Pediatric Coronavirus Infection and Immunity: Common Cold Coronaviruses and SARS-CoV-2

by

Aaron M. Frutos

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Epidemiological Science)
in the University of Michigan
2023

Doctoral Committee:

Associate Professor Aubree Gordon, Chair
Associate Professor Nancy Fleischer
Dr. Josh Petrie, Marshfield Clinic Research Institute
Assistant Professor Zhenke Wu

Aaron M. Frutos

frutosa@umich.edu

ORCID iD: 0000-0001-6948-3918

Acknowledgements

I am immensely grateful to Dr. Aubree Gordon for her unwavering support and encouragement. This dissertation is a testament of her dedication and mentorship. From my first meeting with her in Fall 2018 as an MPH student, I felt that Aubree cared about my success and recognized what I value most in life. Having her has a mentor when I first arrived at the University of Michigan changed my trajectory in life in so many ways. She has looked out for me and my family, giving us every possible opportunity for increased learning and responsibility. We will be forever grateful for her.

I also want to acknowledge the tremendous work of the entire study team in Nicaragua. None of this work would be possible without them. Their dedication to public health and improving the health of others, even during a global pandemic, is inspiring.

I have been so blessed by the amazing faculty here at the University of Michigan. I want to thank Dr. Nancy Fleischer, Dr. Josh Petrie, and Dr. Zhenke Wu for their service on my dissertation committee and for the constructive discussion we have had. I am grateful for Dr. Sarah Adar, Dr. Andrew Brouwer, Dr. Sung Kyun Park, Dr. Bhramar Mukherjee, and Dr. Eduardo Villamor who repeatedly challenged me and what I thought I knew, helping me to grow as an epidemiologist.

I am thankful for dedicated faculty from Brigham Young University, principally Chantel Sloan-Aagard, Ray Merrill, and Evan Thacker, that provided me early career mentorship experiences which helped me to be well prepared for graduate school at the University of Michigan. Thank you for giving me my first formal experiences with public health and research.

I want to thank my parents, Brenda and Tony Frutos, for encouraging me to tirelessly pursue my academic goals. The value you have placed on education has stayed with me since I was a young kid; I remember having the goal to earn a Ph.D. as I was running around student family housing at the University of Wisconsin-Madison. Thank you for supporting me to achieve a childhood dream.

To my own children, thank you for loving me and helping me to become a better person. You inspired me to focus on the importance of childhood within my work. I hope I can support you in your childhood dreams as I was supported in mine.

Finally, thank you Emily for supporting me through all that comes with a completing Ph.D., especially during a global pandemic. You have always patiently loved and encouraged me. I am grateful for our partnership and family we have grown together. I look forward to the years that come as we continue to work together to love and serve everyone around us. I love you eternally.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

HCoV	Human coronavirus
ccCoV	Common cold coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus Disease 2019
RT-PCR	Reverse transcription polymerase chain reaction
NPICS	Nicaraguan Pediatric Influenza Cohort Study
HICS	Household Influenza Cohort Study
ALRI	Acute lower respiratory infection
LRI	Lower respiratory infection
ELISA	Enzyme-linked immunosorbent assay
RSV	Respiratory syncytial virus
HMpV	Human metapneumovirus
SAR	Secondary attack risk
95% CI	95% confidence interval
MIS-C	Multisystem inflammatory syndrome in children
PASC	Post-acute sequelae SARS-CoV-2 infection

Abstract

The epidemiology of human coronaviruses (HCoV) is not well understood, particularly among children. The emergence of SARS-CoV-2 and the resulting, ongoing global pandemic highlight our limited understanding. Better understanding of both common cold coronavirus (ccCoV) and SARS-CoV-2 epidemiology among children will provide insight into how SARS-CoV-2 will transition to endemicity.

To examine fundamental questions about HCoVs we used data from the Nicaraguan Pediatric Influenza Cohort Study (2011-2016, 2020-2021) and the Household Influenza Cohort Study (2020-2022). First, we characterized the burden and seasonality of ccCoVs in children in Chapter 2. In Chapter 3 we compared symptoms and severity of ccCoV infections and SARS-CoV-2 among children. We next evaluated the association of SARS-CoV-2 infection-induced immunity with infectivity and protection against infection.

In Chapter 2 we observed that ccCoVs spread annually with the greatest burden among those aged 0-1. We found that prior infection was associated with slight protection against lower respiratory infection (LRI) among the youngest children. In Chapter 3 we characterized the symptom presentation of ccCoVs and SARS-CoV-2 infections and found that they were similar in symptom presentation. Interestingly, in children SARS-CoV-2 infections were as severe or less severe than ccCoV infections. In Chapter 4, we showed that infection-induced immunity was associated with protection against infection and decreased infectivity among adults and adolescents; however, while less infectious than adults, infection-induced immunity was not associated with decreased infectivity among children. By increasing our understanding of

pediatric HCoV infection and immunity we can better prepare for SARS-CoV-2's transition to endemicity where children will increasing represent the greatest proportion of primary SARS-CoV-2 cases.

Chapter 1 Introduction

1.1 Human Coronaviruses

Since their discovery in 1965, multiple human coronaviruses (HCoVs) associated with illness have been characterized.[1,2] NL63, 229E, OC43, and HKU1 are HCoVs that are endemic globally and generally associated with the common cold.[3-6] It has been proposed that these four HCoVs be referred to as common cold coronaviruses (ccCoVs).[7] Since 2002, three zoonotic coronaviruses, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2, have emerged in humans and caused severe disease. However, unlike SARS-CoV-2, the spread of SARS-CoV-1 was fairly rapidly contained; while cases of MERS-CoV are still occurring, they are sporadic and to date have not caused large-scale outbreaks.[3] Because of the high transmissibility of SARS-CoV-2 combined with many asymptomatic or mild cases, SARS-CoV-2 was not contained and is currently transitioning to endemicity.[8]

1.1.1 Common Cold Coronaviruses

Pediatric infections with ccCoVs are common, particularly under two years of age.[3-6,9,10] By age six, children have likely been infected with each ccCoV, sometimes multiple times.[11] Common cold coronaviruses generally spread annually with transmission occurring primarily during winter months in temperate locations;[4-6,12] in other climates, transmission varies throughout the year.[9,13-15]

Pediatric ccCoV infections usually present with upper respiratory symptoms typical of the common cold: fever, cough, rhinorrhea, nasal congestion, or sore throat. However, ccCoVs can cause more severe presentations such as croup and lower respiratory infections (LRI) (with presentations like pneumonia or bronchitis).[3-6,16]

1.1.2 SARS-CoV-2

During SARS-CoV-2's transition to endemicity, children will represent the greatest proportion of primary SARS-CoV-2 infections.[8] While SARS-CoV-2 incidence in children is comparable to that in adults, children have less severe presentations and are more likely to be asymptomatic.[17] Like with ccCoVs, SARS-CoV-2 can cause pediatric LRI. Pediatric SARS-CoV-2 infections are as or less likely to be associated with hospitalization and pneumonia as influenza virus.[18-20]

1.1.3 Repeat Coronavirus Infections

Respiratory viruses can infect someone repeatedly, with most adults having had many infections with the same type of virus in their lifetimes. Repeat ccCoV infections are frequent; in adults, repeat infection can occur as little as six months later.[21] Other work in adults and children showed that symptomatic reinfections occurred, on average, over 1.5 years apart.[22] In children, waning antibodies following ccCoV infection may explain frequent reinfection.[23] SARS-CoV-2 reinfections have been well documented, especially with emergence of variants capable of immune escape.[24-26]

1.2 Dissertation Aims

1.2.1 Aim 1- Determine the burden and seasonality of symptomatic endemic coronavirus infection in a pediatric cohort in Managua, Nicaragua

In Chapter 2, we will discuss Aim 1 of the dissertation, which focused on determining the burden and seasonality of ccCoV infection among a community-based, pediatric cohort in Managua, Nicaragua. Using data from 2011-2016, we examine the incidence of symptomatic ccCoV infections among children aged 0-14. We identify seasonal patterns for each of the four ccCoVs, model ccCoV incidence using an age-period-cohort approach, and evaluate the risk of specific symptoms between primary and secondary ccCoV infections.

1.2.2 Aim 2- Compare symptom presentation and severity of endemic coronavirus infections with SARS-CoV-2 infections in children in Managua Nicaragua

In Chapter 3, we compare symptoms and severity of ccCoV and SARS-CoV-2 infections in children. Although it was known that SARS-CoV-2 infection is less severe in children compared to adults,[17] no prior work had compared pediatric SARS-CoV-2 infections with pediatric ccCoV infections to evaluate whether SARS-CoV-2 resembles ccCoVs in children. Using the same community-based, pediatric cohort and identical testing criteria, we compare symptom presentation and the risk of LRI between ccCoV infections (2011-2016) and SARS-CoV-2 infections. We also compare symptom presentation by age and ccCoV species.

1.2.3 Aim 3- Investigate the effect of infection-induced immunity on SARS-CoV-2 transmission in a community-based household transmission study

Finally, we investigate the effect of infection-induced immunity on SARS-CoV-2 transmission in a community-based household transmission study, highlighting the differences between adults and children. Using data (2020-2022) from a community-based household cohort with an embedded transmission study, we use a statistical transmission model to investigate factors associated with household SARS-CoV-2 infectivity and susceptibility. As children and

adults have distinct immune responses to ccCoV infection,[27] we investigate, stratified by age, the association between infection-induced immunity and symptom presentation with infectivity; we also evaluate the association between infection-induced immunity and the risk of SARS-CoV-2 infection by age.

