Epidemiology of Human Metapneumovirus Infection in a Community-Based Setting, Seattle, WA, USA

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Abstract

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Abstract

Objective: To better quantify human metapneumovirus (hMPV) prevalence, associated risk factors, and genomic epidemiology in a community setting.

Methods: This is a secondary analysis using data from the Seattle Flu Study (SFS). SFS was a community-based observational surveillance study for respiratory infections among clinical and community populations across the greater Seattle area from 2018-2022. SFS utilized prospective cross-sectional sampling, prospective cohort sampling, and retrospective cross-sectional sampling to obtain clinical and community samples representative of the greater Seattle area. Whole genome sequencing of samples was performed for a select number of samples meeting viral load cutoff criteria (Relative cycle threshold < 20). Clinical samples were matched to community samples based on respiratory season to allow for comparison of subtypes across sampling methods.

Results: Of the 52,036 community samples collected, 247 (0.47%) tested positive for hMPV. 74% of hMPV positive samples were collected prior to the COVID-19 pandemic and 47% were among those under the age of 18. The most commonly reported symptoms were cough and rhinorrhea. The presence of additional symptoms varied by age group, with fever more commonly reported among those under 5 and headaches more commonly reported among those over 65. In our multivariable model, lower income (aOR: 2.05 95% CI: 1.12 – 3.74), larger household size (aOR: 2.94 95% CI: 1.44 – 6.00), and international travel (aOR: 2.71 95% CI: 1.23 – 5.97) were found to be significant risk factors for testing positive. Testing from January 2019 through February 2020 was associated with an increased odds of testing positive for hMPV compared to testing from March 2020 through July 2022 (aOR 3.02 95% CI: 1.90 – 4.81).

There was co-circulation of multiple hMPV subtypes across community and clinical samples. Community sampling captured subtypes A2b, A2c, B1 and B2 whereas clinical sampling captured A2b, B1, and B2. There was variation of subtype prevalence by age, with those under 18 having a larger proportion of A2b subtypes in community settings compared to clinical

settings. Subtype prevalence changed after the start of the COVID-19 pandemic, with the proportion of B1 subtype increasing after the start of the pandemic. This change was observed in both clinical and community settings.

Percent positivity for hMPV was highest prior to the start of the COVID-19 pandemic. Clinical samples had higher percent positivity compared to community samples (1.74% vs 0.47%). The highest percent positivity across community and clinical samples was among clinical samples who resided in the Southwest Seattle Public Use Microdata Area (PUMA) (5.3%). This PUMA is an area with a large proportion of low socioeconomic status (SES) census tracts.

Conclusion: In a study of individuals with respiratory illness in community settings, risk factors for testing positive included lower income, larger household size, and international travel. Testing prior to the start of the COVID-19 pandemic was also associated with an increased odds of testing positive. The COVID-19 pandemic had an impact on both frequency and diversity of hMPV subtypes in clinical and community settings. Percent positivity among clinical and community samples were highest prior to the start of the COVID-19 pandemic, and the highest percent positivity was in a PUMA with a large proportion of low SES census tracts.

Background and Significance:

Human metapneumovirus (hMPV) is responsible for a large burden of acute respiratory disease, particularly in young children.[1–3] hMPV infection in young children has been associated with severe outcomes like hospitalization and bronchiolitis.[1–3] By the age of five, it is estimated that most children will have had at least one hMPV infection.[4,5] Approximately 5-7% of children hospitalized with respiratory tract infections have hMPV infections. Medically attended hMPV infection in children has been well characterized since the discovery of hMPV in 2001.[1,2,4] Most current literature is focused on hospitalized children, and there is little information about infections in children who do not seek medical care. Reinfections can occur throughout one's lifetime, yet there is also limited literature on hMPV infection in adults. Of the existing literature on hMPV in adults, the majority is focused on medically attended adult populations. [6,7] To date there are no known studies that evaluate hMPV infection among community populations, specifically among individuals who may not seek healthcare. It is important to better understand the community epidemiology of hMPV to allow for further characterization of hMPV infection in non-clinical settings.

Other respiratory pathogens, like influenza and respiratory syncytial virus (RSV), have been well characterized in both clinical and community settings, which has assisted with vaccine implementation. Several hMPV vaccines are currently in clinical development, with a target population of young children. It is important to better understand the community epidemiology of hMPV to assist with vaccine implementation. Characterizing the prevalence of hMPV in a community setting, specifically among various age groups, can assist with vaccine recommendations across age groups when a vaccine becomes available. Understanding risk factors associated with hMPV infections in a community setting can also assist with prioritizing certain groups for vaccination. Gathering and analyzing pre-vaccine epidemiological and genomic data will also allow for comparisons between pre and post vaccine implementation, specifically in community settings.

It is also important to better understand the genomic epidemiology of circulating hMPV strains in community and clinical settings to quantify the burden of each subtype in various populations. Further understanding the relationship between subtype and infection severity may assist with vaccine development. Previous literature has conflicting accounts of the relationship between hMPV subtypes and infection severity.[8,9] In addition, characterizing where hMPV subtypes appear first, in community or clinical settings, can help to determine the potential utility of a vaccine in various populations and settings.

Finally, the impact of the COVID-19 pandemic on hMPV community transmission has yet to be studied. Of the existing literature evaluating hMPV transmission before and after the start of the COVD-19 pandemic, much of it is geography dependent and focused on clinical settings. Pre-pandemic, a study from the Netherlands observed that from January 2016 to April 2020 there was little change in hMPV incidence among hospitalized children.[10] In the United States the CDC

reported a decrease in hMPV positivity among lab tested samples starting in March 2020 and lasting through May 2021.[11] In Israel, there was a sharp decrease in hMPV transmission in March 2020, and a peak in transmission during March 2021.[12] These studies base their findings on medically attended hMPV infection and only include samples up until 2021. There is also no current literature assessing how the COVID-19 pandemic impacted community transmission of hMPV and hMPV subtypes. Analyzing the trends of hMPV circulation before and after the start of the COVID-19 pandemic can help quantify the impact that the COVID-19 pandemic had on circulating hMPV strains. Similarly, mapping the geospatial distribution of hMPV infections both before and after the COVID-19 pandemic can allow for further analysis of the impact of mitigation measures on the prevalence of hMPV.

To fill the current gaps in literature we aimed to estimate the prevalence of hMPV in community settings and to explore risk factors associated with community infection. We also aimed to compare genomic epidemiology and percent positivity across time between clinical and community samples.

Methods

<u>Study Design and Setting</u>

This was a secondary analysis using data from the Seattle Flu Study (SFS). SFS was an observational surveillance study for respiratory infections among clinical and community populations across the greater Seattle area from 2018-2022. [3] SFS utilized prospective cross-sectional sampling and prospective cohort sampling, and retrospective cross-sectional sampling to obtain clinical and community samples representative of the greater Seattle area. The study collected samples from individuals residing in King County, Snohomish County, Pierce County, Skagit County, and Island County. Individuals with a reported home address outside of King, Snohomish, Pierce, Skagit, and Island County were excluded from this analysis.

<u>Study Subjects and Data Collection (Community Setting)</u>

There were two major sample sets for the study: community and clinical samples. Community samples were defined as samples collected across various community settings, including childcare, workplaces, schools, and at home. Sampling locations were scattered across the surveillance counties, with most sampling locations located in King County.

Community samples came from participants in SFS and included individuals of all ages who resided in one of the surveillance counties (King, Snohomish, Pierce, Skagit, and Island). Inclusion criteria included experiencing two or more new or worsening acute respiratory illness symptoms. Individuals who were incarcerated, a ward of the state, or who were unable to give informed consent were excluded. Participants who had previously enrolled in the SFS in the last 14 days were also excluded. Participants of all ages were able to participate as there was no age

limitation. Individuals who enrolled in the study completed a questionnaire including questions regarding symptoms, recent travel, and household information. Participants were recruited from flyers, digital advertising, press releases, and in-person advertising via approaching potential participants in the various community sampling arms (e.g. parents at participating childcare facilities). The Seattle Flu Study was approved by the University of Washington Human Subjects Institutional Review Boards (UW IRB) and this secondary analysis was exempt from additional IRB review.

One portion of community sampling included the prospective enrollment of children in a childcare setting. For childcare enrollments, research assistants would obtain informed consent from a legally authorized representative (e.g. parent or guardian). The prospective childcare cohort included a baseline mid-nasal swab collected by a research assistant. Parents filled out weekly symptom questionnaires, and if parents of participating children reported that the child had more than one acute respiratory symptom, a research assistant at the participating childcare settings would collect a nasal swab from the child.

