a non-mRNA COVID-19 vaccine, or had unknown vaccination status at the time of testing. Within 90-day intervals, we included the first SARS-CoV-2 positive swab from each individual as test-positive cases and the first negative swab from each individual without any positive swabs as test-negative controls. The same participant therefore could contribute both case and control swabs to the analysis. We used the test-negative design[66] and logistic regression implemented via GEE with an independent working correlation structure and robust standard errors[67] to estimate relative VE of primary series and monovalent booster vaccination compared to primary series only against symptomatic SARS-CoV-2 infection during the entire analysis period and during periods when the Delta and Omicron VOCs were the dominant VOCs circulating in the university community. Analyses were adjusted for age, calendar time, prior SARS-CoV-2 infection, and testing frequency (a proxy for test-seeking behavior, continuous measure of the number of swabs completed per participant during the analysis period). Calendar time was included in the model as 3-month indicator variables for the overall analysis, 1-month indicator variables for the Delta period-specific analysis (September to November 2021) and 2-month indicator variables for the Omicron period-specific analysis (January to July 2022) in order to capture changes in VOCs, test-positivity, and testing demand in the university community. Relative VE was calculated as (1 – adjusted odds ratio).

Secondary analyses included estimating differences in relative VE by prior SARS-CoV-2 infection status and by time since receiving the monovalent booster dose (less than six months versus greater than six months). Models including indicator variables for having received a booster dose and being in the strata listed above were used to estimate stratum-specific effects. Models including indicator variables for not having been boosted and for having been boosted and being in one particular stratum were also fitted, and the Wald P-value corresponding to the latter indicator was used to test for differences between strata.

As sensitivity analyses, we estimated relative VE among all swabs collected in the study (not restricted to those who were symptomatic at the time of testing), included additional health behavior covariates in the regression model (receipt of seasonal influenza vaccine, frequency of wearing a face mask in public, frequency of social distancing, and frequency of hand washing), and stratified the primary analysis by university affiliation (students versus faculty and staff). Finally, to assess potential for residual confounding in this observational study, we estimated the association between symptomatic SARS-CoV-2 infection and seasonal influenza vaccination, for which influenza vaccination is a negative control[68] with no expected biological association.[69]

Results

From September 2021 to July 2022, 123,913 swabs were collected and 39,346 (32%) were obtained from individuals symptomatic at the time of testing and eligible for inclusion in the primary VE analysis (**Figure 1.1**). Among those from individuals who reported being symptomatic, 17,501 were excluded due to unknown COVID-19 vaccination status at the time of testing, or a vaccination status that was not included in the analysis (unvaccinated, partially vaccinated, received the primary series <6 months before the test [i.e., ineligible for a booster dose], or received a non-mRNA vaccine). A maximum of one swab per individual per 90-day period was included in the analysis and an additional 10,012 repeat swabs from individuals who had taken multiple tests within a 90-day interval were excluded. A total of 2,218 test-positive cases (59% received a monovalent booster dose) and 9,615 test-negative controls (62% received a monovalent booster dose) from 9,066 unique individuals were included in the primary analytic sample.

A median of 1 (range: 1 to 4) swab was included in the analysis per individual (maximum one swab included in the analysis per individual per 90-day interval), and individuals included in the study tested between 1 and 72 times (median: 5, interquartile range (IQR): 3, 9) during the analysis period (**Table 1.1**). Over half of individuals (67%) were female, most were White (56%) or Asian (28%), and very few (3%) reported one or more comorbidities considered to be risk factors for severe COVID-19[70]. Median age was 21 years (IQR: 19, 32) and majority of individuals (71%) were students, of whom 25% were on-campus residents and 8% were fraternity or sorority members (social organizations that have experienced previous COVID-19 outbreaks on campus and have chapter housing with upwards of 50 members and large, frequent social events[18]). At the time of testing, 68% of swabs were from individuals who received the Pfizer (BNT162b2) primary series and 32% were from individuals who received the Moderna (mRNA-1273) primary series (**Supplementary Table 1.1**). Among swabs from those who received a monovalent booster dose, 59% and 41% were from individuals who received the Pfizer (BNT162b2) booster dose and Moderna (mRNA-1273) booster dose, respectively. Among individuals responding to questionnaire items regarding reasons for receiving vaccines, self-reported reasons for not receiving an annual influenza vaccine were similar between those who received the COVID-19 primary series only and those who received the primary series and monovalent booster dose at the time of testing (**Supplementary Table 1.2**). Reported reasons for receiving one or more COVID-19 vaccines were also similar, but slightly more individuals who received the primary series only reported receiving a COVID-19 vaccine because it was required for work or school (8%) compared to those who received the primary series and monovalent booster dose (6%).

Enrolled participants who did not test during the analysis period (i.e., only tested before or after the analysis period or enrolled but never tested; N=13,172, 33% of all participants) were generally similar to those who tested once or more during the analysis period (26,865 (67%)), but among those who tested once or more, there was a slightly greater proportion of students and a smaller proportion of staff compared to those who never tested (78% versus 73% and 15% versus 20%, respectively). (**Supplementary Table 1.3**). Also among all swabs, those from individuals with unknown vaccination status at the time of testing (N=22,877, 18% of all swabs) were from people who were generally demographically similar to people with known vaccination status at the time of testing (N=101,036, 82%). However, a greater proportion of swabs from individuals with unknown vaccination status were from students (83%) and males (40%) and a smaller proportion were from faculty (4%) and staff (13%) compared to swabs from individuals with known vaccination status at the time of testing (71% from students, 8% from faculty, 21% from staff, and 35% from males; **Supplementary Table 1.4**). The proportion of HCT swabs collected from individuals with unknown vaccination status at the time of testing declined only slightly over the analysis period (19% of all swabs collected from September 2021 through January 2022 to 18% from January to July 2022) (**Supplementary Figure 1.1**).

Days since symptom onset at testing was similar for cases and controls (median: 2, IQR: 1 to 3 for both) and a median of 3 (IQR: 2 to 6) and 2 (1 to 3) symptoms were reported among cases and controls, respectively (**Table 1.2**). More cases than controls had COVID-19-like illness (self-reported feeling feverish or sweats with cough and/or increased trouble with breathing; 23% versus 6%, respectively) and influenza-like illness (self-reported feeling feverish or sweats with cough and/or sore or itchy/scratchy throat; 29% versus 9%). The most commonly reported symptoms were sore throat (71% and 52% for cases and controls, respectively), runny or stuffy nose (56% and 47%), and cough (55% and 29%). At the time of testing, 9% of cases and 15% of controls had a prior probable SARS-CoV-2 infection. Fifteen of 208 (7%) test-positive cases with a prior probable SARS-CoV-2 infection were confirmed by genomic sequencing.

Estimated adjusted relative VE of the primary series and monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection was 40.3% (95% CI: 33.1-

46.7%) during the overall analysis period (**Table 1.3**). Estimated relative VE was greater when the monovalent booster dose was received within six months of testing (40.7%, 95% CI: 33.4-47.2%) compared to greater than six months (27.1%, 95% CI: 8.2-42.1%), but this difference was not statistically significant (P=0.06). Estimated relative VE was also greater among individuals without any known prior probable SARS-CoV-2 infection (41.1%, 95% CI: 33.6-47.8%) versus among those with a prior probable infection (33.4%, 95% CI: 8.5-51.5%, P<0.001). When stratified by university affiliation, estimated adjusted relative VE of the primary series and monovalent booster dose versus primary series only against symptomatic SARS-CoV-2 infection was 32.9% (95% CI: 24.2-40.6%) among students and 58.5% (95% CI: 42.7-70.0%) among faculty and staff (**Supplementary Table 1.5**). During the period of Delta circulation (September 9, 2021–November 30, 2021, when 100% (N=198) of sequenced study samples were of the Delta variant and nine days before the first Omicron detection in a study sample) only 35 test-positive cases were captured, and we were unable to accurately estimate relative VE. During the period of Omicron circulation (January 1, 2022–July 18, 2022, when 99.8% (N=2901/2906) of sequenced study samples were of the Omicron variant) estimated relative VE was 46.3% (39.4-52.4%).

When analyses were conducted among all swabs collected during the study period (i.e., not restricted to swabs from symptomatic individuals), relative VE estimates were slightly attenuated but similar (33.7%, 95% CI: 27.2-39.6%; **Supplementary Figure 1.2** and **Supplementary Table 1.6**). Results did not meaningfully change when adjusting for health behavior covariates, including influenza vaccination, in the model (**Supplementary Table 1.7**).

Our influenza vaccine sensitivity analysis aimed to assess potential for residual confounding in our primary analysis and included 90% of swabs evaluated in the primary analysis from individuals who self-reported 2021–2022 seasonal influenza vaccination. After adjusting for COVID-19 vaccination, age, calendar time, prior SARS-CoV-2 infections, and testing frequency, we estimated 17.7% (95% CI: 7.5-26.7%) influenza VE against symptomatic SARS-CoV-2 infection (**Supplementary Table 1.8**).

Discussion

We utilized data from a university-wide testing program to estimate relative VE of COVID-19 monovalent booster doses in a university population comprised primarily of young adult students with few comorbidities. We incorporated detailed symptom data from cases and controls and adjusted analyses for prior SARS-CoV-2 infections, which is often not possible in studies using routine COVID-19 testing data that are not longitudinal and do not capture results from other testing programs or at-home test results. We estimated relative VE of the primary COVID-19 vaccine series and a monovalent booster dose compared to primary series alone to be 40% from September 2021 to July 2022 and 46% during the period of Omicron circulation on campus. We also found that relative VE decreased over time since receipt of monovalent booster dose and was higher for individuals without any known prior SARS-CoV-2 infection. By reporting relative VE of COVID-19 booster doses this study can inform use of different COVID-19 vaccination strategies. This study demonstrates the ability of university testing programs to provide VE estimates in a healthy, community population.

Other general population studies that estimate relative VE of the monovalent booster dose relative to primary series only have not captured large samples of young adults with mild COVID-19. As of October 2023, the View-hub COVID vaccines ongoing systematic review has identified 177 VE studies for monovalent mRNA boosters.[48] Over half of these evaluated VE against emergency department visits, ICU admission, severe disease, hospitalization, or death, and of 44 studies that evaluate symptomatic disease, many were among health care workers, long-term care residents, older adults, or other populations at high risk of severe illness. Overall, results from the current study were similar to other studies estimating relative VE of COVID-19 mRNA monovalent booster doses against Omicron infection in non-university populations, including a general population cohort study in Qatar using national surveillance data (estimated relative VE: 47–49%)[71] and a general population cohort study in the US (51%).[72] Specifically in university populations, our estimated relative VE during the Omicron period was similar to but higher than that estimated in another US university cohort study (65% among faculty and staff and 41% among students in this study versus 41% for employees and 38% for students)[56] and lower than relative VE estimated in a case-control study among university students in Japan (60%),[57] although, study designs and confounders adjusted for vary in these studies and results are not directly comparable. Our finding of higher relative VE among faculty and staff was unexpected and not explained by differences in testing frequency, number of symptoms reported at testing, or days since vaccine receipt, which were similar between students and faculty and staff. It is possible that students experienced a higher rate of SARS-CoV-2 exposures through more frequent in-person activities and congregate living and that students who received a booster dose may have modulated their behavior to take fewer precautions that reduce risk of SARS-CoV-2 infection. It is also possible that confounding by healthcare seeking behaviors is stronger among the relatively older faculty and staff.