Chapter 2 Burden and Seasonality of Primary and Secondary Symptomatic Common Cold Coronavirus Infections in Nicaraguan Children

2.1 Preface

This chapter of my dissertation was published in *Influenza and Other Respiratory Viruses* in 2023 (DOI: 10.1111/irv.13086). In addition to myself, the authors include Angel Balmaseda, Nivea Vydiswaran, Mayuri Patel, Sergio Ojeda, Andrew Brouwer, Rebecca Tutino, Shuwei Cai, Kevin Bakker, Nery Sanchez, Roger Lopez, Guillermina Kuan, and Aubree Gordon.

2.2 Abstract

The current SARS-CoV-2 pandemic highlights the need for an increased understanding of coronavirus epidemiology. In a pediatric cohort in Nicaragua, we evaluate the seasonality and burden of common cold coronavirus (ccCoV) infection and evaluate likelihood of symptoms in reinfections.

Children presenting with symptoms of respiratory illness were tested for each of the four ccCoVs (NL63, 229E, OC43, HKU1). Annual blood samples collected before ccCoV infection were tested for antibodies against each ccCoV. Seasonality was evaluated using wavelet and GAM analyses, and age-period effects were investigated using a Poisson model. We also evaluate the risk of symptom presentation between primary and secondary infections

In our cohort of 2,576 children followed from 2011-2016, we observed 595 ccCoV infections and 107 cases of ccCoV-associated lower respiratory infection (LRI). The overall

incidence rate was 61.1 per 1,000 person-years (95% CI: 56.3, 66.2). Children under two had the highest incidence of ccCoV infections and associated LRI. ccCoV incidence rapidly decreases until about age six. Each ccCoV circulated throughout the year and demonstrated annual periodicity. Peaks of NL63 typically occurred three months before 229E peaks and six months after OC43 peaks. Approximately 69% of symptomatic ccCoV infections were secondary infections. There was slightly lower risk (RR: 0.90, 95% CI: 0.83, 0.97) of LRI between secondary and primary ccCoV infections among participants under the age of 5.

ccCoV spread annually among children with the greatest burden among ages 0-1. Reinfection is common; prior infection is associated with slight protection against LRI among the youngest children.

2.3 Introduction

The SARS-CoV-2 pandemic underscores the need for understanding human coronavirus epidemiology. The four common cold coronaviruses (ccCoVs), NL63, 229E, OC43, and HKU1, are generally associated with upper respiratory tract infections, [3,6,7,9] but have also been associated more severe lower respiratory tract infections. [4,15,16,28] Following the detection of NL63 and HKU1 in the 2000s, OC43 is the most frequently detected globally while 229E is the least and is primarily detected among individuals with severe infections or weakened immune systems. [4,5,9,10,12,13,15,29-32] ccCoVs are split into two, genetically similar groups, alpha (NL63 and 229E) and beta (OC43 and HKU1); [3] prior work has identified cross-reactive antibodies within and between groups.[27] However, it is unclear whether immunity to one of the ccCoV, whether within alpha and beta groups or across, protects against infection with another.

Younger children have higher rates of symptomatic and severe illness associated with ccCoV infection compared to older children and adults. [4,12,33] By age three most children have

had their first ccCoV infection and by age six, children typically have antibodies against each of the four ccCoV types. [11,23] ccCoV infections occur repeatedly throughout life, suggesting the lack of long-lasting sterilizing immunity produced by natural infection. [21] Declining antibody levels following primary ccCoV infection may explain frequent ccCoV reinfection in children.[23] The clinical significance of primary vs secondary ccCoV infections in children is not well understood.

Many large ccCoV studies lack a well-defined study population and rely on reporting from hospitals, healthcare systems, and passive surveillance networks; these studies detect and report on the epidemiology of more severe ccCoV infections. [4-6,10,15,28,30,31] Studies conducted in temperate locations report consistent annual seasonal peaks during winter months, similar to other common respiratory pathogens; ccCoV spread in other climates, however, does not appear to follow similar patterns and drivers of ccCoV seasonality remain unknown.[4-6,9,10,12,14,15]

Here we describe the incidence and seasonality of symptomatic ccCoV infections and evaluate risk of symptom presentation of between primary and secondary ccCoV infections in a community-based pediatric cohort in Managua, Nicaragua, from 2011-2016.

2.4 Methods

The Nicaraguan Pediatric Influenza Cohort (NPICS) is an ongoing prospective cohort study of children aged 0-14 years in Managua, Nicaragua which has a tropical, urban environment. Previous work has detailed descriptions of study protocols. [34] Briefly, children aged 0-12 were enrolled in 2011 and newborns are enrolled monthly. Parents agreed to bring enrolled children to the study health center, Health Center Sócrates Flores Vivas, at the first signs of a fever. Children age out of the cohort on their 15th birthday. This analysis uses data collected January 1, 2011-December 31, 2016.

Study personnel collected nasal and oropharyngeal swabs (oropharyngeal only if under 6 months) from participants if they met any one of the testing criteria: 1.) fever (temperature of 37.8 °C or greater) or feverishness and cough, sore throat and/or rhinorrhea; 2.) fever or feverishness and under two years old; 3.) severe respiratory symptoms (i.e., pneumonia, chest indrawing, wheezing, apnea, etc.) evaluated by a study physician; 4.) and hospitalization with respiratory symptoms or sepsis. Laboratory personnel at the University of Michigan tested samples using reverse transcriptase-polymerase chain reaction (PCR) for the four seasonal ccCoVs following the CDC protocol.[35] Respiratory symptoms are recorded from each clinic visit by study physicians as well as from symptom diaries by parents/guardians. Participants with diagnosed cases of pneumonia, bronchiolitis, bronchitis, or bronchial hyperreactivity were considered to have lower respiratory tract infections (LRI). [36]

Blood samples were collected annually from participants between February and April each year. To evaluate the frequency of secondary ccCoV infections, blood samples that were collected within one year before an ccCoV PCR+ infection were tested for IgG antibodies to the spike protein for each ccCoV via an enzyme-linked immunosorbent assay (ELISA) following previously developed protocols [37]. Results from blood samples may be paired with multiple PCR+ infections if a participant had multiple PCR+ infections within a year.

Person-time was calculated as the number days between the participants' enrollment and exit from the study. Exit dates were determined as participants' fifteenth birthday for NPICS, the day the participant withdrew from the study, or was lost to follow-up. In cases of loss-to-follow-up, the midpoint between the date of last contact with the participant and the start of the annual survey collection as the exit date was used. Participants did not contribute person time for 28 days following a PCR positive sample. Person-time was calculated for all ccCoV infections and

separately for each coronavirus. To identify significant seasonal patterns, wavelet analyses with pink noise and a log +1 transformation were used. To examine if the seasonality of one ccCoV impacted the seasonality of another, a cross-wavelet analysis was conducted. Using time-series data, wavelets can be used to identify periodic signals; cross-wavelet analysis allows us to evaluate the temporal relationship between two time-series [38-40] A generalized additive model was used to identify peak months for each group and type. To calculate incidence rates, a Poisson model was used. Crude rates and rates adjusted for period and for age were calculated; age was adjusted for using B-splines; age-period provided better model fit than age-cohort, or period-cohort. Crude and fitted incidence rates were displayed using hexamaps to visualize age-period-cohort (APC) trends.[41]

To evaluate differences in symptom presentation between primary and secondary infections, symptom presentation risk was compared among those with blood samples collected within a year before a ccCoV PCR+ infection. Secondary infections were defined as PCR+ infections following a previous PCR+ infection with the same ccCoV type or presence of type-specific IgG spike antibodies before infection. PCR+ infections in children under the age of 1 without a collected blood before infection were considered primary infections. Risk ratios were calculated from a generalized estimating equation log-binomial model adjusted for age linearly; this model was restricted to participants under the age of 5.

We used R version 4.1.1 to create figures as well as conduct the wavelet, GAM, and incidence analyses. All other analyses occurred in SAS version 9.4 (SAS Institute Inc.).

2.5 Results

From 2011-2016 there were 2,576 NPICS participants who contributed 7,309 person-years. On average about 3% of participants withdrew from the study or were lost to follow-up

per year (range 2-6%) and over the 6 years there were six deaths (Figure A.1). Approximately 50% of participants were female. There were between 1,436 and 1,776 active participants each month. (Figure A.2).

Study personnel collected 9,018 respiratory samples of which 8,803 (97.6%), had sufficient sample remaining to test all four ccCoVs. 610 (6.8%) were positive for ccCoVs. We detected 595 distinct coronavirus infections and 28 ccCoV coinfections (sample positive for two or more ccCoVs) among 476 participants. OC43 was the most common ccCoV detected (n=323; 52.9%) followed by NL63 (n=163; 26.7%), 229E (n=86; 14.1%), and HKU1 (n=69; 11.3%) (Table 1). There were 107 cases of ccCoV-associated LRI and 23 hospitalizations.