Another portion of community sampling included cross-sectional sampling. Cross-sectional samples were collected from three study arms including kiosks, SwabNSend, and the Seattle Coronavirus Assessment Network (SCAN). Community cross-sectional participants would enroll in SFS during the time of their respiratory sample collection. Kiosks ran from November 2018 to February 2020 and included testing kiosks at public locations like college campuses, workplaces, and other public sites. A research assistant walked participants through a consent form to obtain informed consent. For participants under the age of 18, informed consent was obtained from a legally authorized representative. Kiosk participants would present to a kiosk if they were symptomatic, enroll in SFS, and have a mid-nasal swab collected by a study staff member.

SwabNSend was conducted from October 2019 through March 2020 and allowed for remote testing. Participants would go through an electronic consent form by themselves prior to swabbing and answering questionnaires. Symptomatic individuals could request a test kit sent to their home, where they would enroll in SFS, self-collect an anterior nasal swab and return the sample back to the lab by mail.

SCAN was conducted from March 2020 through July 2022 and was similar to SwabNSend as it also allowed for remote testing for symptomatic individuals. SCAN participants also went through an electronic consent form by themselves prior to swabbing and answering questionnaires. SCAN was a larger version of SwabNSend to assist with the high demand for respiratory virus community testing during the COVID-19 pandemic. SCAN was restricted to King County residents. Community members could request a testing kit be sent through their home through SCAN, and eligible participants would then enroll and swab themselves at home. SCAN testing kits were also sent back to the lab by mail.[13]

Figure A. Duration of community based SFS sampling by year

SFS Study	2018	2019	2020	2021	2022
Kiosks					
SwabNSend					
SCAN					

Study Subjects and Data Collection (Clinical Setting)

Clinical samples were defined as residual nasal swabs collected from participating regional clinics and hospitals. Residual clinical samples included all respiratory nasal swab samples submitted for respiratory virus testing, regardless of clinical test results. Limited demographic information including age, sex, and census tract was shared. Samples collected outside of the surveillance period (2018-2022) and samples collected from individuals who resided outside of the surveillance counties were excluded from this analysis.

Identification of hMPV

All respiratory samples were tested for 28 respiratory pathogens via RT-PCR (Thermo Fisher Open Array platform), including hMPV. A relative cycle threshold (Crt) value was generated for samples tested on the Open Array platform. Crt values were used a proxy for viral load. Samples with Crt value less than 20 were eligible for sequencing. 158 community samples were eligible for sequencing and 787 clinical samples were eligible for sequencing. All 158 eligible community samples were requested to be pulled for sequencing. To allow for comparison between community and clinical genomes, clinical samples were randomly matched to community samples based on respiratory season (Figure A). This resulted in a total of 316 requested samples (158 community, 158 clinical) that were pulled for sequencing.

RNA was extracted using the MagnaPure 96 DNA and viral nucleic acid small volume kit (Roche Diagnostics), with 200µL input and 50µL elution. Extracted RNA was converted to double-stranded cDNA, purified by bead cleanup, enzymatically fragmented, end-repaired, indexed, amplified, and purified again using the QIAseq FX DNA Library Kit (Qiagen). Hybridization capture was performed using the QIAseq xHYB Viral Respiratory Panel (Qiagen) after pooling libraries by sample Crt values, with four samples in each pool. After overnight hybridization with biotinylated probes and subsequent washing to remove unbound fragments, enriched libraries were amplified and then purified by bead clean-up. Library fragment sizes were estimated by TapeStation 4200 D1000 (Agilent) and concentrations were measured by Qubit 4 Fluorometer (Invitrogen). Libraries passing QC were sequenced on Illumina Novaseq 6000 or Nextseq 2000 instruments using a 2x150 read format.

Statistical Methods

Medians and percentages were used to describe the characteristics of people who tested positive versus tested negative for hMPV in a community setting. Symptoms reported among those who tested positive for hMPV were compared to symptoms reported among those who tested negative for all respiratory viruses, and those who tested negative for hMPV but positive for another respiratory virus. A heatmap was created to visualize the proportion of various symptoms reported across age groups (chills, ear pain, sweats, muscle or body aches, trouble breathing, headaches, sore throat, fever, fatigue, rhinorrhea, cough). Age groups were broken down into the following categories: less than 1 year old, 1-4, 5-17, 18-49, 50-64, and 65+.

A risk factor analysis, using logistic regression, was performed to identify risk factors for testing positive for hMPV in a community setting. Known predictors of hMPV related hospitalization including age (continuous variable) and sex at birth (male vs female) were evaluated in univariate models.[4,5] Other variables including sample collection in relation to the start of the pandemic (pre-pandemic meaning January 2018-February 2020 vs during the pandemic meaning March 2020-July 2022), income level (≤50,000, 50,001 – 100,000, 100,001 – 150,000, ≥150,000), residence type (apartment, assisted living/congregate settings, house, no consistent housing/shelter, other), household size, (lives alone, 2 people, 3 people, 4 people, 5 people, 6+ people) children under 5 in the household (yes vs no), comorbidities (none vs at least one), domestic travel in the past 2 weeks (yes vs no), and international travel in the past 2 weeks (yes vs no) were also evaluated. All variables evaluated in a univariable model were included in the multivariable to determine which were independently associated with testing positive for hMPV. Complete case analysis was used. Certain variables, including health insurance status and education status, were not included in our model due to the high percentage of missingness. The missingness primarily occurs in data from SCAN as SCAN participants were not queried about health insurance status and were only asked about education status and children in the household in their enrollment survey as of October 2021.

Proportions and counts were used to compare hMPV subtype prevalence across respiratory season, age group, and sampling method (clinical vs community). Descriptive statistics were also used to compare hMPV subtype prevalence before and after the start of the COVID-19 pandemic, across sampling methods and age group.

Percent positivity maps by Public Use Microdata Areas (PUMAs) were created to visualize the variation of percent positivity across the greater Seattle area using survey and sryvr packages in R. Maps were created across the four respiratory seasons (2018-2019, 2019-2020, 2020-2021, and 2021-2022) and further stratified by age (less than 18 and 18+) to visualize how percent positivity changed over time and across age groups.

R Studio was used for all statistical analyses ((Posit team (2024). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL http://www.posit.co/).

Results

Community Epidemiology of hMPV

From 2018-2022, 330,415 samples were collected as part of SFS. Only 112,035 were tested on Open Array for hMPV due to the start of the pandemic and the prioritization of testing for SARS-CoV-2. Of the 112,035 samples tested for hMPV, 52,036 were from a community setting and 59,999 were from a clinical setting. Most of the community samples tested came from SCAN (n = 40,997), followed by kiosks (n = 7,902), and SwabNSend (n = 3,137). The percent positivity of hMPV was 0.47% among community samples (247/52,036).

The percent positivity for hMPV was highest among SwabNSend (2%), followed by kiosks (1.9%), and SCAN (0.1%). In the 2018-2019 respiratory season the percent positivity for hMPV was 3.31%, 1.40% in 2019-2020 season, 0.03% in the 2020-2021 season, and 0.33% in the 2021-2022 season (Table 1). Children ages 1-4 accounted for 51% of the positives during the 2018-2019 respiratory season. During the 2019-2020 respiratory season 52% of positives were from the 18-49 age group, and throughout the pandemic the 18-49 age group was responsible for most positives (67% in ’20-21 and 44% in ’21-22). Overall, 49% of hMPV positives were among those under 18 years of age, whereas 24% of hMPV negatives were among those under 18 years of age. Seventy-four percent of the hMPV community positives were collected before the COVID-19 pandemic which was defined as starting on March 1st, 2020. Around 5% of people with hMPV and 4% of people without hMPV reported that they sought care for their respiratory symptoms prior to their enrollment in SFS. Due to variation in data storage practices across community cross-sectional sampling (kiosks, SCAN, SwabNSend), there was a high level of missingness for certain variables. Across community samples included in our analysis, around 14-16% of participants were missing data on race, ethnicity, income, housing status, household size, comorbidities, international travel, or domestic travel. About 20-30% were missing data on income, smoking status, or clinical care seeking behavior. Lastly, 83-90% of participants were missing education status, insurance status, or data on children in the household.

Among people with hMPV, the most commonly reported symptoms were cough (86%), rhinorrhea (82%), and fatigue (60%). For those who tested negative for hMPV, but positive for another respiratory virus, the most common symptoms were rhinorrhea (59%), cough (53%), and sore throat (47%) (Table 2). After stratifying hMPV positives by age group, cough and rhinorrhea were still the most commonly reported symptoms across all age groups (Figure 1). Fever was more commonly reported for those <1 and 1-4 years old compared to all other age groups. Fatigue was most common in the 50-64 age group followed by the 18-49 age group. Headaches were most common for those 65+, but not as common for all other age groups.