We estimated higher VE in the first six months after receipt of a monovalent booster dose as compared to six months or more after receipt, but this difference was not statistically significant (P=0.06). This result is consistent with a meta-analysis of 40 studies in the setting of Omicron.[73] In the other US university study, there was not sufficient evidence to conclude that relative VE waned over time, though this study included only one month of testing data.[56] For both individuals with and without a prior SARS-CoV-2 infection, we estimated lower odds of symptomatic SARS-CoV-2 infection among those who received a monovalent booster dose compared to primary series only, however, our finding of higher VE among individuals without any known prior SARS-CoV-2 infection compared to those with a prior infection suggests that the relative increase in protection conferred by the monovalent booster dose is greater for those without any prior SARS-CoV-2 infection. This is consistent with other studies in the setting of Omicron, including findings from a general population case-control study in Qatar.[74] In a test-negative design study of US outpatients, odds of SARS-CoV-2 infection was lower among vaccinated individuals with versus without a prior SARS-CoV-2 infection.[75] In this study of US outpatients, three COVID-19 vaccine doses compared to zero doses was estimated to be 57% (95% CI: 20-76%, N=475) among individuals with a prior SARS-CoV-2 infection, however, no statistically significant association was found among individuals without a prior SARS-CoV-2 infection (23%, 95%

CI: -72-65% and N=215), although the sample size for the no prior SARS-CoV-2 infection group was less than half of the prior SARS-CoV-2 infection group. Our findings also differ from another US community cohort study that did not find a difference in relative VE by prior infection status. The latter study included only 883 participants and had a shorter period of follow-up (approximately 6 months).[72]

In observational VE studies, health care seeking behavior is difficult to fully account for and often is considered to be the most important confounder (in this context, all health behaviors and other factors that influence test and health care seeking).[65] In theory, the test-negative design reduces bias associated with health care seeking behavior by conditioning on those who present for testing.[66,76] However, this is likely an oversimplification of health care seeking behavior, which is multifaceted and likely not fully captured by having sought testing or not.[76] In addition, selection bias in VE studies using the test-negative design can bias VE estimates through collider stratification bias (i.e., when a factor influenced by the exposure and outcome, or influenced by causes of the exposure/ outcome, is conditioned on by design).[76–78] By conditioning on having tested, it is possible to induce collider stratification bias if confounding by health care seeking behavior is not adequately adjusted for (see **Supplementary Figure 1.3**). In this study, in addition to conditioning only on individuals who presented for testing, we restricted the primary analysis to individuals who were symptomatic at testing (and excluded those testing for other reasons) to minimize confounding by test-seeking behavior, although at least one study in support of the test-negative design suggests that using syndrome-negative controls may give similar VE estimates as symptomatic test-negative controls.[65,79]

We also adjusted analyses for age, calendar time, prior SARS-CoV-2 infection, and testing frequency (as a proxy for test-seeking behavior). In sensitivity analyses we included additional health behavior covariates, and these did not meaningfully change VE estimates. In addition, it has been suggested that confounding by health care behavior may be further reduced when comparing booster vaccination to primary series vaccination alone as these individuals likely have more similar health care behaviors compared to unvaccinated individuals.[77] However, during our analysis period, primary series COVID-19 vaccination was mandated by the university whereas booster vaccination was strongly recommended but not mandated, with the exception of health care workers, for which both were mandated. Therefore, it was still important to consider and account for confounding by health care behavior in this study.

Despite these efforts to account for confounding in this analysis, our influenza vaccine sensitivity analysis estimated seasonal influenza VE of 18% against symptomatic SARS-CoV-2 infection. Influenza vaccination is thought to have no to minimal direct protection against SARS-CoV-2 infection and therefore this likely represents some residual confounding in our primary analysis due to "healthy vaccinee bias," where vaccinated individuals are on average at lower risk for infection compared to unvaccinated individuals.[80,81] These results raise questions about validity of COVID-19 VE estimates, not only from this study but also other studies using the test-negative design, particularly those without data on both influenza and SARS-CoV-2 vaccination and infection status that do not account for biases from collinearity of COVID-19 or from not using controls that test negative for both SARS-CoV-2 and influenza viruses.[82–85] As annual COVID-19 vaccine formulations become standard, estimating VE of these vaccines each year is of interest, similar to annual influenza VE studies.[85] The test-negative design is frequently used for such studies because differences in health care seeking behavior between vaccinated and unvaccinated individuals can bias results from influenza/COVID-19 VE cohort studies. Cohort studies can also be more difficult and costly to conduct relative to test-negative design studies that can leverage routine surveillance or testing data.[80] Therefore, new methods development to increase validity of VE estimates and reduce bias from confounding for test-negative design studies is needed, including evaluating the potential for negative control results, such as those estimated in this analysis, to adjust VE estimates for residual confounding and simulation studies to evaluate new approaches.

Our analysis has other limitations. Although participants were invited to test in certain circumstances in accordance with university guidelines, testing was voluntary, and individuals could obtain testing for any reason. Therefore, to a large degree, participants self-selected for testing. In addition, participants who tested but did not report up-to-date vaccination status were excluded from the analysis and may differ from included participants in health behaviors that are related to VE and our results may not be generalizable to the wider university population. Second, our study relied on self-reported vaccination status, which was dependent on participant recall and may result in misclassification.[76] Despite this, participants entered vaccine information in online forms, which may have reduced misclassification due to social desirability bias. In addition, COVID-19 vaccination cards were commonly available to participants during the study period and many individuals retained pictures of their vaccination card on their phone to access restaurants and other venues using vaccine mandates. The ability to conveniently reference vaccination cards may theoretically have improved self-report of vaccination status relative to other non-COVID-19 vaccines. Available data from the US on accuracy of self-reported COVID-19 vaccination status suggest that it may be a reasonable source of vaccine information. Among a community cohort of >40,000 US adults, >90% of survey respondents reported receiving vaccine doses within ±7 days of electronic medical record documentation and among a hospitalized cohort of US adults, 95% agreement was observed between self-report and electronic medical record.[86,87] The accuracy of self-reported vaccination data in this study may be similarly high and we do not believe that misclassification of vaccination status was differential between our comparison groups. Despite this, we did not validate self-reported vaccination data. Third, we were unable to estimate VE compared to unvaccinated individuals, which is typically the standard in VE studies, because a very low proportion of participants were unvaccinated. Finally, known prior SARS-CoV-2 infections were more common among test-negative cases in our study and although it is unknown if this is the case for both symptomatic and asymptomatic infections as we did not collect serologic data, depletion of susceptible individuals may have biased our estimates of relative VE by time since receipt of monovalent booster dose.[88]

Our study captured a large young adult population that is not frequently included in VE studies. Although this population is younger and has fewer comorbidities than the general adult population and is overall at lower risk for severe COVID-19, it is an important population to understand vaccine impact in given congregate living and social factors that increase risk for SARS-CoV-2 transmission.[18,49–53] In addition, individuals with mild and asymptomatic illness may contribute to community transmission, including to more vulnerable populations, as has been documented by genomic sequencing for deaths among skilled nursing facility residents.[89] Mild infections are also associated with missed work and school and risk of long COVID.[18–24] Our study captured detailed symptom data for both cases and controls with mild illness. We also captured data on prior SARS-CoV-2 infections documented from repeat tests within the study and both RT-PCR and rapid antigen tests performed outside of the study and reported through online questionnaires. Therefore, our prior infection data are likely complete, despite our inability to access SARS-CoV-2 testing results from tests performed outside of the study that participants failed to self-report on study questionnaires.

Conclusions

In a university population that was on average younger, had fewer comorbidities, and was overall at lower risk for severe COVID-19 than the general adult population, a monovalent booster dose provided meaningful protection against symptomatic SARS-CoV-2 infection with the Omicron variant over primary series alone. These results can inform university decisions regarding future COVID-19 vaccination recommendations and policies. This study offers a framework for estimating VE for COVID-19 and other vaccinations in university populations.

Table 1.1. Self-reported demographic data on unique participants included in primary analyses who tested for SARS-CoV-2 while symptomatic at least once from September 2021–July 2022 (N=9066)

No. swabs incl. in analysis per participant, Median (IQR)	1 (1, 2)
No. swabs completed per participant during analysis period, Mean (IQR)	5 (3, 9)
Age at enrollment (years), Median (IQR)	21 (19, 32)
Sex assigned at birth, N (%)	
Male	2907 (32%)
Female	6106 (67%)
Other	9 (0.1%)
Unknown	44 (0.5%)
Race, N (%)	
American Indian/Alaska Native	31 (0.3%)
Asian	2511 (28%)
Black	198 (2%)
Native Hawaiian or other Pacific Islander	30 (0.3%)
White	5034 (56%)
Other	281 (3%)
Multiple Races[1]	781 (9%)
Unknown	200 (2%)
Hispanic Ethnicity, N (%)	
Yes	752 (8%)
No	8223 (91%)
Unknown	91 (1%)
One or more comorbidities[2], N (%)	286 (3%)
Affiliation, N (%)	
Student	6451 (71%)
On-campus Resident[3]	2264 (35%)
Fraternity or Sorority Member[3]	533 (8%)
Faculty	679 (8%)
Staff	1881 (21%)
Volunteer	2 (0.02%)
Other	53 (0.6%)
Household Size[4], Median (IQR)	3 (2, 4)
Received 2021–2022 seasonal influenza vaccine, N (%)	
Yes	4885 (54%)
No	3298 (36%)
Don't Know or Unknown	883 (10%)

1 'Multiple Races' categories with N≥10 include: Asian and White (n=470), White and Other (n=60), American Indian/Alaska Native and White (n=59), Black and White (n=50), Asian and Native Hawaiian or other Pacific Islander (n=28), Native Hawaiian or other Pacific Islander and White (n=17), Asian and Black (n=20), Asian, Native Hawaiian or other Pacific Islander, and White (n=18), and Asian and Other (n=10)
2 Comorbidities include cancer, chronic obstructive pulmonary disease, emphysema, diabetes, heart disease (heart failure or heart attack), and immunosuppressed individuals[70]
3 Proportions of on-campus residents and fraternity sorority member are among students and are not mutually exclusive (a student can be included in both categories)
4 Household size defined as the number of people sharing the same kitchen or living space; a household size of 6 was assumed for participants who reported ≥6 household members when calculating median household size

Table 1.2. Characteristics of symptomatic SARS-CoV-2 testing events from September 2021–July 2022 included in primary analyses

	Cases (N=2218)	Controls (N=9615)	Total (N=11833)
COVID-19 vaccination status at the time of testing			
Primary series only[1]	908 (41%)	3666 (38%)	4574 (39%)
Primary series and monovalent booster dose[2]	1310 (59%)	5949 (62%)	7259 (61%)
Time since receipt of monovalent booster dose			
<6 months	1039 (79%)	4911 (83%)	5950 (82%)
≥6 months	208 (16%)	722 (12%)	930 (13%)
Unknown	63 (5%)	316 (5%)	379 (5%)
Self-reported symptoms			
Days since symptom onset, Median (IQR)[3]	2 (1,3)	2 (1,3)	2 (1,3)
Number of symptoms reported, Median (IQR)[4]	3 (2, 6)	2 (1, 3)	2 (1, 4)
COVID-19-like illness[5]	517 (23%)	588 (6%)	1105 (9%)
Influenza-like illness[6]	638 (29%)	880 (9%)	1518 (13%)
Sore or itchy/scratchy throat	1564 (71%)	5103 (53%)	6667 (56%)
Runny or stuffy nose	1234 (56%)	4485 (47%)	5719 (48%)
Cough	1227 (55%)	2798 (29%)	4025 (34%)
Headache	862 (39%)	2967 (31%)	3829 (32%)
Feeling feverish or sweats	682 (31%)	1295 (14%)	1977 (17%)
Feeling more tired than usual	695 (31%)	2518 (26%)	3213 (27%)
Muscle or body aches	569 (26%)	1268 (13%)	1837 (16%)
Chills or shivering	450 (20%)	743 (8%)	1193 (10%)
Nausea or vomiting	178 (8%)	599 (6%)	777 (7%)
Increased trouble with breathing	133 (6%)	349 (4%)	482 (4%)
Diarrhea	90 (4%)	474 (5%)	564 (5%)
Ear pain or ear discharge	94 (4%)	335 (4%)	429 (4%)
Eye pain	94 (4%)	335 (4%)	429 (4%)

Loss of smell of taste	47 (2%)	90 (1%)	137 (1%)
Rash	17 (0.8%)	59 (0.6%)	76 (0.6%)
Prior SARS-CoV-2 infection			
Yes, probable[7]	208 (9%)	1462 (15%)	1670 (14%)
Yes, with genomic confirmation[8]	15 (0.7%)	NA	15 (0.1%)

1 Completion of the primary series only and was eligible to receive a booster dose at the time of testing
2 Received a booster dose at least two weeks prior to testing
3 Symptom onset date unknown for 1041 swabs
4 Number of specific symptoms unknown for 531 swabs
5 Self-reported feeling feverish or sweats with cough and/or increased trouble with breathing
6 Self-reported feeling feverish or sweats with cough and/or sore or itchy/scratchy throat
7 Probable prior infection:

1. For SARS-CoV-2 negative controls, previous positive SARS-CoV-2 PCR or rapid antigen test result
2. For SARS-CoV-2 positive cases when genomic sequencing is not available:
    a. Positive SARS-CoV-2 PCR or rapid antigen test result >90 days prior, OR
    b. Positive SARS-CoV-2 PCR or rapid antigen test result >30 days and ≤90 days prior, AND:
        i. Prior infection was symptomatic with: new or worsening symptoms and negative PCR test result between positive test results, OR since last positive test result, there was a change in local circulation of SARS-CoV-2 showing predominance (>80%) of a new variant/lineage, OR asymptomatic and negative PCR test result between positive test results with known exposure to SARS-CoV-2
        ii. Prior infection was asymptomatic with: new symptoms compatible with acute SARS-CoV-2 infection, OR since last positive test result, there was a change in local circulation of SARS-CoV-2 showing predominance (>80%) of a new variant/lineage, OR asymptomatic and negative PCR test result in between positive test results with known exposure to SARS-CoV-2

8 Prior infection with genomic confirmation: previous positive SARS-CoV-2 PCR test result, AND different SARS-CoV-2 variant of concern/lineage