There was no clear season to ccCoV circulation, with cases presenting in every month of the study period. (Figure 2.1). NL63, 229E, and OC43 circulated annually throughout the study period. NL63 generally peaked in the last six months of the year, but there was no identified general peak month for the other ccCoVs (Figure 2.2 & Figure A.3). Cross-wavelet analysis indicated that 229E generally peaks occur three months before NL63; we also found that NL63 and OC43 peaks occurred approximately 6 months apart from 2011-2013 but shifted to three-months apart from 2014-2015 (Figure A.4).

Overall incidence of symptomatic ccCoV infection was 61.1 per 1,000 person-years (95% CI: 56.3, 66.2). Incidence was highest among the youngest participants and sharply decreased with increasing age. The incidence rate among those aged 4 (63.9, 95% CI: 47.7, 85.5) was less than a third of the rate among those less than one year old (217.4, 95% CI: 183.6, 257.6) and the rate among those aged 8 (22.0.3, 95% CI: 13.3, 36.5) is about a third of the rate among those aged 4. This age pattern is similar for each ccCoV type (Figure 2.3). Incidence rates between males and females were similar (Table A.1). ccCoV-associated lower respiratory

infection (LRI) incidence was 8.9 per 1,000 person-years (95% CI: 7.2, 11.0). LRI incidence was also highest among the youngest participants, with all ccCoV-associated LRI incidence following a similar age pattern as symptomatic infection incidence (Figure 2.4). There was also no difference in ccCoV-associated LRI incidence by sex (Table A.1).

Although it has been shown that people are repeatedly infected with ccCoVs, we hypothesized that the breadth of immunity would increase as children accumulate exposures to the same type, resulting in a decrease in the incidence of cases. APC analysis suggests that incidence declines sharply until around age six when incidence rates decline more slowly (Figure 2.5 & Figure A.5). At age six, ccCoV incidence is less than 30% of infant ccCoV incidence. Symptomatic infections are relatively uncommon in individuals older than 10 years old. Periods that had higher incidence for a specific ccCoV (ex. NL63 in 2013, HKU1 in 2015) had higher incidence among all participants, including older children (Figure A.6). Compared to others, cohorts with lower incidence at under one year of age old (ex. NL63 and 2011 cohort, OC43 and 2013 cohort) had generally higher incidence at age one.

To determine whether an infection was a primary or secondary ccCoV infection we tested 406 blood samples that were collected within one year before 434/595 (72.9%) ccCoV PCR+ infections. 108/161 (67%) of PCR+ infections without a blood sample collected within one year were among participants less than one year old. Overall, most children experiencing an infection had had at least one prior ccCoV infection. OC43 antibodies were detected most frequently (n=316, 77.8%) then NL63 (n=286, 70.4%), HKU1 (n=278, 68.5%) and 229E (n=257, 63.3%). 300 infections (69.1%) occurred in participants who had preexisting antibodies against the infecting ccCoV type, ranging from 73.5% of OC43 infected participants to 63.1% of 229E acute

infections (Table 2.2). Seropositivity was over 50% for each ccCoV for those aged 2 and older (Figure A.7).

By age 5, 96.7% of symptomatic infections were type-specific secondary infections (range 95.0% to 100.0% by type). Indeed, for the most common types, OC43 and NL63, 54.6% and 67.7%, of symptomatic infections are secondary infections by age 2; for 229E and HKU1 only 14.3% and 36.4% are secondary infections by age 2. We then examined the severity of primary versus secondary ccCoV infections, adjusted for age. Because almost all ccCoV symptomatic infections were secondary infections by age 5, we limited this analysis to participants under the age of 5. The risk of ccCoV-associated LRI was lower among secondary infections compared to primary infections (RR: 0.90, 95% CI: 0.83, 0.97). We found that the risk of cough (RR: 1.12, 95% CI: 1.04, 1.20) and rhinorrhea (RR: 1.13, 95% CI: 1.06, 1.2) were slightly higher among those with secondary infections compared to primary infections. We found no difference in risk of measured fever, congestion, or hospitalization by serostatus prior to infection (Table 2.3).

2.6 Discussion

This study investigates the burden and seasonality of symptomatic ccCoV infections in a community-based pediatric cohort. This study is the longest running pediatric cohort in Central or South America that has evaluated the burden of ccCoV infections. Like other studies in non-temperate locations, we observed ccCoV infections throughout the year, [14,42-45] with alternating spread of different ccCoV types. While there was no distinct ccCoV season in Managua, Nicaragua, each ccCoV type exhibited annual periodicity. We found that the two alpha coronaviruses, 229E and NL63, peaks generally do not occur at the same time. Other studies in temperate locations found that while NL63 and 229E did spread at the same time, years with a

high prevalence of 229E coincided with low levels of NL63. [5,46] These results may indicate the presence of short-term, sub-group specific cross-reactive immunity.[47-53]

Consistent with other respiratory infections and previous research, younger children had a higher incidence of symptomatic ccCoV infections and ccCoV-associated LRI than older children, especially within the first two years of life. [4-6,10,12,29,54] We note a clear pattern of rapidly decreasing incidence of symptomatic infection until about age six at which point nearly all infections are secondary. Additionally, ccCoV reinfection is very common among children; [23] we found that by age five, almost all symptomatic infections were secondary, not primary infections. Among those under five years of age, there was slightly lower risk of ccCoV-associated LRI for secondary infections compared to primary infections after adjusting for age. Similarly, frequent reinfection with SARS-CoV-2 has also been observed among children. [26] In a household transmission study, infection-induced immunity was not associated with protection against SARS-CoV-2 infection for children. [55] These findings suggest that while protection against ccCoV-associated LRI develops following a primary infection, protection against symptomatic infections wanes quicker early in life,[23] but may build, lasting longer, over several exposures.

We also observed some years that had high ccCoV type-specific incidence rates across all ages. We expect that ccCoV type-specific genetic diversity, frequently detected among children [56], may explain these high incidence years. Additionally, birth cohorts that experienced lower rates of symptomatic infections for a particular ccCoV type as infants, had higher rates of symptomatic illness at age one compared to other cohorts; this was likely a result of both annual ccCoV spread and an absence of type-specific immunity acquired before the one year of age.

The main strength of this study is the size and duration of the prospective cohort. With over 9,000 respiratory samples collected and over 7,000 person years, we observed almost 600 ccCoV infections, exclusively among children. The six years of data provides sufficient power to evaluate seasonality statistically, identify annual periodicity, as well as evaluate the frequency of repeat ccCoV infections. The consistent cohort age structure and limited loss-to-follow-up allowed us to identify age-period-cohort trends of symptomatic ccCoV illness.

We do note some limitations in this study. Respiratory swabs were only collected when a participant presented at the clinic with symptomatic illness, thus likely missing some mild cases and underestimating the true incidence of both ccCoV infections and the frequency of reinfections in the population. However, testing participants' blood samples for ccCoV antibodies, did reveal that the majority of symptomatic ccCoV infections were reinfections. Additionally, we did not examine genetic variation in ccCoVs which may help to explain seasonal variation as well as the frequency of reinfections.

Although ccCoV infections occur repeatedly throughout childhood, our understanding of coronavirus epidemiology in early life is limited. We show that ccCoV infections spread continuously throughout the year in a pediatric population in Nicaragua, with frequent reinfections; however, history of prior infection did convey protection against ccCoV-associated LRI among those under five. Future research should focus on the early-life development of coronavirus immunity to, the contributions of viral evolution and immunity to coronavirus reinfections and immune correlates of protection against coronaviruses.

Table 2.1: Study Participants and Samples Collected by Year

		2011	2012	2013	2014	2015	2016	Total
Participants		1579	1653	1790	1950	1895	1874	2576
Female (%)		795 (50.3)	830 (50.2)	903 (50.5)	973 (49.9)	946 (49.9)	944 (50.3)	1307 (50.7)
Person-years		1480	1531	1599	1684	1676	1717	9687
Age	0	93	63	117	123	112	109	616
	1	127	109	57	109	119	122	642
	2-4	330	319	349	325	353	361	2037
	5-9	597	576	525	551	559	597	3404
	10-14	345	464	551	577	533	528	2998
Respiratory Samples		1423	1448	1583	1650	1566	1348	9018
CcCoV PCR+ (%)		94 (6.6)	111 (7.7)	113 (7.1)	100 (6.1)	109 (7.0)	83 (6.2)	610 (6.8)
NL63 (%*)		15 (16.0)	29 (26.1)	43 (38.5)	34 (34.0)	24 (22.0)	18 (21.7)	163 (26.7)
229E (%*)		19 (20.2)	13 (17.4)	13 (11.5)	7 (7.0)	9 (8.3)	15 (18.1)	86 (14.1)
OC43 (%*)		54 (57.4)	74 (66.7)	37 (32.7)	58 (58.0)	54 (49.5)	46 (55.4)	323 (52.9)
HKU1 (%*)		7 (4.3)	1 (0.9)	22 (19.5)	6 (6.0)	26 (23.9)	7 (8.4)	69 (11.3)