In our risk factor analysis, age, testing prior to the COVID-19 pandemic, income, household size, comorbidities, children in the household, and international travel were all statistically significant variables in univariable models (Table 3). Testing prior to the COVID-19 pandemic, larger household size, having young children in the household, and international travel were all

associated with a higher odds of testing positive. Older age and reporting 1+ comorbidity were associated with a lower odds of testing positive.

In our multivariable model only testing prior to the COVID-19 pandemic (adjusted OR: 3.02, 95% CI: 1.90 – 4.81), lower income (aOR: 2.05, 95% CI: 1.12 – 3.74), larger household size (aOR: 2.94, 95% CI: 1.44 – 6.00), and international travel (aOR: 2.71, 95% CI: 1.23 – 5.97) remained statistically significant variables (Table 3). All were associated with an increased odds of testing positive for hMPV.

Percent Positivity

We also evaluated whether there was differential percent positivity by PUMA within the Seattle metropolitan area. This analysis included clinical samples as well as the community samples described above that were tested for hMPV (n = 112,035). Clinical samples came from Seattle Children's Hospital (n = 25,910), Public Health Seattle King County Testing Centers (n = 16,672), Harborview Medical Center (n = 13,248), University of Washington Medical Center Northwest (n = 2,590), and University of Washington Medical Center (n = 1,579). A total of 1291 samples were positive for hMPV for an overall percent positivity of 1.15%. The percent positivity among clinical samples was 1.74%, higher than the percent positivity among community samples (0.47%) described above.

The geospatial analysis suggests that there was differential percent positivity by region and by clinic versus community samples. (Figure 5). In the 2018-2019 respiratory season clinical samples had the highest percent positivity in the West Seattle Beacon Hill PUMA (5.3%). The highest percent positivity for community samples during the 2018-2019 respiratory season was in the Northwest PUMA. Clinical sample percent positivity remained high in 2019-2020 season, but community percent positivity decreased during the 2019-2020 season. Both clinical and community percent positivity remained low in the 2020-2021 and 2021-2022 season.

To further analyze the geospatial percent positivity trends, community and clinical percent positivity graphs were stratified by age (under 18 vs 18+) and respiratory season. In the 2018-2019 season, clinical percent positivity was driven by those under 18, primarily in the West Seattle Beacon Hill PUMA (Figure 6a). In the 2018-2019 season, community percent positivity was also driven by those under 18 in the Northwest PUMA and West Seattle Beacon Hill PUMA (Figure 6b). For the 2019-2020 season, percent positivity was driven by those under 18 in the Northeast PUMA for both community and clinical samples. Percent positivity was similar across PUMAs and age groups after the 2019-2020 respiratory season, likely due to the start of the pandemic.

Sequencing Results

The median Crt value among hMPV community and clinical positives was 14.8. For hMPV positive children the median Crt value was 14.3 and it was 16.6 for positive adults. Across age

groups, children had the lowest Crt values (Figure 2). Among community samples with a low Crt value (< 10), trouble breathing was more commonly reported (59%) compared to all community positives (35%).

Out of the original 316 samples, 233 (74%) were successfully sequenced. There were 8 co-infections identified and 225 single infections identified. Of the 225 single infections, 224 were hMPV and 1 was enterovirus. We excluded the one person who had a single enterovirus infection, which left us with a total of 232 hMPV sequences to analyze (Figure B). The most common co-infection with hMPV was human coronavirus (63%). Other co-infections included human adenovirus (25%) and enterovirus (12%). Of the identified co-infections, 88% were under the age of 17 years old and 75% were among clinical samples.

Thirty-seven (16%) of the successfully sequenced samples were from the 2018-2019 respiratory season, 159 (68%) were from the 2019-2020 season, 3 (1%) were from the 2020-2021 season, and 33 (14%) were from the 2021-2022 season. There were no community samples sequenced from the 2018-2019 season as these samples were unable to be located and pulled for sequencing. As a result, the 2018-2019 season only has clinical sample sequences, whereas the remaining seasons of 2019-2020, 2020-2021, and 2021-2022 have a mix of clinical and community sample sequences. Eighty samples were identified as subtype A2b, 3 were identified as A2c, 72 were identified as B1, and 77 were identified as B2. Subtypes A1 and A2a were not identified in our sample. Community samples captured subtypes A2b, A2c, B1, and B2 whereas clinical samples captured subtypes A2b, B1, and B2 (Figure 3a). The number of subtypes present by respiratory season were approximately equal for 2019-2020 and 2021-2022 (Figure 3b). The proportion of subtypes present by age group were approximately equal, with some exceptions (Figure 3c).

Of the sequenced samples there were 70 community samples and 94 clinical samples that were collected before the start of the COVID-19 pandemic. After the start of the COVID-19 pandemic there were 28 community samples and 40 clinical samples collected. The proportion of subtypes present in clinical and community settings were approximately equal before the start of the COVID-19 pandemic, apart from community samples capturing A2c (Figure 4a). After the start of the COVID-19 pandemic, the proportion of B1 increased in both community and clinical settings. In pre-pandemic clinical settings 24/94 (24%) samples were B1 and after the start of the pandemic 19/40 (47%) samples were B1. In pre-pandemic community settings 17/70 (24%) samples were B1 and after the start of the pandemic 13/28 (46%) were B1.

Stratifying community and clinical samples by age and pandemic status revealed some noticeable differences (Figure 4b and 4c). Pre-pandemic, most age groups across clinical and community settings captured multiple subtypes. After the start of the pandemic, more age groups only had two subtypes circulating, rather than the 3 or 4 observed pre-pandemic. This difference was particularly noticeable in community samples (Figure 4c).

Discussion

Community Epidemiology of hMPV

In a study of individuals with respiratory illness in community settings, risk factors for testing positive in a community setting included testing prior to the start of the COVID-19 pandemic, lower income, larger household size, and international travel. The COVID-19 pandemic had an impact on both frequency and diversity of hMPV subtypes in this population. Percent positivity among clinical and community samples was highest prior to the start of the COVID-19 pandemic

Most of our community samples that tested positive for hMPV were collected prior to the start of the pandemic (74%). This was expected as previous studies have documented a decline in non-SARS-CoV-2 respiratory virus circulation after the emergence of SARS-CoV-2.[14] Our study demonstrated that working age adults ages 18-49 substantially contribute to the community burden of hMPV. A previous study also found that infections were most common among adults ages 18-40, although this study had a much smaller sample size (n = 24) and was focused on healthcare seeking adults, rather than individuals of all ages.[7] Based on our findings, there may be value in vaccinating working age adults who regularly have contact with those at risk for more severe outcomes (children, older adults, immunocompromised) since working age adults account for a large portion of hMPV positives.

Our study also documented hMPV symptom profiles and variation across age groups. All age groups reported a high percentage of cough and rhinorrhea (over 80%). Notably, fever was not as frequently described in adults with hMPV infection compared to children (74% for 1-4 vs 50% for 18-49). All adults aged 65+ reported headaches compared to only 53% of adults aged 18-49. Only one other study has reported symptom variation by age and included younger adults (18-39) compared to older adults (65+). In 1999-2001 in Rochester, New York, younger adults reported a higher percentage of hoarseness, whereas older adults reported a higher percentage of dyspnea and wheezing.[7] Our study did not include these specific symptoms, but we noticed similar percentages of sore throat across adults ages 18-49 and adults 65+ (78% vs 83%). We also noticed similar percentages of trouble breathing in adults ages 18-49 and adults 65+ (41% vs 33%). The Rochester study included participants in both outpatient and inpatient settings but the sample size was small: 11 adults aged 18-39 and 13 adults aged 65+ (versus 91 adults aged 18-49 and 6 adults aged 65+ in this study). No other study to our knowledge has compared symptom profiles between children and adults.

In our univariable models young age, testing prior to the pandemic, low income, international travel, children in the household, and a large household size (greater than 5) were all statistically significant risk factors for testing positive. Age has been previously described as a risk factor for hMPV related hospitalization, while having young children in the household and household crowding have been previously described as risk factors for the acquisition of respiratory viral infections in clinical settings; we find that these are risk factors for testing positive in a community setting as well.[15,16]

In our multivariable model, an income level of $50,001-100,000 compared to ≥150,000, household size of 5 compared to living alone and international travel remained significant risk factors for hMPV infection (Table 3). Our analysis indicates that a lower income level and a larger household size may be risk factors of testing positive for hMPV. Having children in the household was only significant in our univariable model, despite being previously described as a risk factor for the acquisition of respiratory viruses. The decision to do a complete case analysis, in addition to the high percent of missingness for the children in the household variable, may have attributed to the unexpected result of children in the household only being significant in the univariable model. Having children in the household may still be an important factor to consider when an hMPV vaccine becomes available, despite our findings here. This relationship should be further evaluated in future studies.