Table 1.3. Association between symptomatic SARS-CoV-2 infection and prior COVID-19 vaccination (primary series and monovalent booster dose versus primary series only)

Dominant SARS-CoV-2 Variant	Test-positive cases (N)	Test-negative controls (N)	Odds Ratio (95% CI)		Adjusted Vaccine Effectiveness (%)[1]
			Crude	Adjusted[2]	
Overall	2218	9615	0.89 (0.81, 0.98)	0.60 (0.53, 0.67)	40.3 (33.1, 46.7)
Months Since Monovalent Booster Dose					
<6 months	2155	9299	0.85 (0.77, 0.94)	0.59 (0.53, 0.67)	40.7 (33.4, 47.2)
≥6 months			1.16 (0.98, 1.38)	0.73 (0.58, 0.92)	27.1 (8.2, 42.1)
Prior SARS-CoV-2 Infection					
None	2218	9615	0.91 (0.82, 1.00)	0.59 (0.52, 0.66)	41.1 (33.6, 47.8)
≥1			0.61 (0.47, 0.78)	0.67 (0.49, 0.92)	33.4 (8.5, 51.5)
Delta[3]	35	2056	0.78 (0.24, 2.58)	1.04 (0.32, 3.38)	-3.9 (-238.0, 68.0)
Omicron[4]	2071	6680	0.42 (0.38, 0.47)	0.54 (0.48, 0.61)	46.3 (39.4, 52.4)

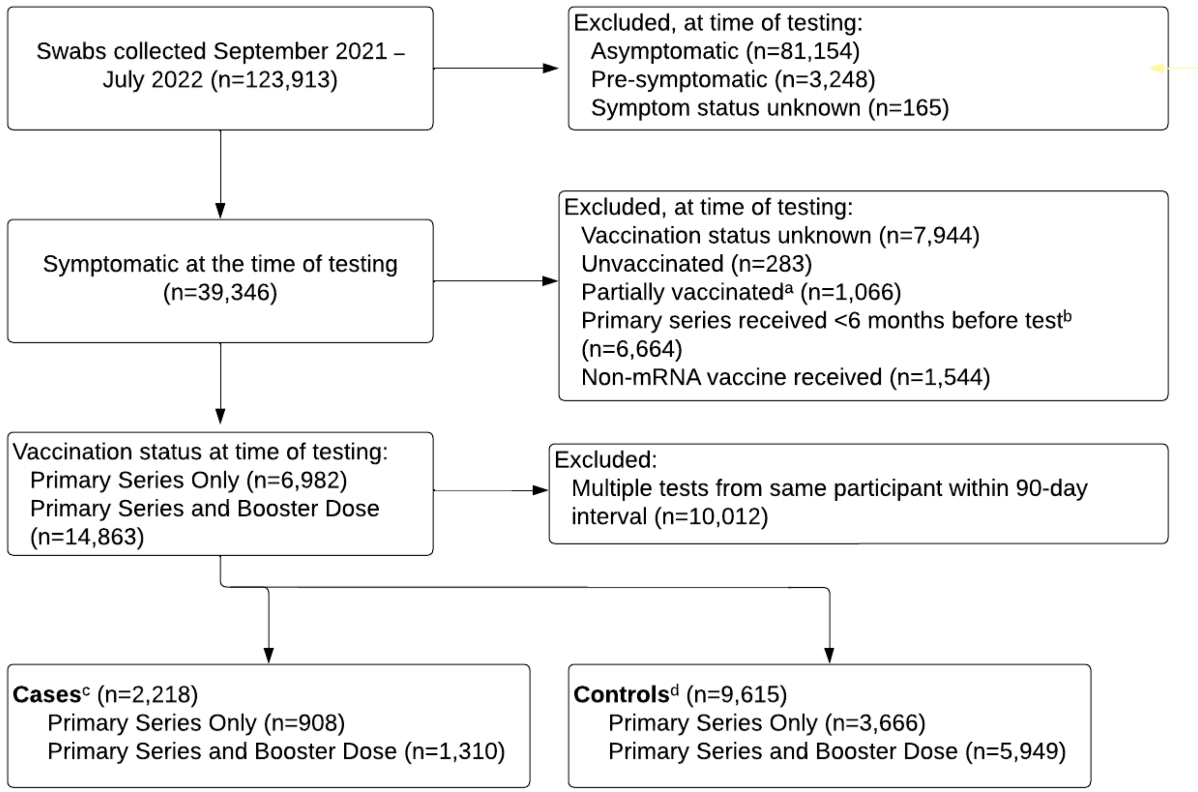
1 Adjusted vaccine effectiveness = (1 – the adjusted odds ratio)

2 Logistic regression of SARS-CoV-2 infection status (cases versus controls) on COVID-19 vaccination status (primary series and monovalent booster dose versus primary series only) adjusted for age, calendar time, prior SARS-CoV-2 infections (yes versus no), and testing frequency (proxy for test-seeking behavior) implemented via generalized estimating equations (GEE)[67]

3 September 9, 2021–November 30, 2021, when 100% of sequenced study samples were of the Delta variant and nine days before the first Omicron detection in a study sample

4 January 1, 2022–July 18, 2022, when 99.8% of sequenced study samples were of the Omicron variant

Figure 1.1. Flowchart of Husky Coronavirus Testing Study swabs included in primary vaccine effectiveness analysis (symptomatic at the time of testing only)



a Last vaccine dose (primary series or booster) received within two weeks of testing or one of two-dose primary series received
b Ineligible for booster dose
c First positive test from each participant within 90-day intervals
d First negative test from participants without a positive test within 90-day intervals

Supplementary Table 1.1. Vaccine manufactures/ products self-reported by participants for COVID-19 testing events from September 2021–July 2022 included in primary analyses

Manufacturer/ product	COVID-19 vaccination status at the time of testing	
	Primary series only[1] (N=4574)	Primary series & monovalent booster dose[2] (N=7259)
Primary series		
Moderna (mRNA-1273)	1440 (32%)	2292 (32%)
Pfizer (BNT162b2)	3128 (68%)	4957 (68%)
Mixed (Moderna and Pfizer)	6 (0.1%)	10 (0.1%)
Booster dose		
Moderna (mRNA-1273)	NA	2984 (41%)
Pfizer (BNT162b2)		4275 (59%)

1 Completion of the primary series only and was eligible to receive a booster dose at the time of testing
2 Received a booster dose at least two weeks prior to testing

Supplementary Table 1.2. Vaccine beliefs reported by vaccination status at the time of testing for testing instances included in the primary analysis (from 9066 unique participants)

	Primary series only[1] (N=4574)	Primary series and monovalent booster dose[2] (N=7259)
Received 2021–2022 seasonal influenza vaccine, N (%)		
Yes	1810 (40%)	4832 (67%)
No	2325 (51%)	1714 (24%)
Primary reason for <u>not</u> receiving seasonal influenza vaccine, N (%)		
None of the below, prefer not to say, or no response	384 (17%)	253 (15%)
Among those that reported reason(s) for not receiving seasonal influenza vaccine:	N=1941	N=1461
Planning to receive the flu vaccine	1272 (66%)	936 (64%)
Do not have time to get vaccinated	298 (15%)	193 (13%)
Not worried about getting sick with the flu	132 (7%)	127 (9%)
Not required for work or school	106 (5%)	90 (6%)
Not offered at a convenient location	49 (3%)	57 (4%)
Concerns about vaccine safety or effectiveness	36 (2%)	25 (2%)
Not covered by health insurance	27 (1%)	17 (1%)
Not recommended by a doctor or health care worker	21 (1%)	16 (1%)
Don't Know or Unknown	439 (10%)	713 (10%)
Self-reported reasons for received ≥1 COVID-19 vaccine, N (%)		
None of the below, don't know, prefer not to say, or no response	2164 (47%)	1405 (19%)

Among those that reported reason(s) for receiving ≥1 COVID-19 vaccine:	N=2410	N=5854
Want to protect themselves/ others from COVID-19	276 (11%)	660 (11%)
Required for work or school	189 (8%)	377 (6%)
Required for travel	77 (3%)	145 (2%)
Live in shared or congregate housing	72 (3%)	111 (2%)
Doctor or other health professional recommended it	52 (2%)	131 (2%)
Work in a congregate setting[3]	28 (1%)	68 (1%)
Live in a multigenerational household	24 (1%)	65 (1%)
Having a high-risk medical condition	15 (1%)	57 (1%)
Health care personnel, but does not work directly with COVID-19 patients	11 (0.5%)	22 (0.4%)
Work in a health care setting, not health care personnel	8 (0.3%)	40 (1%)
Work involves direct contract with COVID-19 patients	6 (0.2%)	24 (0.4%)
Live in a long-term care facility	2 (0.1%)	4 (0.1%)
Being ≥65 years of age	0 (0%)	28 (0.5%)

1 Completion of the primary series only and was eligible to receive a booster dose at the time of testing
2 Received a booster dose at least two weeks prior to testing
3 E.g., agriculture, food processing, grocery store, K-12 (educators & staff), childcare, corrections, prisons, jails or detention centers, public transit, fire, law enforcement

Supplementary Table 1.3. Self-reported demographic data on unique participants enrolled in the Husky Coronavirus Testing study who did versus did not test for SARS-CoV-2 ≥1 time during the analysis period

	Did not test (N=13172)	Tested ≥1 time (N=26865)	Overall (N=40037)
No. swabs completed per participant during analysis period, Mean (Range)	0 (NA)	3 (1, 72)	1 (0, 72)
Age at enrollment (years), Median (Range)	23 (14, 83)	20 (15, 84)	21 (19, 29)
Sex assigned at birth, N (%)			
Male	5272 (40%)	10320 (38%)	15592 (39%)
Female	7805 (59%)	16404 (61%)	24209 (60%)
Other	5 (0.04%)	20 (0.1%)	25 (0.1%)
Unknown	90 (1%)	121 (1%)	211 (1%)
Race, N (%)			
American Indian/Alaska Native	71 (1%)	107 (0.4%)	178 (0.4%)
Asian	3704 (28%)	9133 (34%)	12837 (32%)
Black	411 (3%)	627 (2%)	1038 (3%)
Native Hawaiian or other Pacific Islander	56 (0.4%)	85 (0.3%)	141 (0.4%)
White	6954 (53%)	13165 (49%)	20119 (50%)
Other	485 (4%)	865 (3%)	1350 (3%)

Multiple Races	1104 (8%)	2176 (8%)	3280 (8%)
Unknown	387 (3%)	707 (3%)	1094 (3%)
Hispanic Ethnicity, N (%)			
Yes	1153 (9%)	2159 (8%)	3312 (8%)
No	11856 (90%)	24370 (91%)	36226 (91%)
Unknown	163 (1%)	336 (1%)	499 (1%)
One or more comorbidities[1], N (%)	444 (3%)	701 (3%)	1145 (3%)
Affiliation, N (%)			
Student	9596 (73%)	21075 (78%)	30671 (77%)
On-campus Resident[2]	586 (6%)	7208 (34%)	7794 (25%)
Fraternity or Sorority Member[2]	651 (7%)	2398 (11%)	3049 (10%)
Faculty	827 (6%)	1517 (6%)	2344 (6%)
Staff	2600 (20%)	4105 (15%)	6705 (17%)
Volunteer	23 (0.2%)	18 (0.1%)	41 (0.1%)
Other	126 (1%)	150 (1%)	276 (1%)
Household Size[3], Median (Range)	2 (1, 6)	3 (1, 6)	3 (1, 6)

1 Comorbidities include cancer, chronic obstructive pulmonary disease, emphysema, diabetes, heart disease (heart failure or heart attack), and immunosuppressed individuals[70]
2 Proportions of on-campus residents and fraternity sorority member are among students and are not mutually exclusive (a student can be included in both categories)
3 Household size defined as the number of people sharing the same kitchen or living space; a household size of 6 was assumed for participants who reported ≥6 household members when calculating median household size