* Does not sum to 100 because of codetections

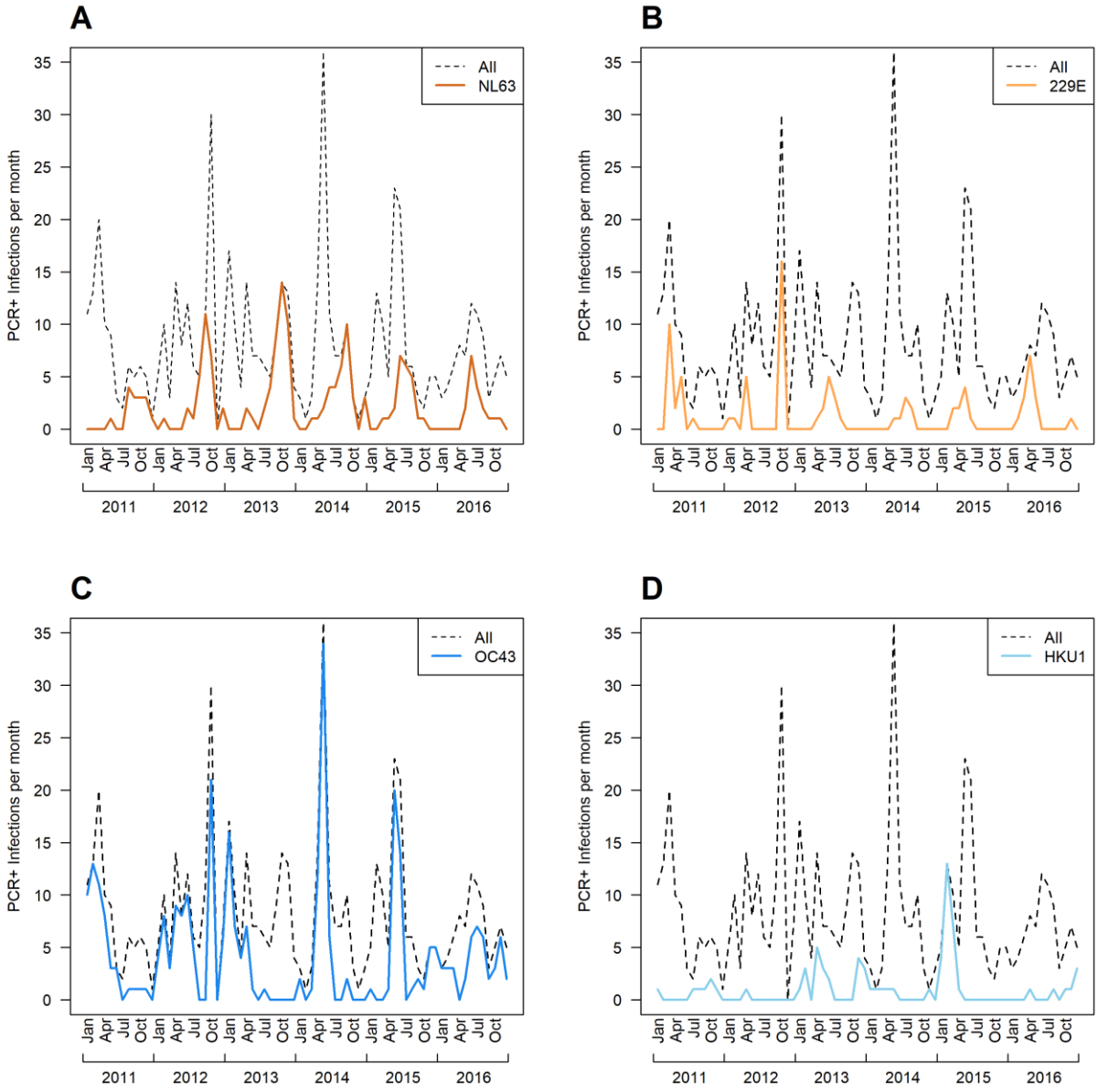

Figure 2.1: Monthly ccCoV PCR+ Infections by Type, 2011-2016

Symptomatic ccCoV infections over time. Dashed line represents monthly sum of all ccCoV PCR+ infections during the study period

A: NL63 PCR+ infections, B: 229E PCR+ infections, C: OC43 PCR+ infections, D: HKU1 PCR+ infections

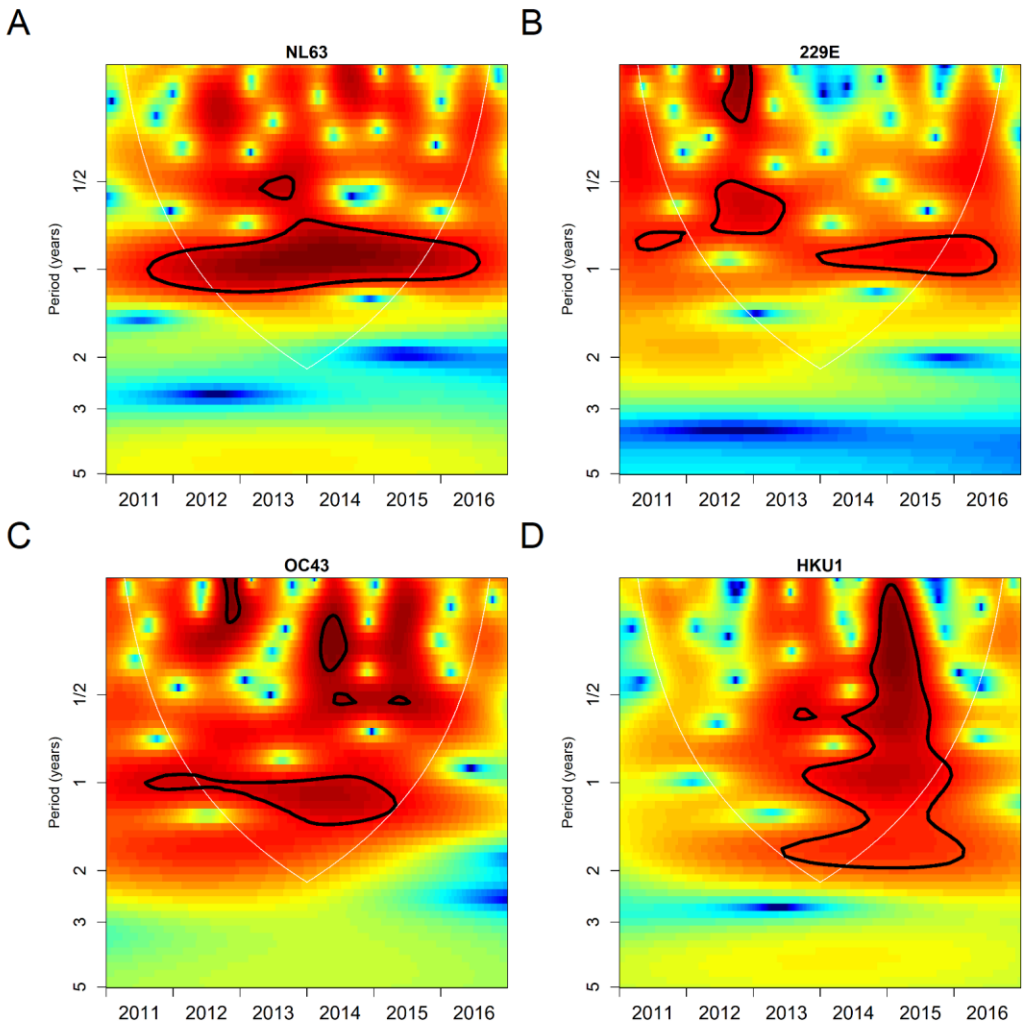

Figure 2.2: Wavelet Analysis by ccCoV Type

Wavelet analysis conducted separately by ccCoV type to identify type-specific periodicity. Red represents dominant periods and the area circled in black lines represent significant periodicity. Only the area within the light grey semi-circle (the wavelet cone of influence) can be interpreted.

A: NL63 wavelet analysis, B: 229E wavelet analysis, C: OC43 wavelet analysis, D: HKU1 wavelet analysis