Percent Positivity

According to the City of Seattle's Racial and Social Equity Composite Index, there are a higher number of sociodemographic disadvantaged census tracts in the SW Seattle PUMA compared to the NW Seattle PUMA.[17] In our geospatial analysis, the percent positivity for clinical and community samples varied by PUMA. Clinical samples had the highest percent positivity in the Southwest (SW) Seattle PUMA. After stratifying by age, we were able to see that those under 18 in the SW Seattle PUMA were driving percent positivity in clinical samples.

For community samples under 18, the SW Seattle PUMA and the Northwest (NW) Seattle PUMA had the highest percent positivity. Despite the high percent positivity among community samples in the NW Seattle PUMA, this area did not have a substantially high clinical percent positivity. It is possible that the difference in sociodemographic status between these PUMAs contributes to the SW Seattle PUMA being overrepresented in clinical percent positivity. This adds to the already existing literature that suggests that socioeconomic status is associated with severe respiratory infection outcomes like hospitalization.[18,19] Interventions to reduce respiratory infection severity and burden should therefore be targeted towards areas with low socioeconomic status.

Sequencing

This study is also the first to describe the percent positivity of hMPV subtypes in a community setting as well as a clinical setting and across age groups. Other studies have primarily focused on hMPV subtype prevalence in a clinical setting.[20–23] Understanding the variation of subtype prevalence in community settings and across age groups may assist with vaccine development and implementation. Across clinical and community settings, subtypes A2b, B1, and B2 were the most common co-circulating subtypes. This suggests that it may be strategic to focus vaccine development on bivalent or multivalent vaccines to address the co-circulating nature of hMPV subtypes.

Based on our data, there appears to be little variation of subtype by age; this may facilitate vaccine implementation as a multi-variant vaccine may be able to be deployed across age groups. Our study found an approximately equal proportion of subtypes across clinical and community settings. Previous studies have suggested that infection with a certain subtype may be related to more severe outcomes, like hospitalization. [8,9] Assuming that clinical samples were associated with more severe infections, our findings of approximately equal subtype proportions across clinical and community settings differ from previous studies, which have suggested a relationship between infection severity and subtype. Previous studies that have suggested a relationship between infection severity and subtype were among hospitalized children under the age of three. We may not have observed this relationship since we included samples from individuals of all ages in both clinical and community settings. Further analysis should be done to evaluate if there is a relationship between infection severity and subtype.

Our data also allows us to look at the variation in hMPV subtype before and after the COVID-19 pandemic, stratified by age group; this has not been previously evaluated in the United States. Both community and clinical samples had a decrease in the proportion of B2 subtypes and an increase in the proportion of B1 subtypes. Both clinical and community samples had a decrease in the number and proportion of B2 subtype infections among those under 18 years of age. This may have been in part due to stay at home orders and the closing of schools and daycare facilities during the pandemic. It is unclear why stay at home orders may have led to a decrease in the proportion of B2 infections in those under 18, but not B1 or A2b infections.

Other studies have found varying results regarding subtype variation before and after the start of the COVID-19 pandemic. One study from Israel found that in 2021 subtype B1 became dominant in their hospitalized patient population.[12] Our study found an increase in the proportion of B1 subtype infections after the pandemic, but unlike the Israel study B1 was not dominant but rather co-dominant with subtype A2b in community and clinical settings. Another study from South Korea found that after the pandemic subtypes A2b and B2 co-circulated.[24] This differs from what was found in our study. Lastly, a study from Spain found that A2c was dominant in the reemergence of hMPV after the pandemic, which again differs from our findings.[21] Our results suggest that hMPV subtype dominance and prevalence may be dependent on country post-pandemic.

This study has some limitations. Firstly, there was a high level of missingness in certain variables, which limited the statistical power of our study to find meaningful differences between those who tested positive vs negative for hMPV and prevented us from analyzing some characteristics for which data were missing on most people. Our decision to perform a complete case analysis due to the high level of missingness may also impact our ability to find meaningful differences across groups and may impact the generalizability of our findings.

Secondly, only a subset of clinical samples were sequenced for our genomic analyses. It is possible that our sequenced clinical samples do not represent the true variation of hMPV subtypes in Seattle's clinical settings from 2018-2022. Thirdly, there was a limited number of

community samples that could be sequenced, as all our community samples from the 2018-2019 season could not be located and pulled from our partner labs. This may limit our ability to truly understand hMPV subtype variation prior to the pandemic since we have no sequenced community samples from 2018-2019. Lastly, convenience sampling may be an important limitation of our study, particularly for our geospatial analysis. There may be certain areas or populations that were under sampled and are therefore not appropriately represented in our analyses.

Despite these limitations, our study has several strengths and may provide new insights about hMPV epidemiology. Firstly, our sample size of hMPV positives was larger than in previous studies and spanned across multiple age groups, which allowed for granular comparisons across groups. Secondly, our study occurred before and during the COVID-19 pandemic, which allowed us to evaluate the trends of hMPV over this important period. Lastly, our study includes both community and clinical samples that contribute to both percent positivity estimates and genomic analyses; few hMPV studies to date have combined such samples.

Our study describes the epidemiology and genomic epidemiology of community-based hMPV infection. We found that adults ages 18-49 account for a large percentage of positives in a community-based setting, which may influence vaccine policy when a vaccine becomes available. Young children, older adults and immunocompromised individuals should likely get vaccinated, and vaccinating healthy working adults that regularly come in contact with high-risk individuals may also benefit those at highest risk for severe disease. Our study also allows us to evaluate percent positivity across multiple years and geographic locations. We observed the highest hMPV percent positivity in those under 18 years of age in an area of low SES. Vaccine and therapeutic interventions may want to target areas of low SES as these areas appear to account for a disproportionate amount of severe disease burden. Lastly, our genomic analysis suggested that multiple subtypes of hMPV circulated in both pre and post pandemic settings. A multi-subtype vaccine may be the best approach to reduce disease severity associated with hMPV infection. Our large amount of missing data limits our ability to identify relevant risk factors for an entire population. Future studies should continue to evaluate the relationship of risk factors for testing positive in a community setting to further guide vaccine implementation when a vaccine becomes available.

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Appendix:

Table 1: Distribution of demographic variables among community samples tested for hMPV (N = 52,036)

	Community Samples	
	hMPV-Positive (n = 247) n, %	hMPV-Negative (n = 51,789) n, %
Age (years)		
<1	13 (5)	574 (1)
1-4	69 (28)	4,067 (8)
5-17	39 (16)	7,629 (15)
18-49	91 (37)	28,935 (56)
50-64	15 (6)	5,524 (11)
65+	6 (2)	2,377 (5)
Missing	14 (6)	2,683 (5)
Female Sex	146 (59)	28,185 (54)
Race		
American Indian/Alaska Native	0 (0)	268 (<1)
Asian	23 (9)	7,600 (15)
Black/African American	3 (1)	1,499 (3)
Native Hawaiian	2 (1)	534 (1)
Multiracial	11 (4)	3,120 (6)
Other	15 (6)	2,162 (4)
White	98 (40)	28,487 (55)
Missing	95 (38)	8,119 (16)
Hispanic	31 (13)	4,145 (8)
Pandemic[1]		
Pre-COVID	187 (76)	7,040 (14)
COVID	60 (24)	44,749 (86)
County		
King	183 (74)	48,070 (93)
Snohomish	33 (13)	885 (2)
Pierce, Island, Skagit	1 (<1)	293 (<1)
Missing	30 (12)	2,541 (5)
Respiratory Season		
2018-2019	86 (35)	2,514 (5)
2019-2020	121 (49)	8,498 (16)
2020-2021	11 (4)	32,166 (62)