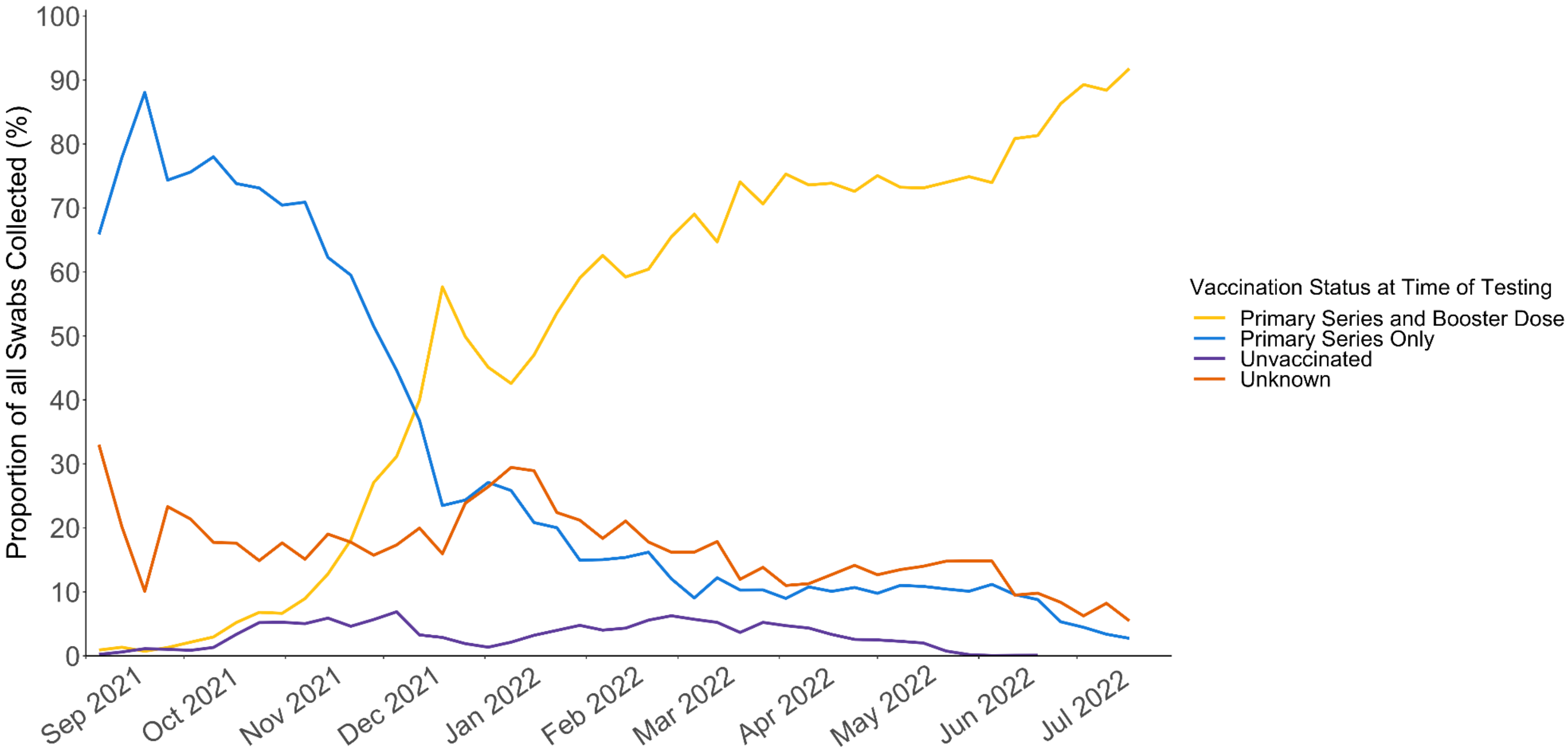
Supplementary Table 1.4. Self-reported demographic data from individuals for all Husky Coronavirus Testing Study swabs during the analysis period for those with known versus unknown vaccination status at the time of testing

	Vaccination Status Known (N=101036)	Vaccination Status Unknown (N=22877)
No. unique participants[1]	20410	7613
No. swabs completed per participant during analysis period, Mean (Range)	3 (1, 72)	5 (1, 55)
Age at enrollment (years), Median (Range)	21 (15, 83)	20 (16, 84)
Sex assigned at birth, N (%)		
Male	34115 (34%)	9219 (40%)
Female	66315 (66%)	13488 (59%)
Other	68 (0.1%)	4 (0.02%)
Unknown	538 (1%)	166 (1%)
Race, N (%)		
American Indian/Alaska Native	339 (0.3%)	109 (1%)
Asian	30949 (31%)	6907 (30%)

Black	1952 (2%)	599 (3%)
Native Hawaiian or other Pacific Islander	220 (0.2%)	65 (0.3%)
White	54264 (54%)	11715 (51%)
Other	2561 (3%)	679 (3%)
Multiple Races	8299 (8%)	1928 (8%)
Unknown	2452 (2%)	875 (4%)
Hispanic Ethnicity, N (%)		
Yes	6531 (7%)	1758 (8%)
No	93275 (92%)	20639 (90%)
Unknown	1230 (1%)	480 (2%)
One or more comorbidities[2], N (%)	3002 (3%)	582 (3%)
Affiliation, N (%)		
Student	71254 (71%)	18940 (83%)
On-campus Resident[3]	27667 (39%)	3334 (18%)
Fraternity or Sorority Member[3]	7510 (11%)	4908 (26%)
Faculty	7712 (8%)	893 (4%)
Staff	21449 (21%)	2857 (13%)
Volunteer	37 (0.04%)	37 (0.2%)
Other	584 (1%)	150 (1%)
Household Size[4], Median (Range)	3 (1, 6)	3 (1, 6)

1 The same participant may be included in both columns of the table if ≥1 swab with vaccination status known and ≥1 swab with vaccination status unknown
2 Comorbidities include cancer, chronic obstructive pulmonary disease, emphysema, diabetes, heart disease (heart failure or heart attack), and immunosuppressed individuals[70]
3 Proportions of on-campus residents and fraternity sorority member are among students and are not mutually exclusive (a student can be included in both categories)
4 Household size defined as the number of people sharing the same kitchen or living space; a household size of 6 was assumed for participants who reported ≥6 household members when calculating median household size

Supplementary Figure 1.1. Vaccination status at the time of testing for all Husky Coronavirus Testing Study swabs collected during the analysis period



Supplementary Table 1.5. Association between symptomatic SARS-CoV-2 infection and prior COVID-19 vaccination (primary series and monovalent booster dose versus primary series only) stratified by university affiliation (students versus faculty and staff[1])

Affiliation	Dominant SARS-CoV-2 Variant	Test-positive cases (N)	Test-negative controls (N)	Odds Ratio (95% CI)		Adjusted Vaccine Effectiveness (%)[2]
				Crude	Adjusted[3]	
Students	Overall	1810	6342	1.08 (0.97, 1.19)	0.67 (0.59, 0.76)	32.9 (24.2, 40.6)
	Delta[4]	27	1544	0.48 (0.06, 3.55)	0.72 (0.10, 5.32)	28.3 (-432.0, 90.34)
	Omicron[5]	1700	4293	0.53 (0.47, 0.60)	0.59 (0.52, 0.68)	40.6 (32.4, 47.7)
Faculty and Staff	Overall	400	3209	1.10 (0.84, 1.43)	0.41 (0.30, 0.57)	58.5 (42.7, 70.0)
	Delta[4]	7	503	1.53 (0.29, 8.02)	1.39 (0.30, 6.32)	-38.7 (-532.0, 69.6)
	Omicron[4]	364	2341	0.36 (0.26, 0.50)	0.35 (0.24, 0.51)	64.7 (48.8, 75.7)

1 Sensitivity analysis stratified by affiliation excludes 70 samples from individuals with "other" affiliation and 2 samples from volunteers

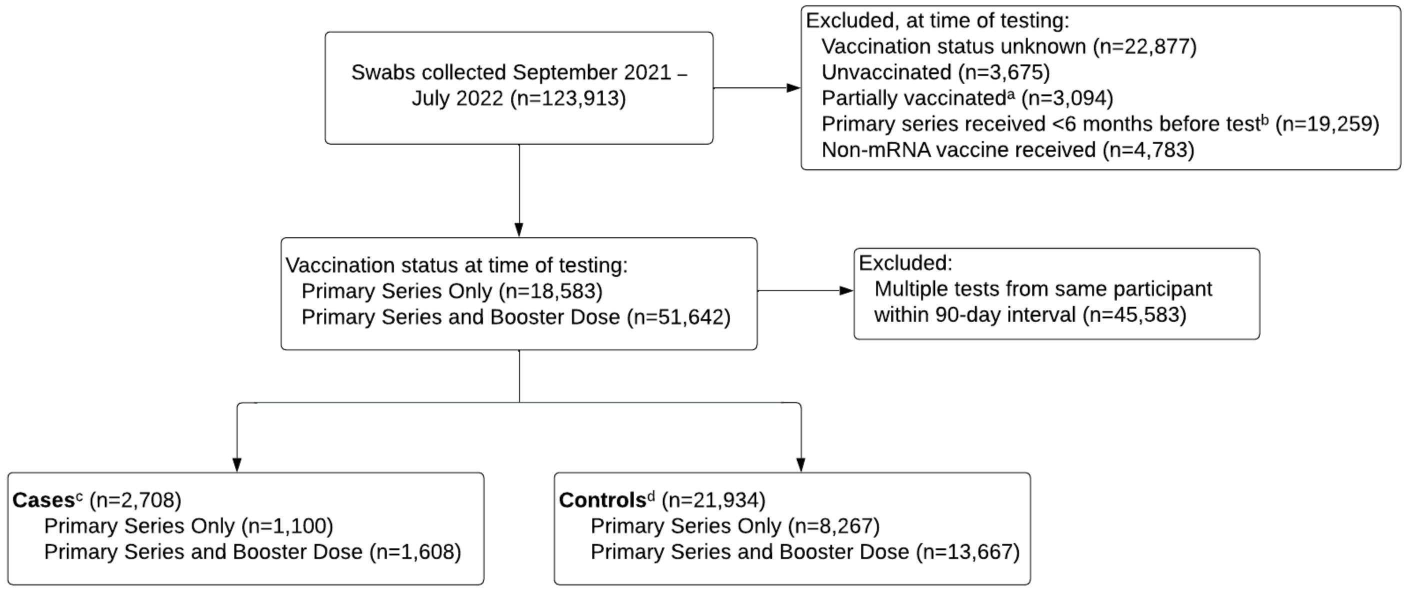
2 Adjusted vaccine effectiveness = (1 – the adjusted odds ratio)

3 Logistic regression of SARS-CoV-2 infection status (cases versus controls) on COVID-19 vaccination status (primary series and monovalent booster dose versus primary series only) adjusted for age, calendar time, prior SARS-CoV-2 infections (yes versus no), and testing frequency (proxy for test-seeking behavior) implemented via generalized estimating equations (GEE)[67]

4 September 9, 2021–November 30, 2021, when 100% of sequenced study samples were of the Delta variant and nine days before the first Omicron detection in a study sample

5 January 1, 2022–July 18, 2022, when 99.8% of sequenced study samples were of the Omicron variant

Supplementary Figure 1.2. Flowchart of Husky Coronavirus Testing Study swabs included in vaccine effectiveness sensitivity analysis (all swabs; not restricted to those symptomatic at the time of testing)



a Last vaccine dose (primary series or booster) received within two weeks of testing or one of two-dose primary series received
b Ineligible for booster dose
c First positive test from each participant within 90-day intervals
d First negative test from participants without a positive test within 90-day intervals

Supplementary Table 1.6. Association between any SARS-CoV-2 infection (i.e., symptomatic, pre-symptomatic, and asymptomatic infection) and prior COVID-19 vaccination (primary series and monovalent booster dose versus primary series only)

Dominant SARS-CoV-2 Variant	Test-positive cases (N)	Test-negative controls (N)	Odds Ratio (95% CI)		Adjusted Vaccine Effectiveness (%)[1]
			Crude	Adjusted[2]	
Overall	2708	21934	0.88 (0.82, 0.96)	0.66 (0.60, 0.73)	33.7 (27.2, 39.6)
Delta[3]	46	4106	0.55 (0.17, 1.76)	0.61 (0.18, 2.04)	39.2 (-104.0, 81.8)
Omicron[4]	2531	15366	0.47 (0.43, 0.52)	0.59 (0.54, 0.65)	40.9 (34.8, 46.4)

1 Adjusted vaccine effectiveness = (1 – the adjusted odds ratio)
2 Logistic regression of SARS-CoV-2 infection status (cases versus controls) on COVID-19 vaccination status (primary series and monovalent booster dose versus primary series only) adjusted for age, calendar time, prior SARS-CoV-2 infections (yes versus no), and testing frequency (proxy for test-seeking behavior) implemented via generalized estimating equations (GEE)[67]
3 September 9, 2021–November 30, 2021, when 100% of sequenced study samples were of the Delta variant and nine days before the first Omicron detection in a study sample
4 January 1, 2022–July 18, 2022, when 99.8% of sequenced study samples were of the Omicron variant

Supplementary Table 1.7. Association between symptomatic SARS-CoV-2 infection and prior COVID-19 vaccination (primary series and monovalent booster dose versus primary series only): sensitivity analyses including additional health behavior covariates[1] over the entire analysis period

Additional Covariate	Test-positive cases (N)	Test-negative controls (N)	Adjusted[2] Odds Ratio (95% CI)	Adjusted Vaccine Effectiveness (%)[3]
Primary analysis restricted to observations with known values for all additional covariates[1]	1928	8720	0.58 (0.51, 0.65)	42.0 (34.6, 48.7)
Seasonal influenza vaccination[4] (received; not received)			0.62 (0.55, 0.70)	38.1 (29.7, 45.5)
Wearing a face mask in public[4] (always; sometimes; never)			0.58 (0.51, 0.65)	42.3 (34.9, 48.9)
Social distancing[4,5] (always; sometimes; never)			0.58 (0.52, 0.66)	41.8 (34.3, 48.5)
Hand washing[4,6] (always; sometimes; never)			0.58 (0.51, 0.66)	41.9 (34.4, 48.6)
All additional covariates above			0.62 (0.55, 0.70)	38.0 (29.6, 45.4)