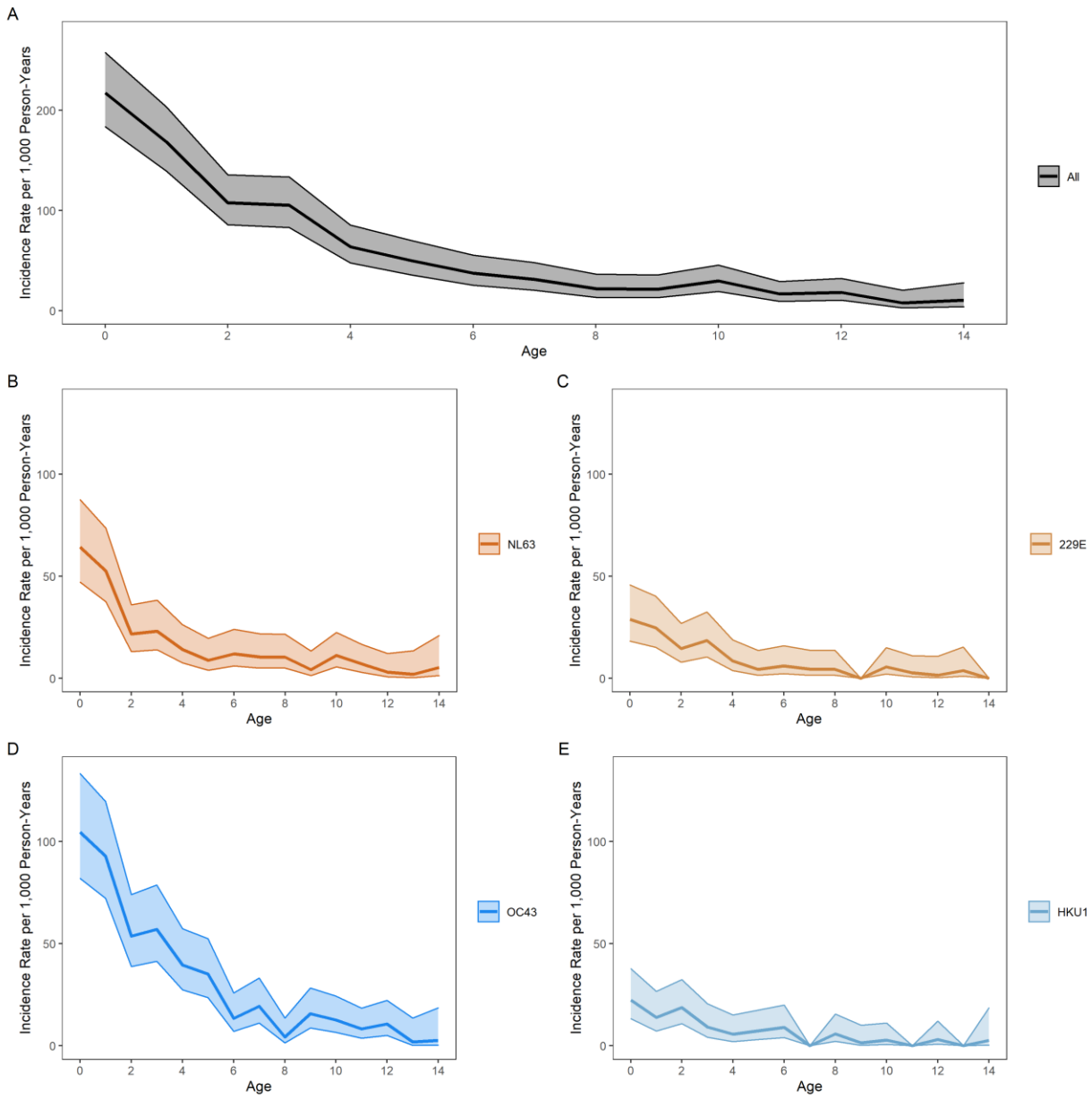

Figure 2.3: ccCoV Incidence Rates by Age, Type

Incidence rates (per 1,000 person-years) of PCR+ ccCoV infections for all ccCoV infections and by type using one year age groups. Shaded area represents 95% confidence intervals.

A: All ccCoV, B: NL63, C: 229E, D: OC43, E: HKU1

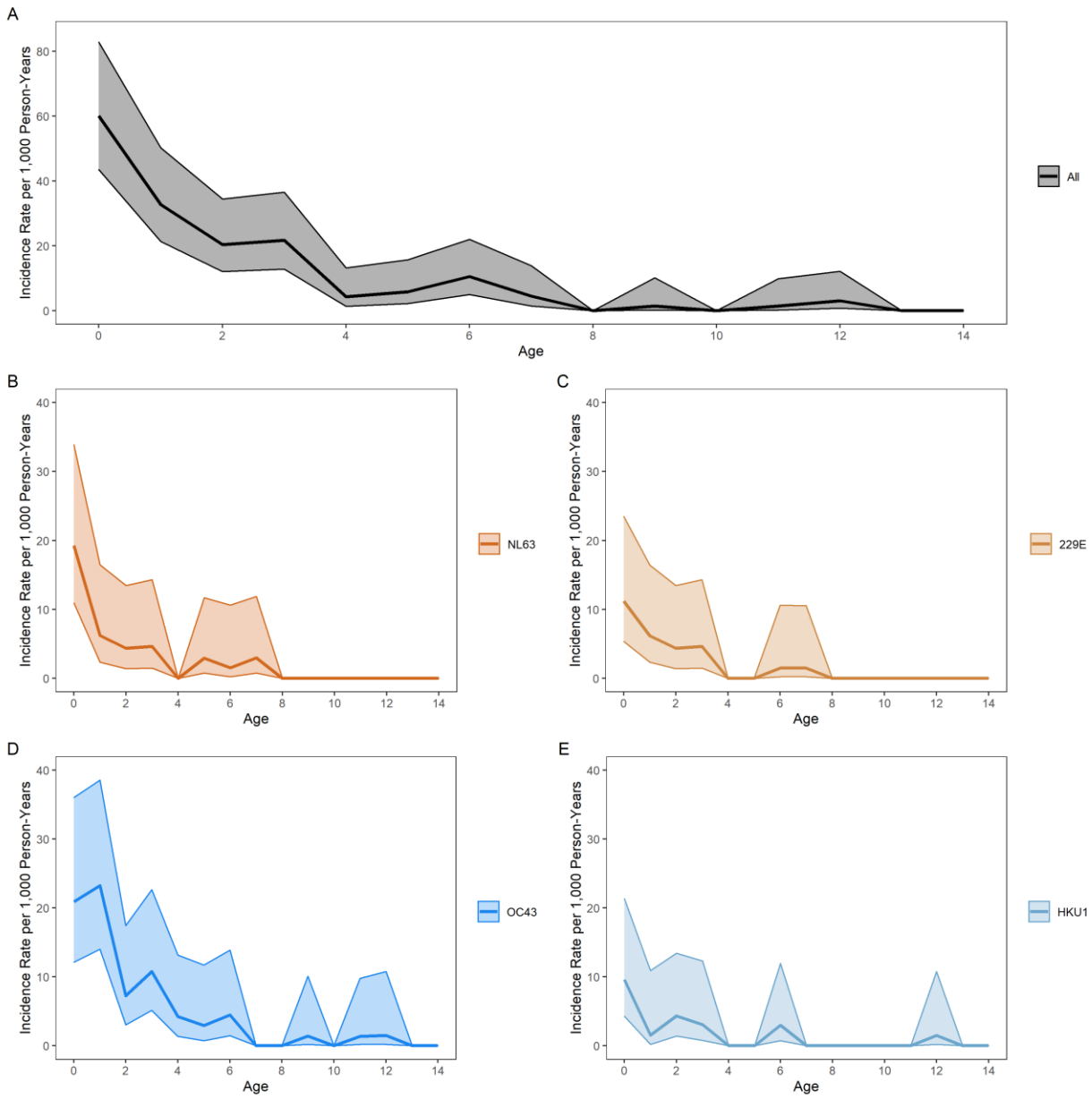

Figure 2.4: ccCoV-Associated Lower Respiratory Infection Incidence Rates by Age, Type

Incidence rates (per 1,000 person-years) of PCR+ ccCoV-associated lower respiratory infections (LRI) for all ccCoV infections and by type using one year age groups. Shaded area represents 95% confidence intervals.

A: All ccCoV-associated LRI, B: NL63-associated LRI, C: 229E-associated LRI, D: OC43-associated LRI, E: HKU1-associated LRI