2021-2022	27 (11)	8,070 (16)
2022-2023	2 (<1)	216 (<1)
Education Level		
Less than High School diploma[2]	13 (5)	850 (2)
High School / GED	6 (2)	778 (2)
Some College	23 (9)	1,542 (3)
Bachelor's Degree	30 (12)	2,528 (5)
Advanced Degree	30 (12)	2,269 (4)
Prefer not to say	2 (1)	612 (1)
Missing	143 (58)	43,210 (83)
Income Level		
<= 50,000	32 (13)	7,874 (15)
50,001 – 100,000	41 (17)	4,437 (9)
100, 001 – 150,000	19 (8)	7,055 (14)
>150,000	35 (14)	13,190 (25)
Prefer not to say	19 (8)	6,903 (13)
Don't Know	8 (3)	2,044 (4)
Missing	93 (38)	10,286 (20)
Health Insurance Status		
Government	28 (11)	1,080 (2)
Private	82 (33)	3,594 (7)
Other	1 (<1)	84 (<1)
Uninsured	5 (2)	122 (<1)
Prefer not to say	4 (2)	98 (<1)
Missing	127 (51)	46,811 (90)
Type of Residence		
Apartment	33 (13)	8,338 (16)
Assisted living/Congregate Setting[3]	1 (<1)	412 (<1)
House	107 (43)	35,131 (68)
No consistent housing/Shelter	1 (<1)	140 (<1)
Other	3 (1)	555 (1)
Missing	102 (41)	7,213 (14)
House Size		
Lives Alone	11 (4)	4,208 (8)
2 people	34 (14)	11,632 (22)
3 people	22 (9)	9,181 (18)
4 people	28 (11)	12,101 (23)
5 people	36 (14)	4,645 (9)
6+ people	21 (8)	2,953 (6)
Missing	95 (38)	7,069 (14)
Children 5 and Under in Household[4]		

Yes	62 (25)	2,330 (5)
No	70 (28)	5,443 (11)
Missing	115 (47)	44,016 (85)
Smoking Status		
Does not smoke	77 (31)	32,554 (63)
Smokes (tobacco or e-cigarettes)	5 (2)	3,269 (6)
Prefer not to say	1 (<1)	695 (1)
Missing	164 (66)	15,271 (30)
Comorbidities		
None	86 (35)	28,386 (55)
At least 1 comorbidity	24 (10)	13,960 (27)
Don't Know/Prefer not to answer	5 (2)	2,119 (4)
Missing	132 (53)	7,324 (14)
International Travel	11 (4)	909 (2)
Domestic Travel	13 (5)	6,103 (12)
Sought Medical Care[5]		
Did not seek care	84 (34)	39,867 (77)
Sought care	12 (5)	2,017 (4)
Missing	151 (61)	9,905 (20)
Flu Shot		
Received	153 (62)	16,560 (32)
Did not receive	74 (30)	11,827 (23)
Missing	20 (8)	23,402 (45)

[1]Anything collected on March 1st, 2020 or after was considered collected after the start of the pandemic

[2]Those who reported having less than a HS diploma may included children still in school

[3]Assisted living and congregate settings includes: adult family homes, assisted living facilities, skilled nursing facilities, long term care or rehab facilities, inpatient of behavioral health residential centers, permanent supportive care or transitional housing centers, and correction facilities

[4]May be some misclassification as SCAN questionnaires asked about children <5 in the household while kiosks and SwabNSend asked about children 0-5 in the household

[5]Did not seek care at time of swab

Table 2: Reported symptoms for community samples tested in the Seattle Flu Study, 2018 – 2022 (N = 268,324)

Symptoms available for community samples tested in the Seattle Flu Study (2018-2022)

	Reported symptoms for those who tested positive for hMPV (n = 247) n, (%)	Reported symptoms for those who tested negative for hMPV, but positive for another respiratory pathogen (n = 19,976) n, (%)	Reported symptoms for those who tested negative for all respiratory pathogens* (n = 248,284) n, %
Cough	215 (87)	10,622 (53)	32,510 (13)
Runny/Stuffy nose	204 (83)	11,764 (59)	41,655 (17)
Fatigue	150 (61)	7,808 (39)	29,508 (12)
Fever	136 (55)	6,320 (32)	13,322 (5)
Sore throat	126 (51)	9,310 (47)	41,908 (17)
Muscle/Body Aches	86 (35)	5,249 (26)	19,968 (8)
Trouble breathing	86 (35)	2,290 (12)	7,280 (3)
Headaches	82 (33)	7,278 (36)	33,982 (14)
Sweats	56 (23)	2,748 (14)	6,774 (3)
Chills	54 (22)	3,819 (19)	9,937 (4)
Ear pain/discharge	48 (19)	1,380 (7)	4,179 (2)
Nausea/vomiting	45 (18)	2,361 (12)	8,818 (4)
Diarrhea	32 (13)	1,932 (10)	9,262 (4)
Loss of taste/smell	3 (1)	1,138 (6)	2,349 (1)
Rash	5 (2)	387 (2)	1,205 (<1)

Figure 1 – Reported symptoms for community samples that tested positive for hMPV, by age group (n = 233)

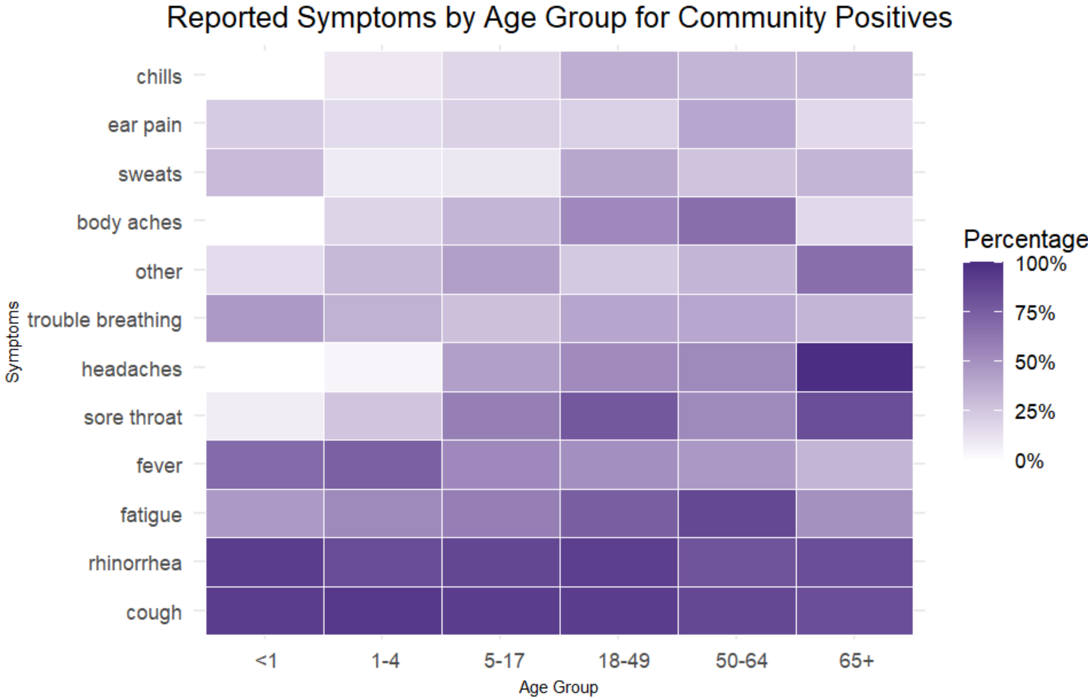


Table 3: Results of univariable and multivariable models assessing risk factors for testing positive in a community setting

Variables	Unadjusted Odds Ratio (95% CI[1], p-value)	Adjusted Odds Ratio (95% CI, p-value)
Age in years	0.96 (0.95 - 0.97, <0.00005)*	0.99 (0.97 – 1.00, 0.30)
Male sex	0.84 (0.64 – 1.10, 0.19)	0.78 (0.48 – 1.28, 0.33)
Pre-pandemic (Jan 2018 –Feb 2020)	19.81 (14.80 – 26.53, <0.00005)*	3.02 (1.90 – 4.81, <0.00005)*
Income (>150,000 reference)		
<= 50,000	1.53 (0.95 – 2.48, 0.08)	0.94 (0.45 – 1.98, 0.87)
50,001 – 100,000	3.48 (2.21 – 5.47, <0.00005)*	2.05 (1.12 – 3.74, 0.02)*
100,001 – 150,000	1.01 (0.58 – 1.78, 0.96)	0.68 (0.31 – 1.50, 0.34)
Housing Type (apt reference)		
House	0.77 (0.52 – 1.14, 0.19)	1.18 (0.63 – 2.19, 0.61)
Household Size (lives alone as reference)		
2 people	1.12 (0.57 – 2.21, 0.75)	N/A[2]
3 people	0.92 (0.44 – 1.89, 0.81)	0.62 (0.30 – 1.30, 0.20)
4 people	0.88 (0.44 – 1.78, 0.73)	0.80 (0.36 – 1.74, 0.57)
5 people	2.96 (1.51 – 5.83, 0.002)*	2.94 (1.44 – 6.00, 0.003)*
6+ people	2.72 (1.31 – 5.65, 0.007)*	1.03 (0.38 – 2.80, 0.95)
At least 1 comorbidity	0.57 (0.36 – 0.89, 0.01)*	1.00 (0.58 – 1.74, 0.99)
Having children in the household (0-5 years old)	2.07 (1.47 – 2.92, <0.00005)*	1.57 (0.91 – 2.69, 0.10)
Traveled outside of the United States in the past 2 weeks	3.71 (2.00 – 6.87, <0.00005)*	2.71 (1.23 – 5.97, 0.01)*
Traveled outside Washington State in the past 2 weeks	0.58 (0.33 – 1.03, 0.06)	0.71 (0.32 – 1.57, 0.39)
[1]CI refers to confidence interval		