1 One or more additional covariates missing for 1185 (10%) of observations included in the primary analysis excluded from this sensitivity analysis
2 Logistic regression of SARS-CoV-2 infection status (cases versus controls) on COVID-19 vaccination status (primary series and monovalent booster dose versus primary series only) adjusted for age, calendar time, prior SARS-CoV-2 infections (yes versus no), testing frequency (proxy for test-seeking behavior), and additional covariate indicated in table implemented via generalized estimating equations (GEE)[67]
3 Adjusted vaccine effectiveness = (1 – the adjusted odds ratio)
4 Reported by participants at any time during the analysis period (either on enrollment, daily, or monthly questionnaires)
5 Reported frequency of trying to stay six feet away from people you do not live with
6 Reported frequency of washing hands with soap or hand sanitizer

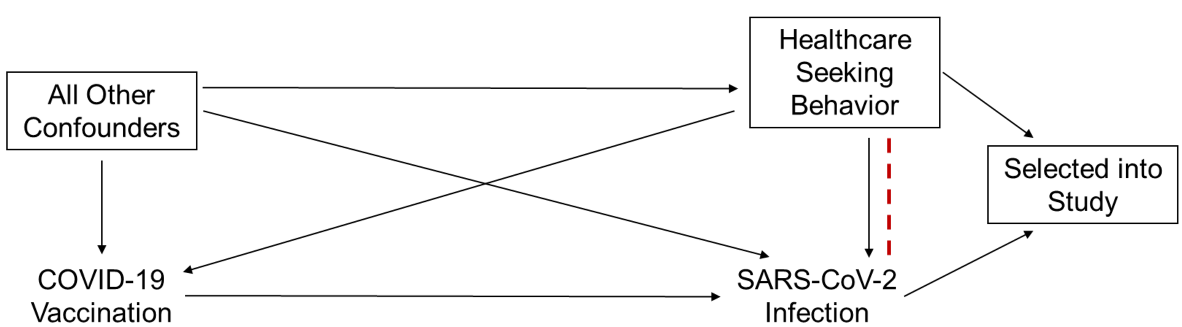
Supplementary Table 1.8. Influenza vaccine sensitivity analysis: association between symptomatic SARS-CoV-2 infection and seasonal influenza vaccination (received versus not received)

Dominant SARS-CoV-2 Variant	Test-positive cases (N)	Test-negative controls (N)	Odds Ratio (95% CI)		Adjusted Vaccine Effectiveness (%)[1]
			Crude	Adjusted[2]	
Overall	1938	8743	0.55 (0.50, 0.61)	0.82 (0.73, 0.92)	17.7 (7.5, 26.7)

Of 11833 swabs included in the primary analysis, seasonal influenza vaccination was self-reported for 10681 (90%). Receipt of a seasonal influenza vaccine was reported for 977 (50%) of test-positive cases 5665 (65%) of test-negative controls.

1 Adjusted vaccine effectiveness = (1 – the adjusted odds ratio)
2 Logistic regression of SARS-CoV-2 infection status (cases versus controls) on seasonal influenza vaccination status (received versus not received) adjusted for COVID-19 vaccination (primary series and monovalent booster dose versus primary series only), age, calendar time, prior SARS-CoV-2 infections (yes versus no), and testing frequency (proxy for test-seeking behavior) implemented via generalized estimating equations (GEE)[67]

Supplementary Figure 1.3. Directed acyclic graph (DAG) of the hypothesized relationship between COVID-19 vaccination status (exposure), risk of symptomatic SARS-CoV-2 infection (outcome), confounding variables to be adjusted for, and selection into the study by seeking voluntary SARS-CoV-2 testing. Confounding variables include age, prior SARS-CoV-2 infection, week of testing, and testing frequency (proxy for health care seeking behavior). Selection bias is represented by conditioning on having self-selected into the study through voluntary testing. Both health care seeking behavior and SARS-CoV-2 infection status influence selection into the study. If health care seeking behavior is not adequately controlled for, this induces collider stratification bias, represented by the red dashed line. When health care seeking behavior is fully controlled for, this path is blocked. Adapted from Sullivan, Tchetgen, and Cowling, 2016[76] and Shi, Li, and Mukherjee, 2023[77].



Chapter 3. RSV Molecular Epidemiology Pre- and Post-COVID-19 Pandemic Periods in Seattle, Washington, USA

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Abstract

Globally, RSV declined to historically low levels following stay-at-home orders in 2020. RSV re-emerged as restrictions lifted, with large inter-seasonal outbreaks and other shifts in epidemiology, including a shift to more severe disease among children of a wider age range than is historically typical. We used data from the SFS, a community-based respiratory virus surveillance study in Seattle, USA to assess clinical characteristics and genomic diversity over three respiratory virus seasons (2019–2020, 2020–2021, and 2021–2022). Individuals with respiratory symptoms were enrolled and nasal swabs were collected. Samples were tested for RSV by RT-qPCR, with WGS performed for a subset. Among

samples collected from children and adults, 1.8% (232/13014) and 0.2% (86/46042) respectively tested positive for RSV-A and 1.1% (139/13016) and 0.3% (119/46043) RSV-B. Positive samples came from at-home self-administered testing (n=253) and kiosk sites located in public spaces of clinical settings (n=270), childcare centers (n=39), workplaces (n=32), a university campus (n=20), and a skilled nursing facility (n=1). Median age for all participants was 33 years (IQR: 23, 48); it was 3 years (IQR: 3, 33) for those who tested positive for RSV. Cough (≥85%) and rhinorrhea (≥85%) were the most common symptoms for both RSV-positive children and adults, with higher semiquantitative viral loads observed in children. From pre- to post-implementation of pandemic restrictions, RSV genomic diversity in Seattle shifted. During 2019–2020, RSV-A and RSV-B co-circulated. Within RSV subtypes, 6 RSV-A and 3 RSV-B clades were observed in SFS (majority clades A.D.1 and B.D.4.1.1). Almost no RSV was observed during 2020–2021. During 2021–2022, RSV-B clade B.D.E.1 was dominant. Multiple sets of identical sequences including both children and adults were observed, with clustering, suggesting transmission between children and adults and trends in genomic diversity trends were similar for all ages. Only three other studies to date have specifically investigated impact of the pandemic on RSV genomic diversity, likely because RSV genomic surveillance is less frequently conducted that that for other respiratory viruses. Trends should continue to be monitored and used to assess potential changes resulting from selective pressure of RSV vaccines and mAbs.

Introduction

RSV is an important cause of morbidity and mortality among young children, immunocompromised individuals, and adults ≥60 years[29,90]. In the US, an estimated 1–2%

of infants are hospitalized with RSV in their first year of life and nearly all children are infected with RSV by two years of age[31,32]. Reinfections with RSV are common throughout childhood and into adulthood, but decrease in severity; however, disease severity increases again among adults ≥60 years[31]. In 2023, RSV prefusion F protein-based vaccines targeting pregnant individuals (Abrysvo, Pfizer) and older adults (Abrysvo and Arexvy, GlaxoSmithKline (GSK)) as well as a general infant population mAb (Nirsevimab; Beyfortus, AstraZeneca and Sanofi) were licensed by the US FDA[34–36]. Several other RSV vaccine and mAb candidates targeting maternal, pediatric, and adult populations are being evaluated in clinical trials[91].

Following stay-at-home orders and non-pharmaceutical interventions implemented in 2020 to reduce transmission of SARS-CoV-2, RSV declined to historically low levels. Expected seasonal peaks in disease did not occur globally during the 2020–2021 respiratory virus season[37]. In the US, the incidence of RSV-associated hospitalization declined from approximately 30 per 100,000 persons in 2018–2019 and 2019–2020 to 0.3 per 100,000 persons in 2020–2021[92]. As pandemic restrictions were lifted, RSV re-emerged, causing larger than expected interseasonal outbreaks in disease in 2021–2022[38]. In the US, RSV typically peaks in December or January, but in 2021–2022, RSV peaked in July. The pandemic may also have altered RSV genomic diversity due to a bottleneck created by reduced RSV transmission during the COVID-19 pandemic[93] . Few studies have assessed RSV genomic diversity from the pre- to post-COVID-19 pandemic periods and no studies to our knowledge have assessed changes in RSV genomic diversity over this period among community-based samples, which may not be well represented by clinical surveillance. In Australia, reductions in RSV genomic diversity were observed among clinical cases from the pre-COVID-19 pandemic period to March 2021, although it is unclear if these changes were sustained over time[44]. In Japan, outpatient data from 2019–2020 showed a decline in the number of RSV-A clusters circulating, but no change in the diversity of RSV-B[94]. In the US, the 2022 off-season surge in RSV was due to several existing RSV lineages and not the emergence of a new, more transmissible RSV lineage, based on clinical cases in Massachusetts, Washington, and Chicago[95–97]. Only one of these US studies specifically analyzed RSV genomic diversity over time, and found evidence of a decline in diversity for RSV-B, but not RSV-A[95].

Analyses of RSV genomic data prior to the widespread introduction of vaccines and mAbs, including describing the impact of the COVID-19 pandemic, are needed in order to establish baseline data for post-licensure vaccine and mAb impact studies. It remains unknown how selective pressure of vaccines and mAbs, which target the F glycoprotein of RSV, will impact genomic diversity of the virus[98,99]. In addition, a better understanding of RSV transmission patterns among individuals of all ages in community settings is needed as most RSV genomic surveillance captures clinical cases only. We used data from SFS, a community-based respiratory virus surveillance study, to describe genomic diversity in community settings over time and by age. Using additional publicly available RSV genomes, we described RSV genomic diversity prior to, during, and following implementation of COVID-19 pandemic restrictions.

Methods

Study Design, Data Collection, and Laboratory Methods

We used data from three community-based respiratory virus surveillance studies conducted as part of SFS from 2018–2022 (**Figure 2.1**). First, participants were cross-sectionally enrolled at community testing kiosks located throughout the city, including childcare centers, a university campus, public spaces of clinical settings (e.g., waiting rooms), skilled nursing facilities, workplaces, transit centers, and other public places (referred to here as, 'Kiosks'). Participants were also cross-sectionally enrolled at home/online as part of 'Swab & Send' from 2019–2020 and Seattle Coronavirus Assessment Network ('SCAN') from 2020–2022. The study design, data collection, and laboratory methods have been previously described[100]. In brief, nasal swabs were collected from participants who reported two or more symptoms of acute respiratory infection (ARI) or cough alone for Kiosks and Swab & Send and at enrollment regardless of symptoms for SCAN. Midturbinate swabs (MTSs) in universal transport media (UTM; November 2018–July 22, 2020) or dry anterior nasal swabs (ANS; July 23, 2020–July 2022) were collected by research staff, self-collected and observed by study staff, or self-collected at home (unobserved) and returned to the laboratory by mail. Demographic, household, and illness data were self-reported by participants for all SFS studies and data were collected and managed using Project REDCap[59,60]. All SFS participants completed informed consent (or assent and parental consent for participants <18 years of age at enrollment).

Nasal swabs were tested using TaqMan RT-qPCR on OpenArray (Thermo Fisher) for the presence of several respiratory pathogens, including RSV, at UW Northwest Genomics Center. WGS was attempted on all RSV-positive samples with relative cycle threshold (Crt) values <20 using a hybrid capture approach[101]. Crt values are an alternative to cycle threshold values used specifically by the OpenArry platform and are based on the amplification curve only rather than all curves for a specific target[102]. A sequencing library was constructed using Illumina TruSeq RNA Library Prep for Enrichment Kit (Illumina), consensus genomes were assembled using a modified iVar bioinformatics pipeline[103], template consensus genomes were created using a custom R script, and finished consensus genomes were created using Bowtie. All genome sequences used in this study were submitted to GenBank[104].

Genomic Analyses

Analyses were conducted separately for RSV-A and RSV-B and included both SFS genomes and publicly available full RSV genomes from around the world in GenBank. Genome quality was assessed and RSV clades were assigned using Nextclade[105]. Genomes with a “bad” quality rating from Nextclade (based on missing data, mixed sites, private mutations, mutation clusters, frameshifts, and premature codon stops), missing collection date, or missing >10% of nucleotides were excluded. Maximum likelihood phylogenetic trees for RSV-A and RSV-B were created using Nextstrain[106]. Sequences were aligned using multiple sequence alignment (MAFFT) relative to a reference genome (GenBank accession KU950499 for RSV-A and KU950676 for RSV-B) and trees included all SFS genomes and a sample of the genomes from Genbank (five genomes randomly selected per country and month from 2005–2023). Time resolved phylogenies were estimated using TimeTree[107]. Trees were visualized using Auspice and the ggtree package in R[108]. Phylogenetic trees were used to assess the relationship between study genomes and between study genomes themselves, as well as publicly available genomes from around the world. All statistical analyses were conducted in R (version 4.1.1, R Core Team, 2021).