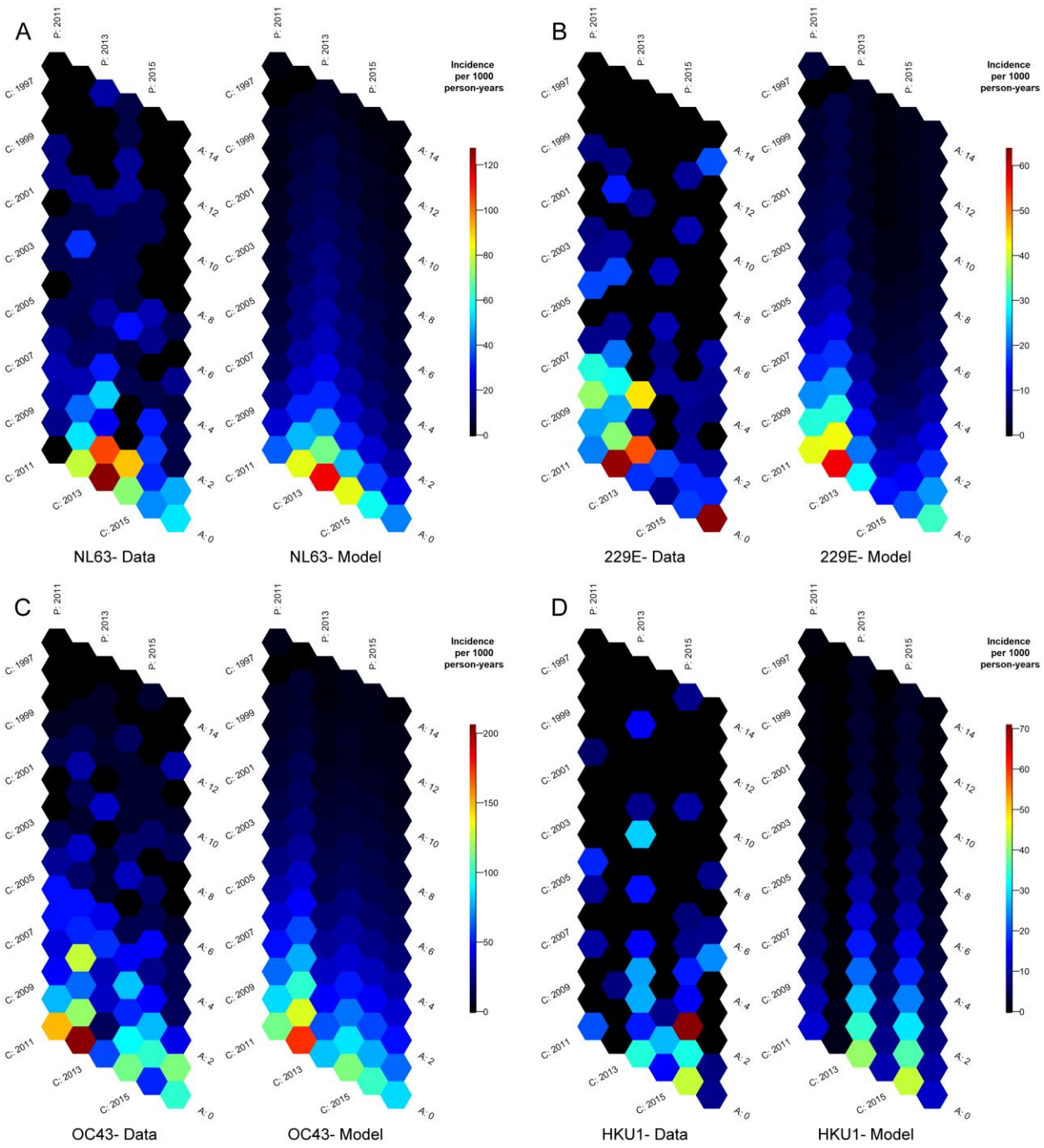

Figure 2.5: ccCoV Incidence Rate Hexamaps

Visualizing ccCoV incidence rates (per 1,000 person-years) by type as a function of age, calendar year, and birth year. Hexamaps with incidence rates from raw data are presented on the left within each ccCoV type's panel. Predicted incidence rates from age-period model are presented on the right within each ccCoV type's panel.

A: NL63, B: 229E, C: OC43, D: HKU1

Table 2.2: ccCoV Antibodies before PCR+ Infection by Year

	2011	2012	2013	2014	2015	2016	Total
Blood samples before ccCoV PCR+	43	84	75	71	78	55	406
NL63 antibodies (%)	29 (67.4)	57 (67.9)	57 (76.0)	55 (77.5)	54 (69.2)	34 (61.8)	286 (70.4)
229E antibodies (%)	28 (65.1)	58 (69.1)	53 (70.7)	43 (60.6)	42 (53.9)	33 (66.0)	257 (63.3)
OC43 antibodies (%)	34 (79.1)	69 (82.1)	67 (89.3)	48 (67.6)	55 (70.5)	43 (78.2)	316 (77.8)
HKU1 antibodies (%)	32 (74.4)	68 (81.0)	57 (76.0)	41 (57.8)	52 (66.7)	28 (50.9)	278 (68.5)
PCR+ Infections	44	92	79	79	81	59	434
Antibodies against ccCoV type (%)	30 (68.2)	70 (76.1)	61 (77.2)	49 (62.0)	54 (66.7)	36 (61.0)	300 (69.1)
NL63	13	21	27	25	18	13	117
NL63 antibodies (%)	10 (76.9)	14 (66.7)	17 (63.0)	17 (68.0)	15 (83.3)	6 (46.2)	79 (67.5)
229E	13	19	10	6	7	10	65
229E antibodies (%)	8 (61.5)	14 (73.7)	7 (70.0)	4 (66.7)	2 (28.6)	6 (60.0)	41 (63.1)
OC43	13	62	25	48	46	32	226
OC43 antibodies (%)	7 (53.9)	51 (82.3)	24 (96.0)	30 (62.5)	32 (69.6)	22 (68.8)	166 (73.5)
HKU1	5	1	18	5	14	7	50
HKU1 antibodies (%)	5 (100)	1 (100)	14 (77.8)	1 (20.0)	7 (50.0)	5 (71.4)	33 (66.0)

Table 2.3: Risk of Symptom Presentation, Secondary vs Primary ccCoV Type Infections

Symptom	Risk Ratio (95% CI)
Measured Fever	0.91 (0.70, 1.18)
Cough	1.12 (1.04, 1.20)
Rhinorrhea	1.13 (1.06, 1.21)
Congestion	1.12 (0.85, 1.47)
Hospitalization	0.92 (0.18, 4.64)
Lower respiratory infection	0.90 (0.83, 0.97)

Chapter 3 SARS-CoV-2 and Endemic Coronaviruses: Comparing Symptom Presentation and Severity of Symptomatic Illness among Nicaraguan Children

3.1 Preface

This chapter of my dissertation was published in *PLOS Global Public Health* in 2022 (DOI: 10.1371/journal.pgph.0000414). In addition to myself, the authors include John Kubale, Guillermina Kuan, Sergio Ojeda, Nivea Vydiswaran, Nery Sanchez, Miguel Plazaola, Mayuri Patel, Roger Lopez, Angel Balmaseda, and Aubree Gordon. This research was published before the recommendation to refer to endemic HCoVs as ccCoVs;[7] thus the phrase endemic HCoV, not ccCoV, is used throughout.

3.2 Abstract

It has been proposed that as SARS-CoV-2 transitions to endemicity, children will represent the greatest proportion of SARS-Co-V-2 infections as they currently do with endemic coronavirus infections. While SARS-CoV-2 infection severity is low for children, it is unclear if SARS-CoV-2 infections are distinct in symptom presentation, duration, and severity from endemic coronavirus infections in children. We compared symptom risk and duration of endemic human coronavirus (HCoV) infections from 2011-2016 with SARS-CoV-2 infections from March 2020-September 2021 in a Nicaraguan pediatric cohort. Blood samples were collected from study participants annually in February-April. Respiratory samples were collected from participants that met testing criteria. Blood samples collected in were tested for SARS-CoV-2

antibodies and a subset of 2011-2016 blood samples from four-year-old children were tested for endemic HCoV antibodies. Respiratory samples were tested for each of the endemic HCoVs from 2011-2016 and for SARS-CoV-2 from 2020-2021 via rt-PCR. By April 2021, 854 (49%) cohort participants were ELISA positive for SARS-CoV-2 antibodies. Most participants had antibodies against one alpha and one beta coronavirus by age four. We observed 595 symptomatic endemic HCoV infections from 2011-2016 and 121 symptomatic with SARS-CoV-2 infections from March 2020-September 2021. Symptom presentation of SARS-CoV-2 infection and endemic coronavirus infections were very similar, and SARS-CoV-2 symptomatic infections were as or less severe on average than endemic HCoV infections. This suggests that, for children, SARS-CoV-2 may be just another endemic coronavirus. However, questions about the impact of variants and the long-term effects of SARS-CoV-2 remain.

3.3 Introduction

As severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transitions to global endemicity, there are many questions about how that will occur. Over time, children will represent the greatest proportion of primary SARS-CoV-2 infections as adults gain immunity from natural infection or vaccination. [8] It is well established that pediatric risk of severe illness and death is much lower than that for adults. [57,58] Differences in immune response between adults and children likely provide children better protection against severe SARS-CoV-2 infections. [27,58,59] Previous research found no differences in severity between SARS-CoV-2 infections and influenza A and B among children. [19,60] However, it is unknown if, in children, SARS-CoV-2 infections are distinct in disease presentation and severity from endemic human coronavirus (HCoV) infections.

As of January 2022, only the Pfizer vaccine (for those age 5 or older) and the Moderna vaccine (for those 12 or older) are recommended by the World Health Organization's Strategic Advisory Group of Experts (SAGE) for use in children and adolescents; multiple other vaccines, including the Cuban Soberana 02 and Abdala, have been approved for use in children by individual countries, including in Nicaragua.[61-63] If SARS-COV-2 infections are more severe, routine pediatric vaccination will be necessary to reduce excess mortality and morbidity. If not, vaccine-induced immunity should prevent severe disease while allowing for transmission to facilitate frequent immune boosting. [8] To determine if SARS-CoV-2 infections have distinct disease presentation from endemic HCoV infections in children, we compare symptomatic SARS-CoV-2 and endemic HCoV infection symptomology and severity in a prospective, community-based pediatric cohort in Managua, Nicaragua from 2011-2016 and 2020-2021

3.4 Methods

Institutional review boards at the Nicaraguan Ministry of Health and the University of Michigan approved this study. Parents/guardians of participants provided written informed consent and participants aged ≥6 years provided verbal assent. The Nicaraguan Pediatric Influenza Cohort Study (NPICS) is a prospective cohort study that began in 2011 and continues today. Children ages 0-14 years who live in District 2 of Managua, Nicaragua and within the catchment area of Health Center Sócrates Flores Vivas were eligible to participate. Participants live in a tropical, urban environment and are representative of children in the larger Managua area. Participants included in this analysis were members of NPICS between 2011-2016 or March 2020 to September 2021. School-aged children attended school throughout the study period.