2 person household was dropped in multivariate model due to complete case analysis - there were not enough individuals in that category with complete data to be included in the multivariate model
*Represents a statistically significant p-value (<0.05)

Figure 2

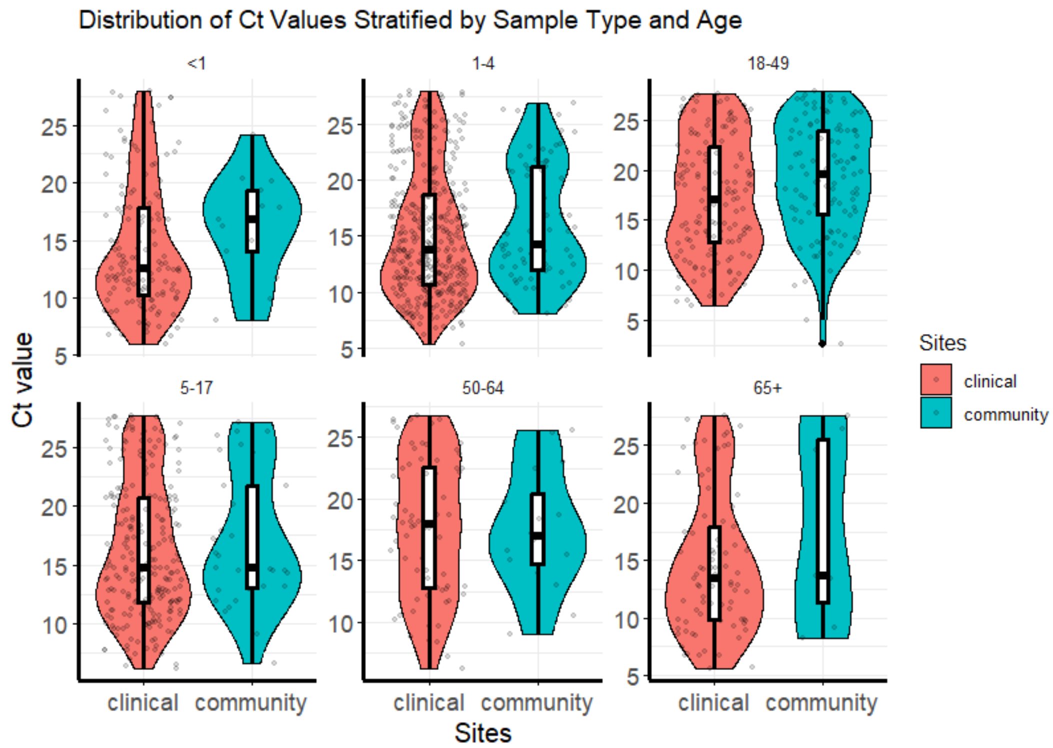


Figure 3a

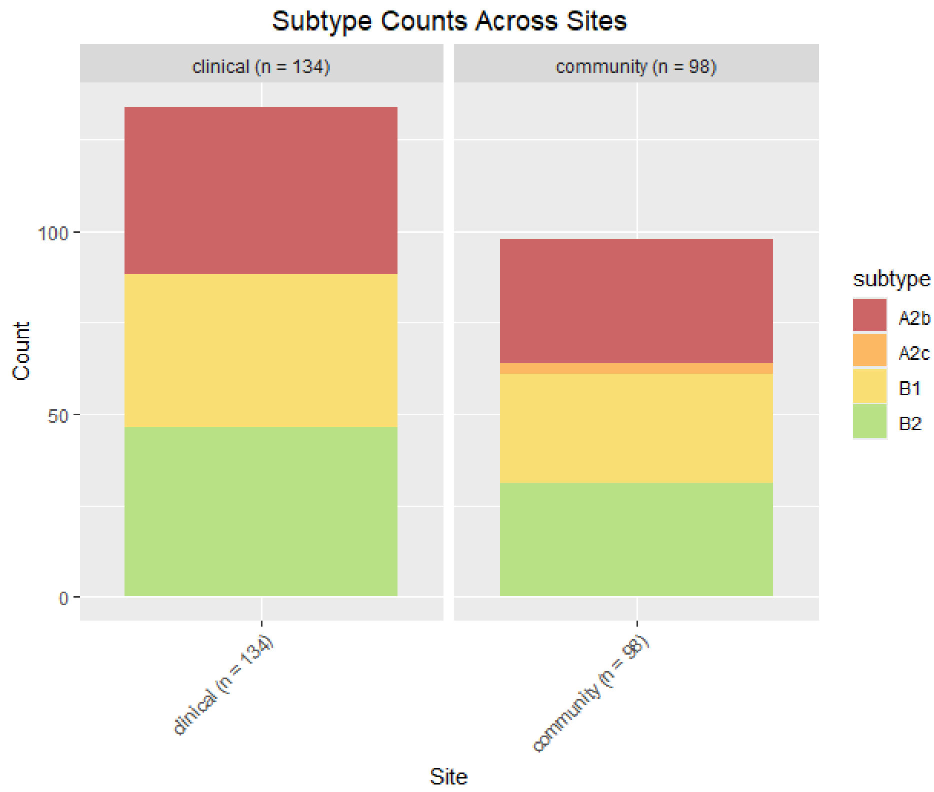


Figure 3b

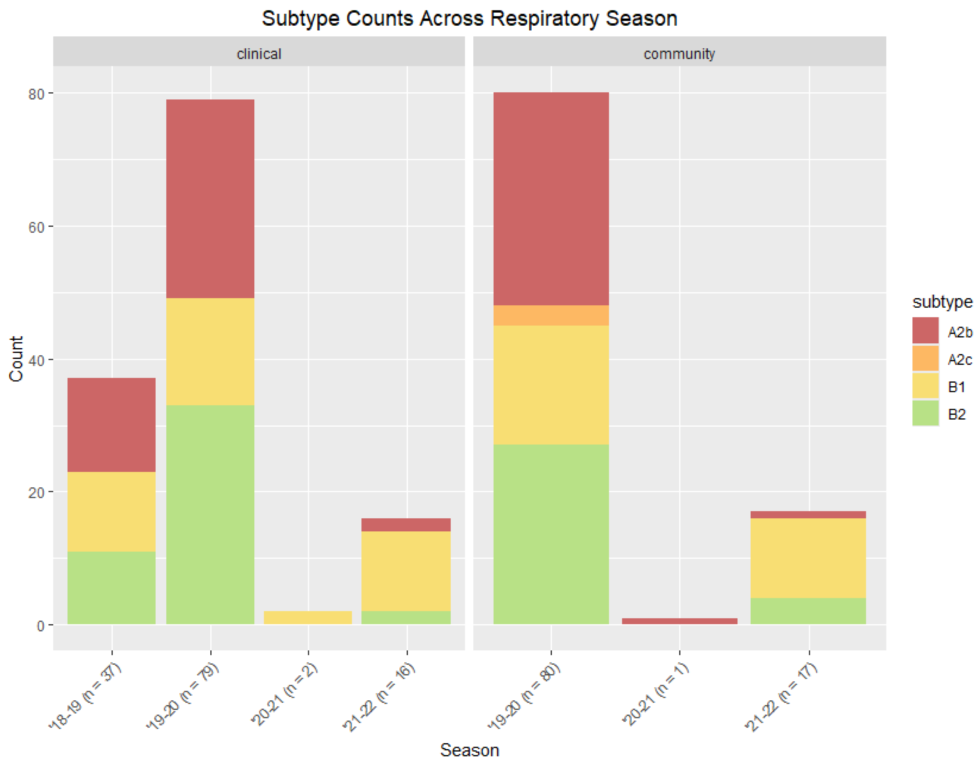


Figure 3c

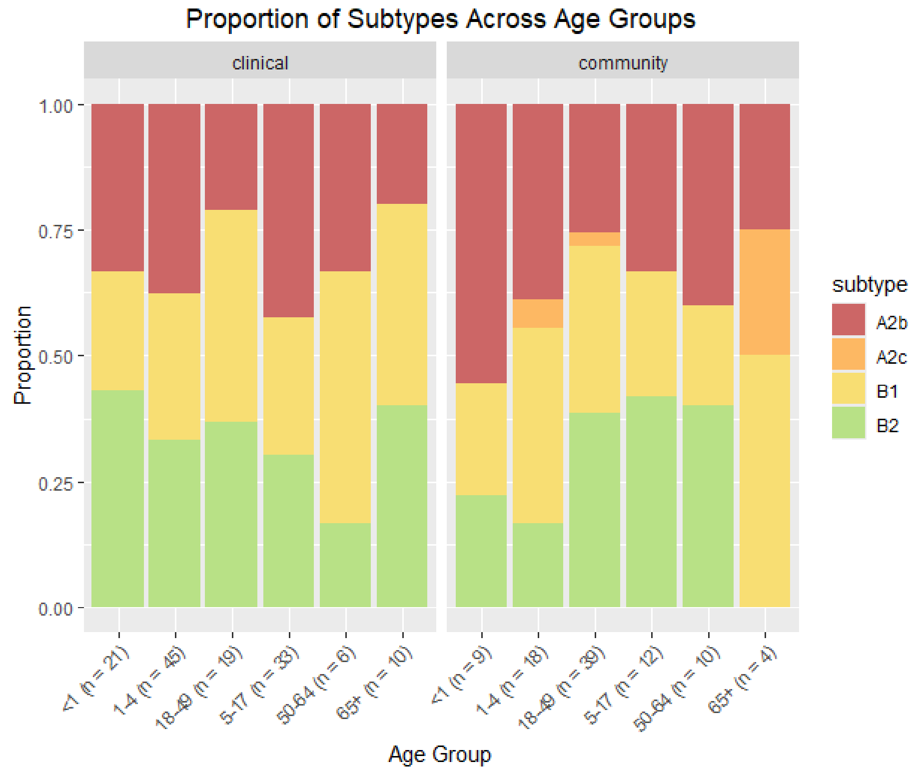


Figure 4a

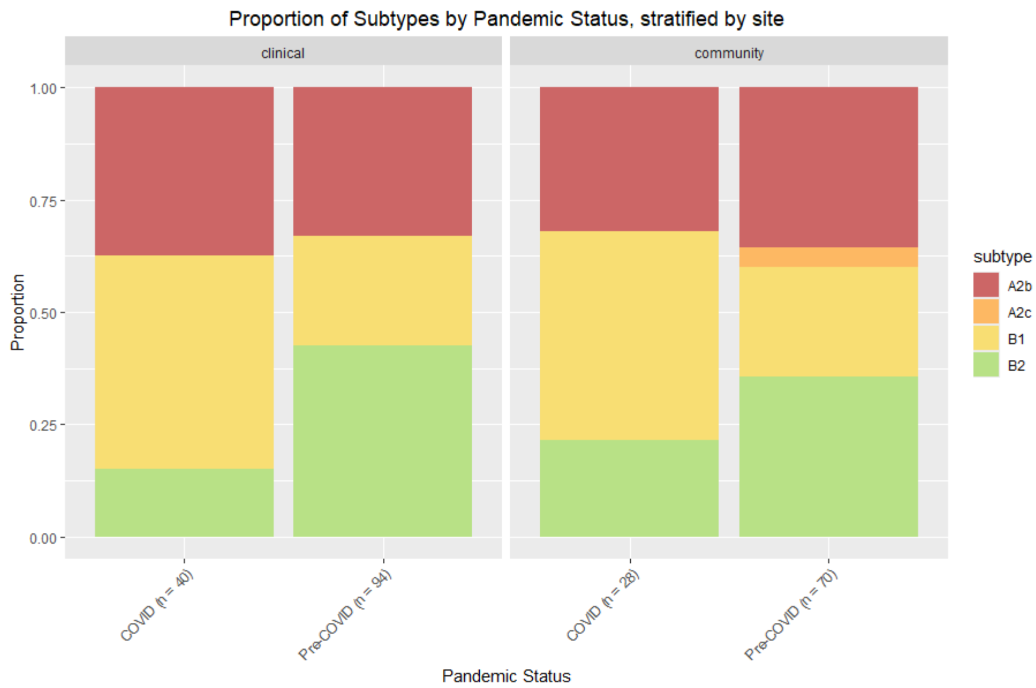


Figure 4b

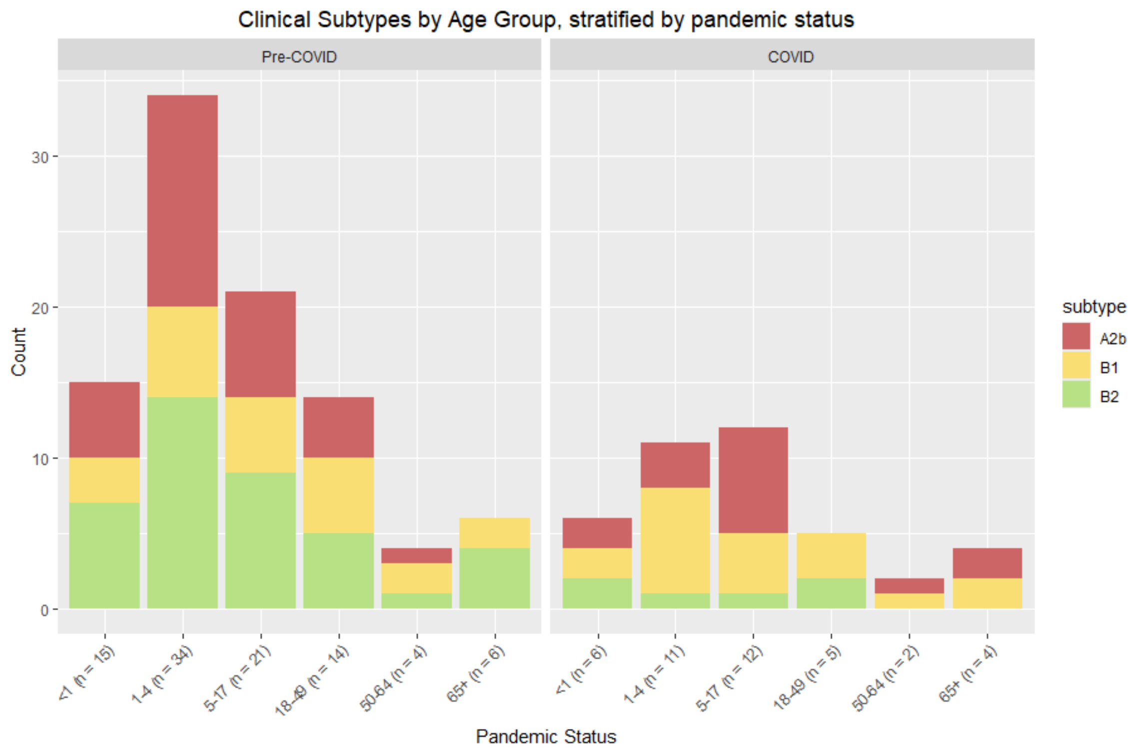