Results

Seattle Flu Study Results

From November 2018 to July 2022, the Kiosks, Swab & Send, and SCAN projects of SFS collected 102253 nasal swabs from individuals of all ages in community settings that were tested for RSV. Median age for all SFS participants was 33 years (IQR: 23, 48) and 3 (IQR: 3, 33) for those who tested positive (**Table 2.1**). Cough (≥85%) and rhinorrhea (≥85%) were the most common symptoms for RSV-positive children and adults. Positive SFS samples came from at-home self-administered testing (n=253) and kiosk sites located in public spaces of clinical settings (n=270), childcare centers (n=39), workplaces (n=32), a university campus (n=20), and a skilled nursing facility (n=1, **Table 2.1**).

Of SFS samples, 1.8% (232/13014) and 0.2% (86/46042) tested positive for RSV-A and 1.1% (139/13016) and 0.3% (119/46043) for RSV-B, among children (<18 years of age) and adults (≥18 years of age), respectively (**Figure 2.2**). Overall, RSV percent positivity was greater prior to the COVID-19 pandemic (November 1, 2018 to March 22, 2020; 8.8% and 1.3% for RSV-A and 2.8% and 0.8% for RSV-B among children and adults, respectively) than following the Washington State stay-at-home order (March 23, 2020 to July 31, 2022; 0.1% and 0.02% for RSV-A and 0.6% and 0.2% for RSV-B among children and adults, respectively). During the 2019–2020 respiratory virus season, RSV-A (2.7% percent positivity) and RSV-B (0.9%) co-circulated, during 2020–2021, almost no RSV was observed (0.03% percent positivity for both RSV-A and RSV-B), and as RSV re-emerged in 2021–2022, RSV-B was dominant (0.2% and 2.2% percent positivity for RSV-A and RSV-B, respectively; **Figure 2.2 and Supplementary Table 2.1**).

Among all RSV-positive samples, median Crt values were lower among children (13.7, IQR: 10.5, 19.6 for RSV-A and 12.6, IQR: 9.5, 21.0 for RSV-B) compared to adults (19.3, IQR: 14.5, 23.1 for RSV-A and 18.2, IQR: 13.3, 23.1 for RSV-B). Median Crt value was slightly lower among dry ANSs (12.8, IQR: 9.3, 19.7; used from July 23, 2020 onward) compared to MTSs in UTM (13.6, IQR: 9.7, 27.9; used prior to July 23, 2020; **Supplementary Table 2.2**).

WGS was attempted for all SFS RSV-positive samples with Crt values <20 (n=233/318 (73%) RSV-A positive samples and 187/258 (72%) RSV-B positive samples). Sequencing was successful for 149 (64% of those attempted) RSV-A and 124 (66%) RSV-B samples, i.e., 47% of all SFS RSV-A and 48% of all RSV-B positive samples were successfully sequenced.

Among SFS genomes, genomic diversity trends were similar for children (n=187 sequenced samples) and adults (n=68), but less prevalent clades (A.D.2.1, A.D.2.2, A.D.5, B.D.E.2, n<5 each) were only observed among children, possibly due to fewer positives among adults (**Figures 2.3 and 2.4, Panel A**). Multiple sets of identical sequences including both children and adults were observed, with clustering, suggesting transmission between children and adults in community settings (**Figure 2.6**).

Global Trends in RSV Genomic Diversity

In addition to the sequenced SFS samples, 1674 RSV-A and 1220 RSV-B whole genome sequences from GenBank were included in genomic analyses (106 and 118 GenBank genomes were excluded due to “bad” quality rating or missing >10% of nucleotides and

531 and 48 were excluded due to missing collection date, for RSV-A and RSV-B, respectively). An additional 1173 RSV-A and 653 RSV-B sequences from Genbank were excluded from maximum likelihood phylogenetic trees during random sampling (n=5 Genbank sequences were randomly selected per country and month from 2005–2023 as background sequences).

Trends observed in genomic diversity in SFS were similar to those in Washington State overall. During the 2019–2020 respiratory virus season in Washington State, RSV-A and RSV-B co-circulated. Within RSV subtypes, 6 RSV-A and 3 RSV-B clades (predominately A.D.1 and B.D.4.1.1) were observed among both SFS genomes and Washington State genomes from Genbank (**Table 2.2, Figures 2.2–2.4 and 2.5, Panels A–B**). Almost no RSV was observed in SFS or Washington State during 2020-2021. In the first season after COVID-19 restrictions were lifted (2021–2022), RSV-B clade B.D.E.1 was dominant among both SFS genomes and Washington State genomes. In the 2022–2023 respiratory virus season, after the conclusion of SFS, RSV-A clade A.D.5.2 was dominant in Washington State, with fewer cases of other RSV-A clades and RSV-B clade B.D.E.2. All RSV clades observed in Washington State from November 2018 to February 2023 were also observed in SFS.

These trends in RSV genomic diversity in Washington State from November 2018 to February 2023 were also similar to those observed in the Northern Hemisphere overall (**Figure 2.5, Panel C**). However, there were notable differences between the Northern and Southern Hemispheres. During 2020–2021 when virtually no RSV was observed in the Northern Hemisphere, available sequence data from the Southern Hemisphere was mostly due to 150 RSV-A clade A.D.1.3 sequences and 8 RSV-B clade B.D.E.4 sequences from Australia (**Figure 2.5, Panel D**). RSV-A clade A.D.1.3 has only been observed in Australia during 2020–2021 and only one case of RSV-B clade B.D.E.4 has been observed outside of Australia during 2020–2021 (United Kingdom in 2019). In addition, while RSV-B clade B.D.E.1 was dominant during 2021–2022 in the Northern Hemisphere, RSV-A clades A.D.1 and A.D.5 were dominant in the Southern Hemisphere and no B.D.E.1 sequences were observed.

Discussion

Using data from a community-based respiratory virus surveillance study in Seattle, USA, we observed that RSV genomic diversity shifted from the pre-COVID-19 pandemic period to the pandemic-related decline in RSV transmission to the post-pandemic restrictions off-season re-emergence. Our community-based surveillance may have allowed us to better capture changes in RSV transmission in the community compared to surveillance of clinical cases alone. Clinical surveillance for the most part captures a narrow portion of all RSV cases that are severe enough to require clinical care and are mostly from high-risk individuals not representative of the wider population. Our study also included individuals of all ages, allowing us to document likely transmission between children and adults in community settings and capture a broader proportion of the population than that which is typically at high risk for clinical RSV disease. Genomic patterns observed in our study were largely similar to those in Washington State, USA and in the Northern Hemisphere, but differences were observed between the Northern and Southern Hemispheres. We know of only three other studies to date that have specifically investigated impact of the pandemic on RSV genomic diversity, likely because RSV genomic surveillance is less frequently conducted than that for other respiratory viruses, including influenza, for which a “bottleneck” effect on genomic diversity during the COVID-19 pandemic has been well documented[109–112].

Our finding that the COVID-19 pandemic disrupted trends in RSV genomic diversity was also observed in Australia, Japan, and Chicago, USA, but specific changes observed vary. In addition to RSV diversity declining in the first year of the COVID-19 pandemic, two distinct, new RSV-A lineages were observed in Australia[44]. These lineages were categorized under clade A.D.1.3 in this study, which has only been observed in Australia since November 2020. Continued surveillance should monitor the potential spread of clade A.D.1.3 beyond Australia. It remains unknown whether the detection of this previously unobserved clade in Australia only is due to limited RSV genomic surveillance, differences between transmission in the Northern versus Southern Hemisphere, or a combination of these. Conversely, in this study and other US studies, all RSV clades observed in the post-COVID-19 pandemic period were also observed in the pre-pandemic period[95–97]. In Japan, seven clusters of RSV-A were observed in 2019–2020 prior to the COVID-19 pandemic versus only two clusters observed following the pandemic through 2022, but only a single monolithic RSV-B cluster was present over the entire study period[94]. One US study, conducted in a different geographic region (Chicago), specifically evaluated changes in genomic diversity among clinical cases following the COVID-19 pandemic and found evidence of a more homogenous population of RSV-B viruses in 2021–2022, but this was not observed for RSV-A[95]. However, the 2021–2022 respiratory virus season in the US was dominated by RSV-B, with very little RSV-A. Therefore, this study may not have been able to adequately assess changes in diversity of RSV-A using data only through 2022. SFS community samples were also only collected through July 2022, and we were similarly unable to assess changes in RSV-A genomic diversity among our study samples because very few cases of RSV-A were captured during the 2021–2022 respiratory virus season. However, publicly available Washington State data from 2022–2023, do suggest a shift from RSV-A clade A.D.1 prior to the COVID-19 pandemic to RSV-A clade A.D.5.2.

Efforts to draw comparisons across studies are complicated by a lack of standardized nomenclature for RSV clades and varying methods used to assess changes in RSV genomic diversity over time across studies[113–115]. For example, the Australia study used the historical RSV-A ON1 strain designation and defined lineages within study sequences[44]. The RSV-A ON1 strain captured all RSV-A in the Australian study and is comparable to the Goya et, al. GA2 clade and sub-lineages, which were reported in the Japan and other US studies described above and are defined using the RSV G gene rather than the whole genome[94,113]. Conversely in this study, Nextclade designations, which are defined using the whole genome, were used to identify seven RSV-A clades, all of which fall under ON1 and GA2. Therefore, conclusions regarding whether genomic diversity changed based on observed number of clades circulating pre- and post-COVID-19 pandemic periods may partially depend on the nomenclature system used.

This analysis has several limitations. First, SFS used convenience sampling at public places in Seattle, USA and may not be representative of the entire metropolitan area. Second, almost all SFS samples were collected from individuals with symptoms of ARI, and we were unable to compare trends in genomic diversity between individuals with symptomatic and asymptomatic infections. Third, we switched from MTSs in UTM to dry ANSs in July 2020. We did not directly compare viral detection rates between MTSs in UTM and dry ANSs, however among RSV-positive swabs in this study, median Crts were slightly lower among dry ANSs and previous data show a slight better ability to detect human nucleic acids from dry ANSs versus MTSs in UTM by RT-qPCR[62]. Fourth, we were only able to generate whole genome sequences for a subset (~47%) of SFS RSV-positive samples. In particular, we did not attempt WGS for RSV-positive specimens with high Crts (>20), corresponding to a low viral load, and among those for which sequencing was attempted, higher Crt values correlated with higher fail rates. Fifth, there is a lack of demographic and clinical data associated with publicly available genomes in GenBank. Therefore, analyses by age were limited to SFS genomes only and we were not able to identify if non-SFS sequences in GenBank represented clinical or community cases. Finally, SFS data appears to have captured multiple members of transmission clusters, which may have resulted in artificially lower genomic diversity among SFS sequences compared to sequences from Genbank which mostly represent individual clinical cases.

Despite these limitations, this is the first study to our knowledge to assess changes in RSV genomic diversity from pre- to post-COVID-19 pandemic periods specifically among community cases. Our study was also strengthened by inclusion of demographic data associated with genomes, such as age. Finally, we generated whole genome sequences for our study samples, versus sequences of the G gene ectodomain only, which may be less informative for inferring transmission and do not inform upon genomic diversity of the F gene, which is targeted by currently available vaccines and mAb.

Conclusions

The COVID-19 pandemic disrupted trends in RSV genomic diversity, although there was geographic heterogeneity in specific changes observed globally. In SFS, RSV-A and RSV-B (majority clades A.D.1 and B.D.4.1.1) co-circulated in 2019–2020 and following implementation of COVID-19 pandemic restrictions no RSV was observed during the 2020–2021 respiratory virus season. In 2021–2022, RSV re-emerged off-season and shifted to predominantly RSV-B (majority clade B.D.E.1)). Using publicly available genomes from Washington State, we observed a subsequent shift to RSV-A (majority clade A.D.5.2) in 2022–2023. SFS surveillance data contributed community-based RSV whole genome sequences to the literature and documented transmission between children and adults in community settings.

Figure 2.1. Seattle Flu Study community-based respiratory virus surveillance study timelines and nasal swab collection methods

Study	2018	2019	2020	2021	2022
Kiosks					
Swab & Send					
SCAN[1]					

Swabs:

Collected by research staff

Self-collected & observed by study staff (as a safety measure following implementation of COVID-19 precautions)

Self-collected at home (unobserved) & returned to the laboratory by mail

1 Seattle Coronavirus Assessment Network

Figure 2.2. RSV-positive cases and RSV percent positivity among community Seattle Flu Study samples. Vertical dashed line indicate March 23, 2020, when the Washington State Stay at Home order went into effect.