Study staff collected blood samples from each study participant between February and April each year. To confirm our assumption that endemic HCoV infection rates are high in the cohort, we tested a random subset of 100 blood samples from four-year-old's from 2011-2016; using protocols for an enzyme-linked immunosorbent assay (ELISA) developed at Mount Sinai [64], we tested samples for IgG antibody response to the spike protein for each of the four endemic HCoVs (alpha: NL63, and 229E, beta: OC43 and HKU1). To confirm that SARS-CoV-2 infections rates were also high, we tested the 2020 and 2021 blood samples in pairs for SARS-CoV-2 IgG antibodies as described previously. If a 2020 annual sample was positive for SARS-CoV-2, the child's 2019 annual sample was also run. Children that were positive in 2019 were not considered SARS-CoV-2 positive. [65]

Parents/guardians agreed to bring participants to the health center at the first signs of fever. Study personnel collected a respiratory swab from participants if they met the testing criteria: feverishness for participants under two years old; measured fever/feverishness and cough, sore throat, or rhinorrhea; severe respiratory symptoms such as apnea or chest indrawing; hospitalization with respiratory symptoms or sepsis. NPICS testing criteria expanded in June 2020 to capture mild symptomatic SARS-CoV-2 cases; however, this analysis is limited to those meeting the original testing criteria to ensure comparability. We used real-time reverse transcription polymerase-chain reaction (RT-PCR) to test respiratory samples from 2011-2016 for each of the four endemic HCoV, influenza A and B, respiratory syncytial virus (RSV, subtypes A and B), and human metapneumovirus (HMpV) and samples from March 2020-September 2021 for SARS-CoV-2 and influenza. [35,66]

Study clinicians record all participant symptoms, prescriptions, and diagnoses during each clinic visit and subsequent medical appointments using standardized forms.

Parents/guardians report participant symptoms for each day since illness onset to study clinicians at the initial and subsequent clinic visits until symptom resolution. We considered symptoms to be associated with an infection if they occurred within 28 days of symptom onset. Study parents/guardians reported on the following symptoms 2011-2016 and 2020-2021: feverishness, cough, rhinorrhea, nasal congestion, loss of appetite, myalgia, arthralgia, and rapid breathing. We considered participants who presented to the clinic with rapid breathing, rhonchi, indrawing, wheezing, or shortness of breath as having abnormal breathing. We defined acute lower respiratory infections (ALRI) as physician diagnosed cases of pneumonia, bronchiolitis, bronchitis, or bronchial hyperreactivity or elevated respiratory rate based on age: ≥ 60 breaths/minute for < 2 months, ≥ 50 breaths/minute for 2-11 months, ≥ 40 breaths/minute for 12-59 months, ≥ 25 breaths/minute for ≥ 60 months [67]. We also evaluated whether participants with ALRI were prescribed antibiotics (amoxicillin, penicillin, other) within 28 days of infection. Data collection forms for the above signs and symptoms were consistent between 2011-2016 and 2020-2021.

To compare risk of symptoms, we calculated symptom specific risk differences between SARS-CoV-2 infections and endemic HCoV infections, overall and stratified by the following age groups: 0-4, 5-9, and 10-14 years. We also stratified the results by sex and endemic HCoV species. Using upset plots, we explored which signs/symptoms tended to present together. We also assessed symptom duration by comparing the time between each specific symptom onset and the last day participants presented with that symptom. We plotted symptom duration using boxplots and compared the distribution of symptom duration between SARS-CoV-2 and endemic HCoVs using the Mann Whitney U test.

We used SAS version 9.4 (SAS Institute Inc.) to calculate risk differences and ratios and R version 4.1.0 to create figures and conduct all other analyses.

3.5 Results

There were 3,220 participants active in NPICS during the included years: 2,576 from 2011-2016 and 1,942 from March 2020-September 2021. On average, there were 1,792 active participants per year. Our assumption was that detected symptomatic infections consist of only a small proportion of total SARS-CoV-2 and endemic HCoV infections that occurred in the cohort children. Specifically, of the 1,942 children in the cohort from March 2020-September 2021, 1,455 in 2020 and 1,743 in 2021 had a blood sample collected that was tested via ELISA for SARS-CoV-2 antibodies. Only 23 (1.6%) tested positive for SARS-CoV-2 antibodies in 2020 while 854 (49%) tested positive in 2021 indicating that SARS-CoV-2 infections were very high in the cohort. We found that 94% of our randomly selected subset of four-year-olds from 2011-2016 had an antibody response to at least one alpha and one beta HCoV, confirming our assumption of high endemic HCoV infection rates in the community. Antibody response prevalence was highest for OC43 (99%) followed by HKU1 (86%), NL63 (83%), and then 229E (74%) (Table 3.1).

Within this cohort we observed high infection rates of both endemic HCoVs and SARS-CoV-2. That there were 595 RT-PCR+ symptomatic endemic HCoV infections from 2011-2016 and 121 RT-PCR+ symptomatic SARS-CoV-2 infections from March 2020- September 2021 again suggests that symptomatic cases represent only a small proportion of overall infections and likely, the more severe infections. Most endemic HCoV, 432 (73%), and SARS-CoV-2, 59 (49%), infections occurred in participants <5 years. Fever, cough, rhinorrhea, and congestion

were the most common symptoms for both endemic HCoVs and SARS-CoV-2 symptomatic infections. Cough, rhinorrhea, and abnormal breathing was more common among endemic HCoV infections while measured fever and headache was more common among SARS-CoV-2 infections. Among SARS-CoV-2 symptomatic infections, 4 (3%) were acute lower respiratory infections compared to 98 (16%) endemic HCoV cases (p=<0.0001; Table 3.2). Among those with ALRI, there was not a difference in antibiotic prescription between SARS-CoV-2 and endemic HCoV infections.

Because the age distribution varied between endemic HCoVs and SARS-CoV-2, with a greater proportion of infections occurring in older children with SARS-CoV-2, we examined signs and symptoms by age group. Across age groups, participants with symptomatic endemic HCoV infections displayed greater risk of cough compared to those with symptomatic SARS-CoV-2 infections. Among participants aged <5 years, symptomatic endemic HCoV infections showed greater risk of rhinorrhea, congestion, abnormal breathing, and ALRI than symptomatic SARS-CoV-2 infections, while SARS-CoV-2 exhibited greater risk of measured fever (Fig 3.1 and Table 3.3). Notably, among those under 5, symptomatic endemic HCoV infection was associated with greater risk of ALRI even after excluding participants that also tested positive for influenza A, influenza B, RSV, or HMpV. We observed no difference in risk hospitalization between SARS-CoV-2 and endemic HCoV symptomatic infections in each age group. These results were consistent after stratifying by sex (Figs B1 and B2), or endemic HCoV species (Figs B3-B6).

For both endemic HCoVs and SARS-CoV-2, we found that cough, rhinorrhea, and sore throat frequently presented together. Loss of appetite appeared in common symptom groupings for endemic HCoVs, while headache was part of more common groupings for SARS-CoV-2 (Fig

3.2). We did find that among participants aged 0-4 years loss of appetite lasted longer and among participants aged 5-9 and 10-14, cough lasted longer for SARS-CoV-2 infections (Fig 3.3 and Table 3.4).

To assess the potential impact of variants on symptoms, we compared symptoms for SARS-CoV-2 infections from 2020 prior to the global emergence of variants and 2021 when delta, gamma, and lambda strains circulated in the cohort area and found no difference in presentation by year. Feverishness, rhinorrhea, cough, headache, and sore throat where the most common symptoms for SARS-CoV-2 cases in both 2020 and 2021. (Fig 3.4). All observed cases of ALRI associated with SARS-CoV-2 occurred in 2020. However, we did find that rhinorrhea lasted longer in SARS-CoV-2 cases from 2021 compared to 2020 (Table 3.5).

3.6 Discussion

This is, to our knowledge, the first study that assesses differences in symptom presentation, duration, and severity between SARS-CoV-2 and endemic HCoV symptomatic infections among children Understanding how SARS-CoV-2 infections compare to endemic HCoV infections is important as SARS-CoV-2 becomes endemic. Other studies have evaluated symptom presentation for endemic HCoV and SARS-CoV-2 infections in children separately.[4,6,29,57,68,69] This work, however, compares medically attended illnesses associated with endemic HCoV and SARS-CoV-2 infections in a large, prospective cohort of children with high infection rates.

In this pediatric cohort, we found that with 854 (49%) participants tested positive for SARS-CoV-2 antibodies in 2021 with only 121 PCR confirmed infections that met the original testing criteria. These results are consistent with results from our community-based household cohort study in the same setting. [65] We also found that most participants had at least one alpha

and one beta endemic HCoV infection by age four suggesting that in this cohort, participants have had at least two endemic HCoV exposures by the age of four. This is similar a previous study that showed that by age six, most children have had infections with each of the four endemic HCoVs with a majority being asymptomatic infections. [11] Thus for SARS-CoV-2 and endemic HCoV, symptomatic infections also represent only a small proportion of all pediatric infections.

Comparing these symptomatic infections, we found that pediatric disease presentation is very similar between endemic HCoVs and SARS-CoV-2, with each frequently presenting with "common cold" symptoms. Consistent with other studies, we found differences in symptom presentation by age; this may be, perhaps, because older children have had more endemic HCoV exposures [3,4,29,57,59]. We also found great variability in symptom duration for SARS-CoV-2 and endemic HCoV infections; symptoms from endemic HCoV and SARS-CoV-2 infections lasted anywhere from 1 day to more than 28. [69] There was a difference in duration of loss of appetite for the youngest participants and cough for those aged 5-14 years suggesting that some symptoms may last longer for SARS-CoV-2 infections. We also found a difference in rhinorrhea duration between SARS-CoV-2 infections in 2020 and 2021, suggesting that SARS-CoV-2 variants may increase symptom duration among children.

Across age groups, risk of ALRI associated with SARS-CoV-2 was the same or lower compared to ALRI associated with endemic HCoV infection. Even when excluding endemic HCoV co-infections with pathogens commonly associated with increased risk of ALRI, the conclusions did not change. [4,68] These results show that, for children, the risk of ALRI and severe illness from SARS-CoV-2 infections is comparable to the risk from endemic HCoV infections at the community level.