Figure 4c

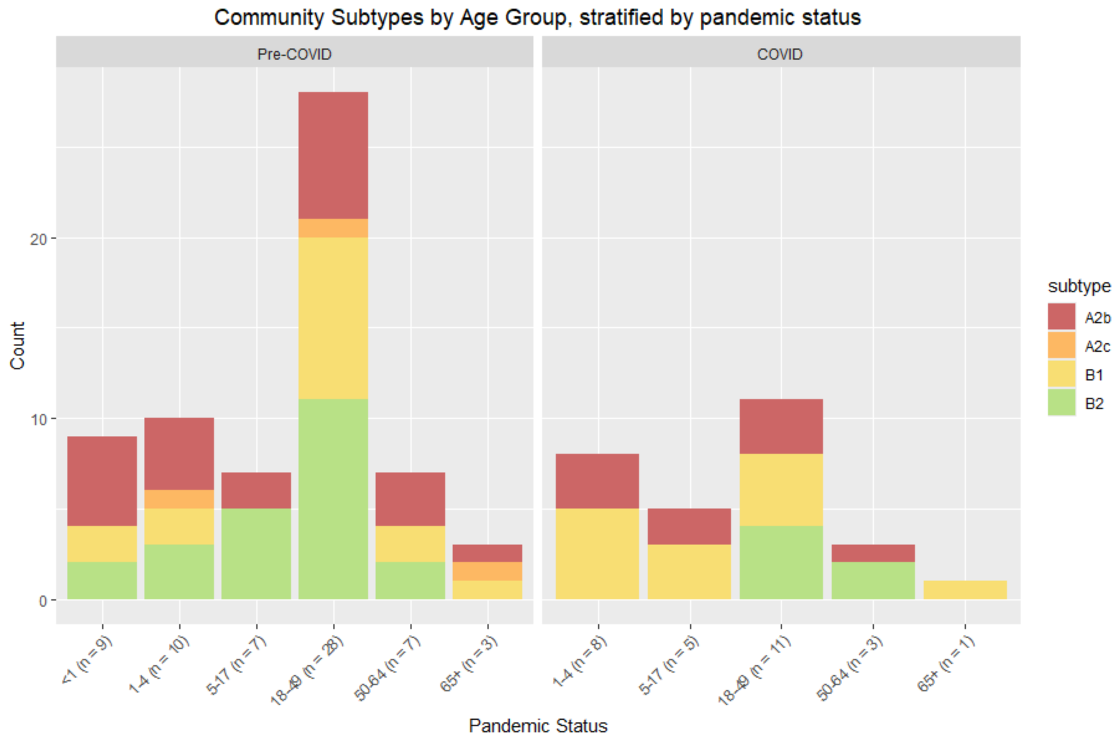
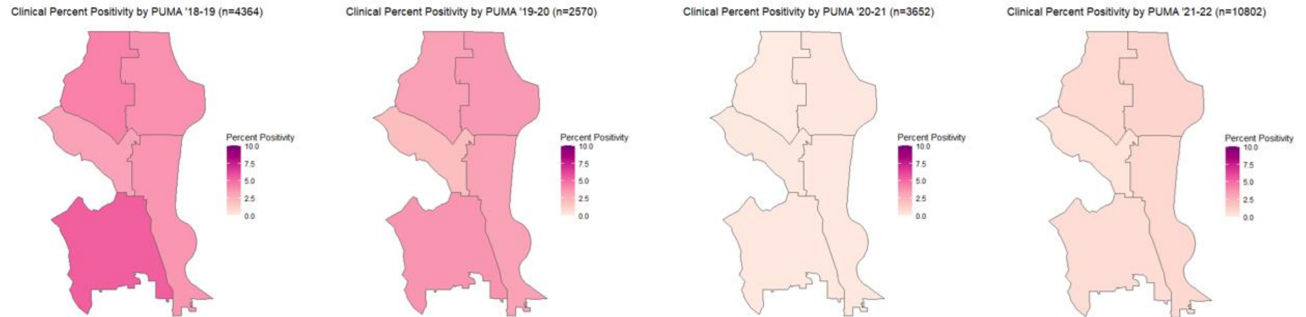


Figure 5 – Clinical and Community Percent Positivity Stratified by Respiratory Season, (2018-2022)

CLINICAL



COMMUNITY

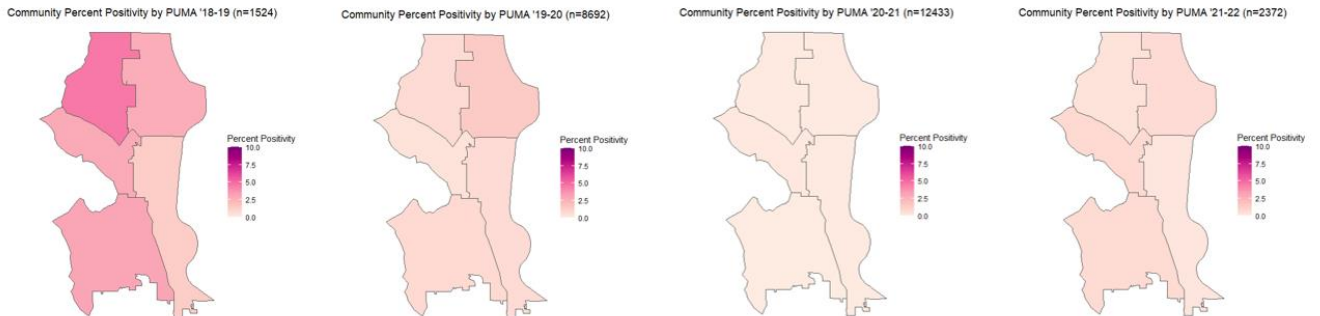
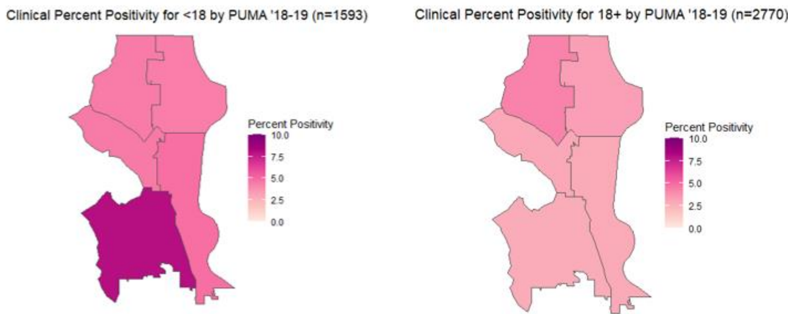


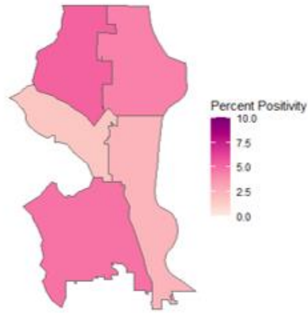
Figure 6a – Clinical Percent Positivity Stratified by Respiratory Season and Age Group (<18, 18+)



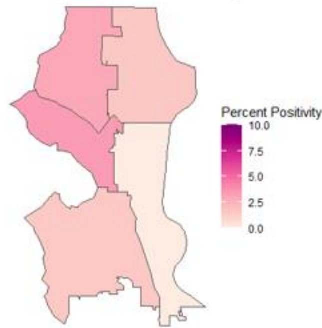
Clinical Percent Positivity for <18 by PUMA '19-20 (n=1171) Clinical Percent Positivity for 18+ by PUMA '19-20 (n=1399)