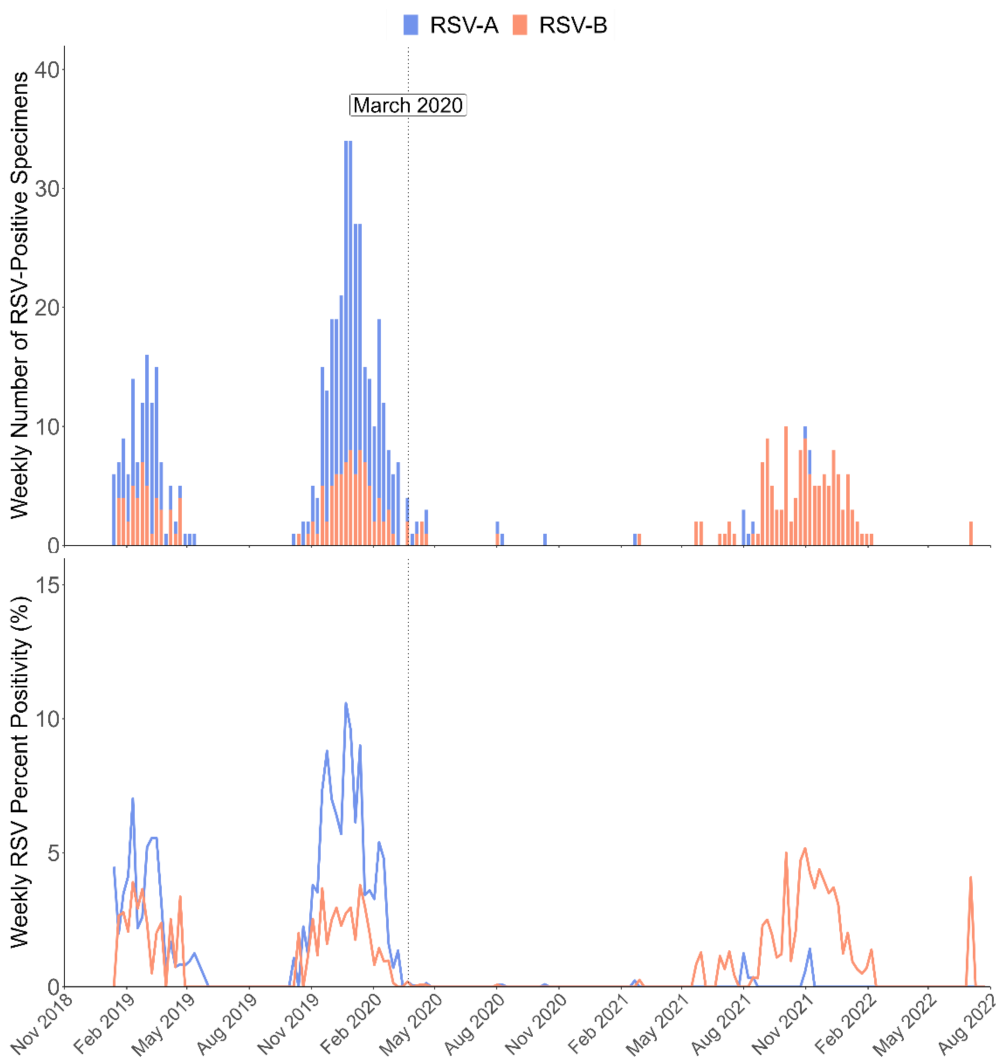


Table 2.1. Description of RSV-positive sequenced Seattle Flu Study samples during the pre- and post-COVID-19 periods

	Pre-COVID-19 Restrictions[1] (N=194)	Post-COVID-19 Restrictions[2] (N=79)	Total (N=273)
Age, N (%)			
<18 Years	142 (73%)	45 (57%)	187 (68%)
≥18 Years	39 (20%)	29 (37%)	68 (25%)
Unknown	13 (7%)	5 (6%)	18 (7%)
Sex, N (%)			
Female	92 (47%)	42 (53%)	134 (49%)
Male	89 (46%)	32 (41%)	121 (44%)
Unknown	13 (7%)	5 (6%)	18 (7%)
Symptom Status, N (%)			
Asymptomatic	0 (0%)	2 (3%)	2 (1%)
Symptomatic	180 (93%)	72 (91%)	252 (92%)
Number of symptoms, Median (IQR)[3]	6 (4, 8)	5 (3, 7)	6 (4, 8)
Cough[3]	177 (98%)	67 (93%)	244 (97%)
Runny or Stuffy Nose[3]	161 (89%)	67 (93%)	228 (90%)
Fatigue[3]	129 (72%)	36 (50%)	165 (65%)
Feeling Feverish[3]	132 (73%)	33 (46%)	165 (65%)
Increased Trouble Breathing[3]	116 (64%)	10 (14%)	126 (50%)
Sore Throat[3]	79 (44%)	43 (60%)	122 (48%)
Headache[3]	52 (29%)	24 (33%)	76 (30%)
Nausea or Vomiting[3]	54 (30%)	15 (21%)	69 (27%)
Muscle or Body Aches[3]	52 (29%)	13 (18%)	65 (26%)
Chills or Shivering[3]	47 (26%)	16 (22%)	63 (25%)
Sweats[3]	48 (27%)	15 (21%)	63 (25%)
Ear Pain or Discharge[3]	39 (22%)	9 (12%)	48 (19%)
Diarrhea[3]	22 (12%)	11 (15%)	33 (13%)
Rash[3]	12 (7%)	5 (7%)	17 (7%)
Unknown	14 (7%)	5 (6%)	19 (7%)
Collection Location			
Childcare center	1 (1%)	0 (0%)	1 (0%)
College campus	4 (2%)	0 (0%)	4 (1%)
Home collection returned by mail	42 (22%)	74 (94%)	116 (42%)
Public space of a clinical building	146 (75%)	0 (0%)	146 (53%)
Workplace	1 (1%)	5 (6%)	6 (2%)
RSV Subtype and Clade, N (%)			
RSV-A	146 (75%)	3 (4%)	149 (55%)
A.D	8 (4%)	0 (0%)	8 (3%)
A.D.1	109 (56%)	1 (1%)	110 (40%)
A.D.2.1	4 (2%)	0 (0%)	4 (1%)
A.D.2.2	4 (2%)	0 (0%)	4 (1%)
A.D.3	20 (10%)	1 (1%)	21 (8%)
A.D.5	1 (1%)	0 (0%)	1 (0%)
A.D.5.2	0 (0%)	1 (1%)	1 (0%)
RSV-B	48 (25%)	76 (96%)	124 (45%)
B.D.4.1	1 (1%)	0 (0%)	1 (0%)
B.D.4.1.1	44 (23%)	8 (10%)	52 (19%)

	Pre-COVID-19 Restrictions[1] (N=194)	Post-COVID-19 Restrictions[2] (N=79)	Total (N=273)
B.D.E.1	0 (0%)	68 (86%)	68 (25%)
B.D.E.2	3 (2%)	0 (0%)	3 (1%)

1 Prior to March 23, 2020, when the Washington State stay at home order went into effect
2 Post-March 23, 2020
3 Number of symptoms and specific symptom types are reported among symptomatic cases only

Table 2.2. Summary of RSV clades observed in Seattle Flu Study, Washington State, and globally

	RSV-A		RSV-B	
	Pre-COVID-19 Restrictions[1]	Post-COVID-19 Restrictions[2]	Pre-COVID-19 Restrictions[1]	Post-COVID-19 Restrictions[2]
RSV Sequences from Seattle Flu Study, November 2018–July 2022				
Sequences, N				
Overall	146	3	48	76
<18 Years	114	3	28	42
≥18 Years	24	0	15	29
Clades, N				
Overall	6	3	3	2
<18 Years	6	3	2	2
≥18 Years	3	N/A	1	2
RSV sequences from Washington State, USA in GenBank, November 2018–February 2023				
Sequences, N	146	60	58	112
Clades, N	6	4	3	2
RSV sequences globally in GenBank, November 2018–February 2023				
Sequences, N	358	711	167	224
Clades, N	8	11	4	5

1 Prior to March 23, 2020, when the Washington State stay at home order went into effect
2 Post-March 23, 2020

Figure 2.3. RSV-A clades observed in Seattle Flu Study (A) by age and over time, (B) maximum likelihood phylogenetic tree over calendar time, and (C) maximum likelihood phylogenetic tree over genetic divergence. Maximum likelihood phylogenetic trees include all Seattle Flu Study sequences and a sample of samples from GenBank. Vertical dashed line indicates March 23, 2020, when the Washington State Stay at Home order went into effect.

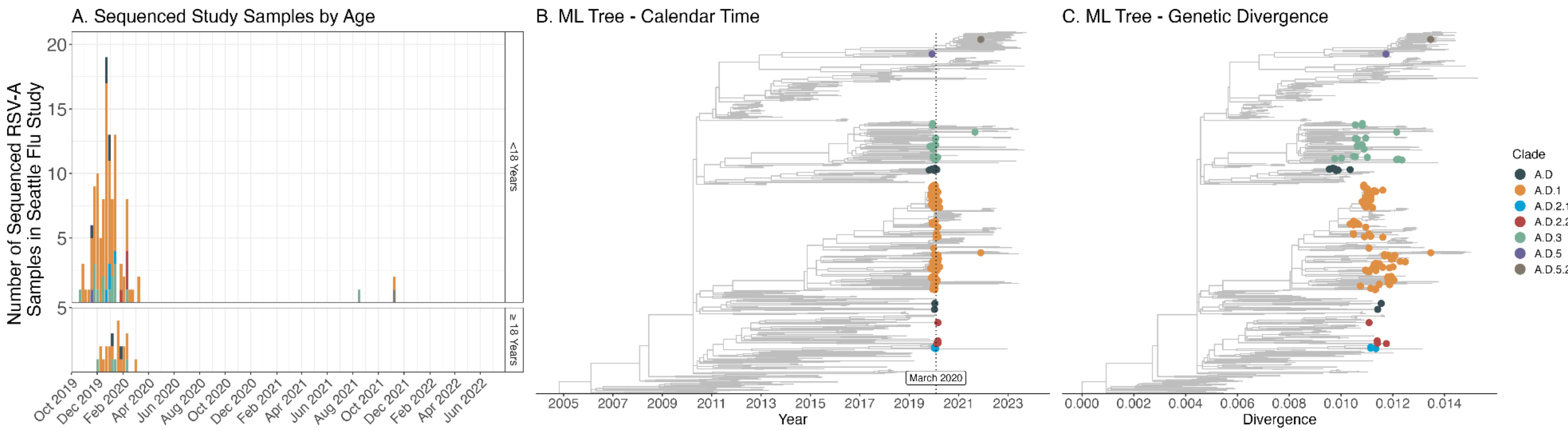


Figure 2.4. RSV-B clades observed in Seattle Flu Study (A) by age and over time, (B) maximum likelihood phylogenetic tree over calendar time, and (C) maximum likelihood phylogenetic tree over genetic divergence. Maximum likelihood phylogenetic trees include all Seattle Flu Study sequences and a sample of samples from GenBank. Vertical dashed line indicates March 23, 2020, when the Washington State Stay at Home order went into effect.