Figure 6b - Community Percent Positivity Stratified by Respiratory Season and Age Group (<18, 18+)

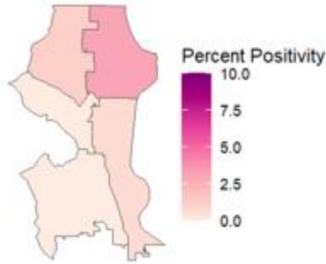
Community Percent Positivity for those <18 by PUMA '18-19 (n=743)



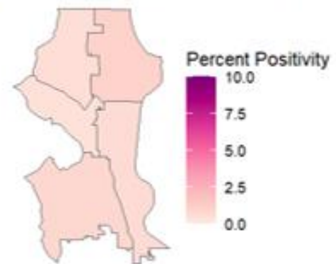
Community Percent Positivity for those 18+ by PUMA '18-19 (n=780)



Community Percent Positivity for those <18 by PUMA '19-20 (n=960)



Community Percent Positivity for those 18+ by PUMA '19-20 (n=7730)



Supplemental Figures:

Figure A

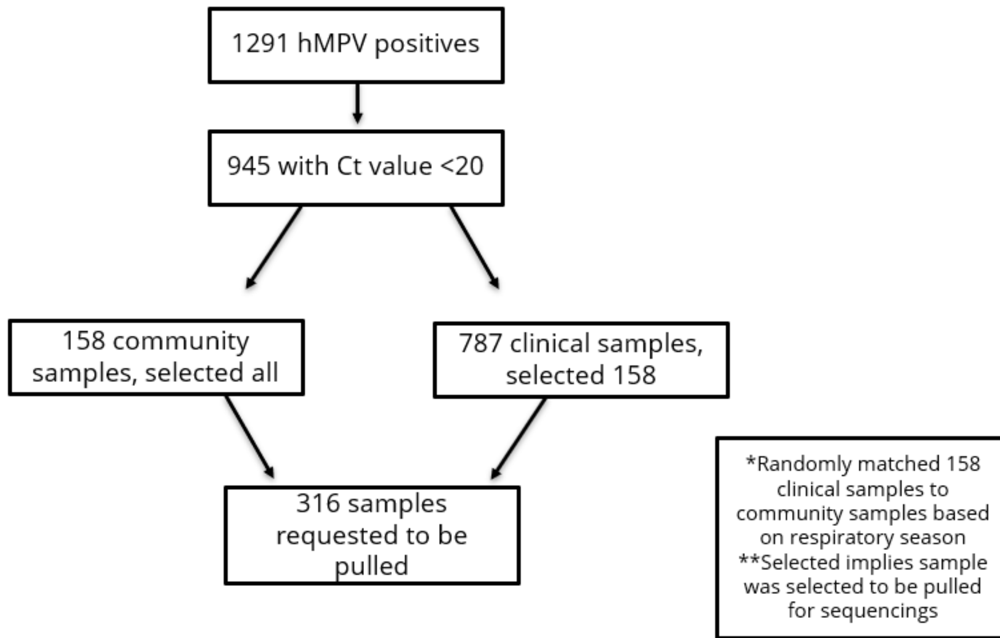


Figure B