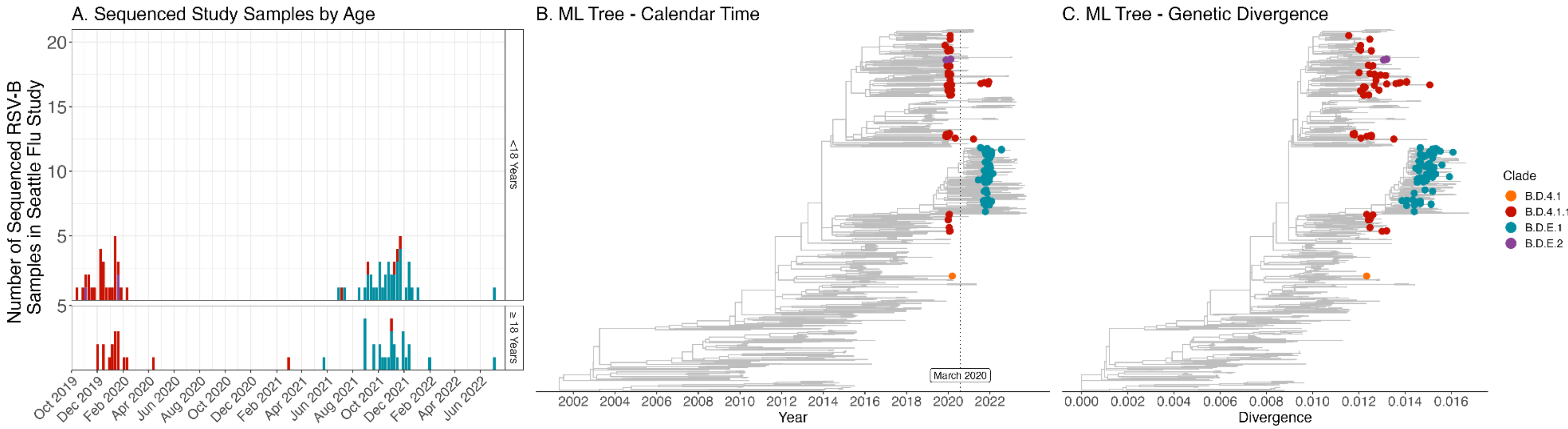
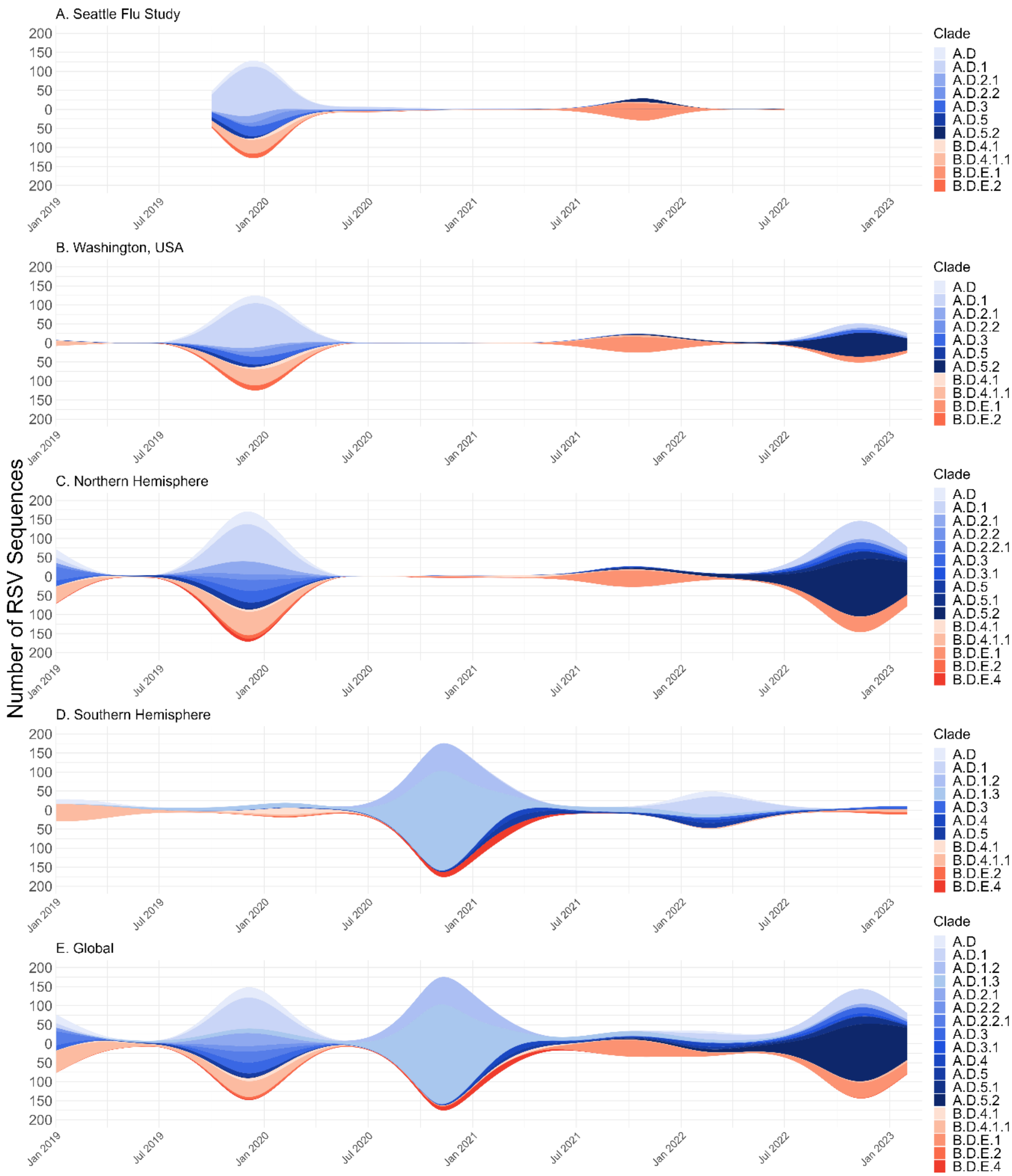
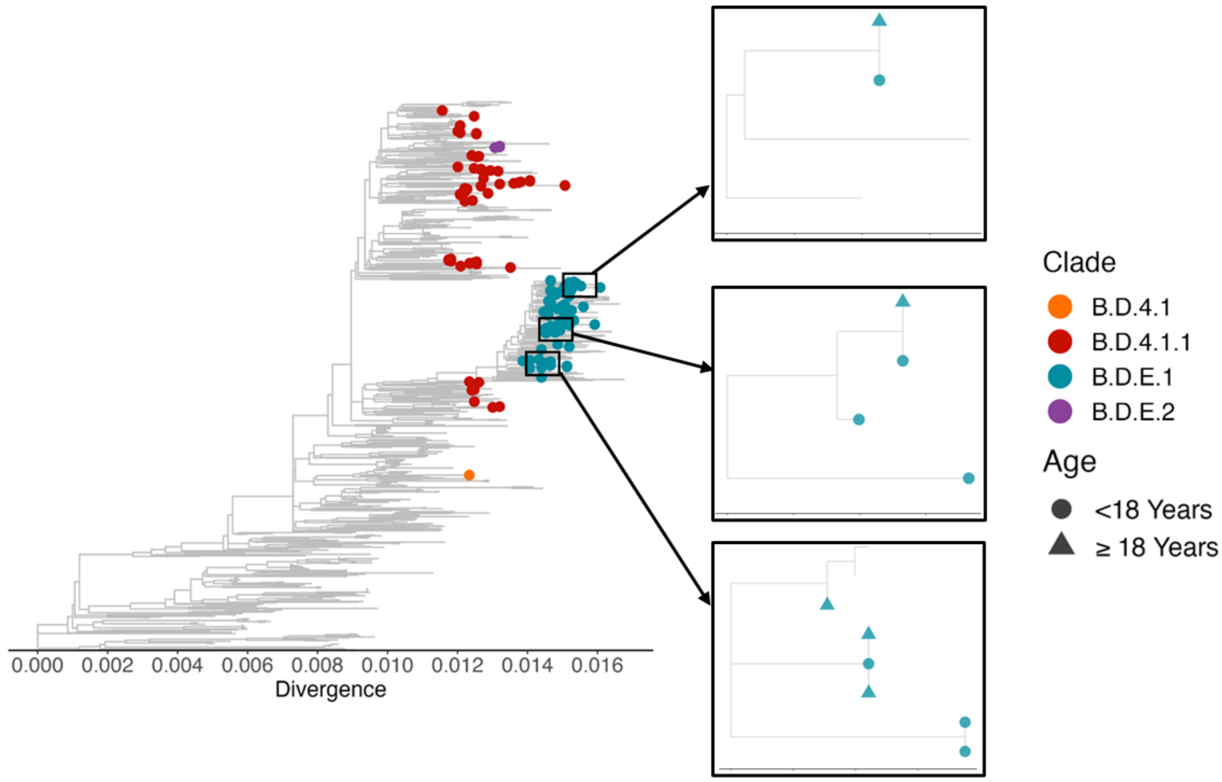


Figure 2.5. RSV clades observed from January 2019 to February 2023 in: (A) Seattle Flu Study (N=273 sequences), (B) Washington, USA (N=376), (C) Northern Hemisphere[1] (N=1190), (D) Southern Hemisphere[2] (N=463), and (E) globally (N=1653). Data in Panels B–E are from GenBank. Total number of sequences is the sum of counts above and below zero on the y-axis and data are smoothed over time.



1 Northern Hemisphere data are from: United States (n=763), United Kingdom (n=95), China (n=87), Netherlands (n=86), Spain (n=79), Philippines (n=29), Austria (n=18), Russia (n=17), Uganda (n=10), France (n=4), Japan (n=1), and Switzerland (n=1).
2 Southern Hemisphere data are from: Australia (n=245), Kenya (n=214), and Zambia (n=4).

Figure 2.6. Example RSV-B clusters with sets of identical sequences including both children and adults observed in Seattle Flu Study. Maximum likelihood phylogenetic tree is over genetic divergence and include all Seattle Flu Study sequences and a sample of samples from GenBank.



Supplementary Table 2.1. RSV-positive cases and RSV percent positivity among community Seattle Flu Study samples by respiratory virus season (July–June) and age

	Respiratory Virus Season		
	2019–2020	2020–2021	2021–2022
RSV-A Positive Samples, N (%)			
Overall	689/25647 (2.7%)	13/43031 (0.03%)	58/33571 (0.2%)
<18 Years	561/6868 (8.2%)	10/13158 (0.1%)	47/11039 (0.4%)
≥18 Years	115/17394 (0.7%)	3/28319 (0.01%)	11/22513 (0.05%)
RSV-B Positive Samples, N (%)			
Overall	237/25647 (0.9%)	14/43035 (0.03%)	725/33571 (2.2%)
<18 Years	152/6868 (2.2%)	6/13160 (0.05%)	547/11039 (5.0%)
≥18 Years	76/17394 (0.4%)	8/28321(0.03%)	178/22513 (0.8%)

Supplementary Table 2.2. Relative cycle threshold values for Seattle Flu Study RSV-positive midturbinate swabs (MTSs) in universal transport media (UTM; November 2018–July 22, 2020) versus as dry anterior nasal swabs (ANSs; July 23, 2020–July 2022)

	MTSs in UTM, N=88068 (November 2018–July 22, 2020)	**Dry ANSs, N=144016 (July 23, 2020–July 2022)**
Crt value, Median (IQR)		
Overall	13.6 (9.7, 27.9)	12.8 (9.3, 19.7)
<18 Years	12.7 (9.5, 18.1)	11.9 (8.8, 18.2)
≥18 Years	18.2 (13.3, 22.7)	16.8 (12.1, 23.3)

Chapter 4. Conclusion

In this dissertation we used data from two community-based respiratory virus surveillance studies to inform gaps in knowledge for two VPDs, COVID-19 and RSV. Collecting and analyzing VPD surveillance data within community settings (rather than from clinical settings alone) is essential for evaluating population-level vaccine impact as clinical cases generally arise from populations at high risk for disease and are not representative of the wider population.

In **Chapter 2**, we estimated relative VE of COVID-19 mRNA vaccine primary series plus a monovalent booster doses versus primary series only using data from HCT, a university COVID-19 testing program. On average, the study population was at low risk for severe COVID-19 but at high risk for SARS-CoV-2 infection given congregate living and frequent social gatherings. Overall booster doses provided increased protection against symptomatic SARS-CoV-2 infection (estimated relative VE=40%) which decreased over time since receipt of monovalent booster dose. VE was higher among individuals without any known prior SARS-CoV-2 infection. Results from this analysis contribute to the evidence base that informs recommendations for COVID-19 vaccine use and support use of COVID-19 vaccines in university populations for prevention of COVID-19 on campus. This study also serves as a framework for VE studies in university populations, which are on average at low risk for severe disease from respiratory pathogens. Such populations are infrequently captured by observational VE studies but are necessary for informed vaccine recommendations as populations at low risk for severe disease may still be at high risk for infection, serve as potential reservoirs for onward transmission, and have a burden of missed work and school associated with VPDs.

In **Chapter 3**, we described RSV genomic diversity prior to, during, and following the COVID-19 pandemic using both SFS data and publicly available genomes from Washington State and global repositories. Using SFS data, a respiratory virus surveillance study, allowed us to link genomic and epidemiologic data to describe trends specifically in community settings over time and by age. We also contributed RSV genomes from community settings to the literature, an important contribution as most RSV genomic surveillance to date captures clinical cases. We observed disruptions in RSV genomic diversity due to the COVID-19 pandemic, but specific trends varied globally. In Washington, USA, RSV-A and RSV-B (majority clades A.D.1 and B.D.4.1.1) co-circulated in 2019–2020. Following implementation of COVID-19 pandemic restrictions, no RSV was observed during the 2020–2021 respiratory virus season. In 2021–2022, RSV re-emerged off-season and shifted to predominantly RSV-B (majority clade B.D.E.1) and subsequently RSV-A (majority clade A.D.5.2) in 2022–2023.

Finally, we hope that results from this work may contribute to the body of research used to inform vaccine policy decisions, advocate for vaccine use in populations where data support addressing disease burden through immunization, and identify limitations of current vaccine programs to inform other public health interventions for both COVID-19 and RSV. Our VE results support use of COVID-19 booster doses among university populations for prevention of COVID-19 among healthy, young adults. Results contribute community-based RSV genomic data to the literature, which primarily captures clinical cases and is limited in comparison to influenza or SARS-CoV-2 sequence data. Results from our RSV genomics analysis also provide data for monitoring of vaccine and mAb impact, including potential resistance to vaccines and mAbs.

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