The main strength of this community-based study is its size and duration. Consistent viral surveillance and symptom evaluation within the same population allow for year-to-year comparisons and facilitates our comparisons of endemic HCoVs and SARS-CoV-2. The high number of participants under the age of five (about 36% of participants during these years), allows us to evaluate SARS-CoV-2 in an age group with little representation in current literature. Additionally, this cohort was already well established when SARS-CoV-2 began circulating in Nicaragua allowing us to quickly incorporate questions regarding its effects on this population.

However, this study does have some limitations. First, using data from this community-based cohort study we were not powered to detect the most severe manifestations of SARS-CoV-2 including death or Multisystem Inflammatory Syndrome in Children (MIS-C) and other rare outcomes. [70,71] Second, our analysis did not include genetic sequencing preventing us from assessing the importance of variants in presentation and severity of SARS-CoV-2 illness. We did compare SARS-CoV-2 symptom presentation, severity, and duration by year and found little or no difference. Additionally, due to the low levels of circulation at the time, SARS-CoV-2 infections were only evaluated for influenza co-infections. We expect that RSV, and HMpV coinfections would also be associated with increased risk of ALRI for SARS-CoV-2; excluding such SARS-CoV-2 coinfections from the ALRI risk comparison would not change our findings. Finally, while our study does not evaluate very mild or asymptomatic illness for endemic HCoVs, our results were consistent with other research, showing that childhood HCoVs are ubiquitous and that symptomatic cases represent only a small proportion of infections, as with SARS-CoV-2. [11]

In this study, we observed that symptomatic SARS-CoV-2 infections at the community level are very similar to symptomatic endemic HCoV infections in symptom presentation.

Among children in a tropical, urban setting with a high SARS-CoV-2 infection rate, symptomatic SARS-CoV-2 infections are on average as or less severe as endemic HCoV infections. These findings support the hypothesis that SARS-CoV-2 may be like another endemic HCoV for children—most children will be asymptomatic with rare cases of severe symptomatic illness. This does not mean SARS-CoV-2 in children is not important. There are many unknowns about the long-term effects and impact of repeat infections among children. [8] Increased transmissibility of emerging variants is also a cause for concern, as it will lead to increased frequency of severe manifestations. Future mutations in the virus needed to be monitored as they may also increase illness severity in children. Despite relatively low risk of severe illness among children, pediatric vaccination that mirrors natural induced immunity against SARS-CoV-2 would further lower individual risk and reduce the number of severe cases and deaths due to SARS-CoV-2.

Table 3.1: Endemic HCoVs ELISA Results, % Positive

	Total (n=100)
Alpha	94%
NL63	83%
229E	74%
Beta	100%
OC43	99%
HKU1	86%

Table 3.2: Study Participants and Symptom Prevalence

	Endemic HCoVs (n=595)	SARS-CoV-2 (n=121)	p-value*
Age Group (%)			<.0001
0-4	432 (73)	59 (49)	
5-9	110 (18)	29 (24)	
10-14	53 (9)	33 (27)	
Symptoms (%)			
Measured fever	272 (46)	68 (56)	0.035
Cough	524 (88)	85 (70)	<0.001
Rhinorrhea	505 (85)	93 (77)	0.030
Congestion	276 (46)	45 (37)	0.064
Sore throat	145 (24)	36 (30)	0.221
Headache	81 (14)	38 (31)	<0.001
Loss of appetite	142 (24)	31 (26)	0.681
Diarrhea	64 (11)	14 (12)	0.793
Hospitalized	23 (4)	6 (5)	0.578
Abnormal breathing	108 (18)	9 (7)	0.004
Acute lower respiratory infection	107 (18)	7 (6)	<0.001
ALRI and prescribed antibiotics†	79 (74)	6 (85)	0.484

* p-value from chi-square test
† % represents % of ALRI cases

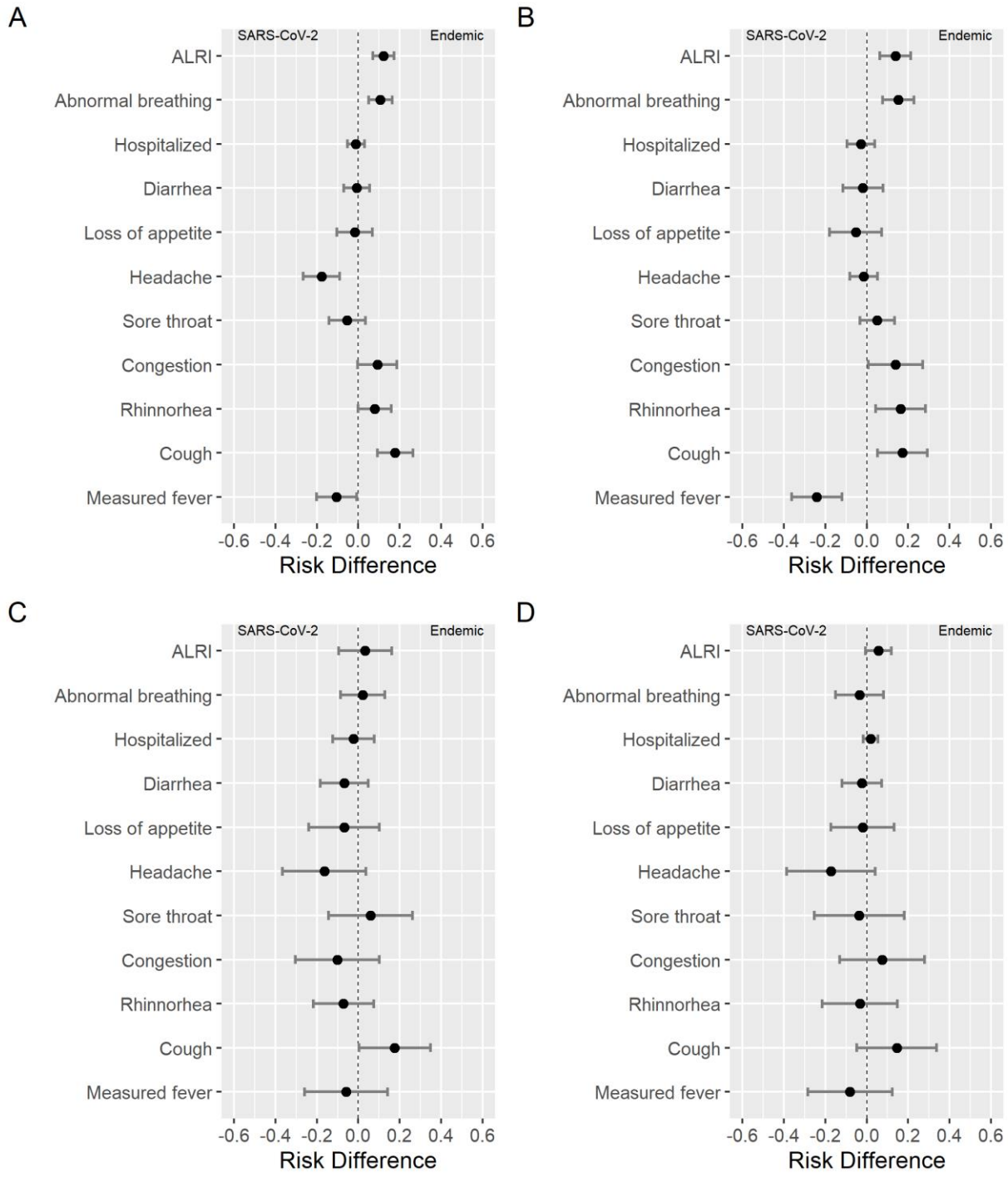

Figure 3.1: Symptom Risk Difference between Endemic HCoVs and SARS-CoV-2

A: All participants. B: Ages 0-4 years. C: Ages 5-9 years. D Ages: 10-14 years.

Table 3.3: Symptom Risk between Endemic HCoV and SARS-CoV-2 by Age Group

0-4				
	Endemic HCoVs (%)	SARS-CoV-2 (%)	Risk Difference (95% CI)	Risk Ratio (95% CI)
Measured fever	218 (50)	44 (75)	-0.24 (-0.36, -0.12)	0.68 (0.57, 0.81)
Cough	382 (88)	42 (71)	0.17 (0.05, 0.29)	1.24 (1.05, 1.47)
Rhinorrhea	378 (88)	42 (71)	0.16 (0.04, 0.28)	1.23 (1.04, 1.45)
Congestion	241 (50)	21 (36)	0.14 (0.09, 0.27)	1.39 (0.97, 1.99)
Sore throat	66 (15)	6 (10)	0.05 (-0.03, 0.14)	1.50 (0.68, 3.31)
Headache	23 (5)	4 (7)	-0.01 (-0.08, 0.05)	0.79 (0.28, 2.19)
Loss of appetite	116 (27)	19 (32)	-0.05 (-0.18, 0.07)	0.83 (0.56, 1.25)
Diarrhea	58 (13)	9 (15)	-0.02 (-0.12, 0.08)	0.88 (0.46. 1.68)
Hospitalized	17 (4)	4 (7)	-0.03 (-0.10, 0.04)	0.58 (0.20, 1.67)
Abnormal breathing	95 (22)	4 (7)	0.15 (0.08, 0.23)	3.24 (1.24, 8.49)
ALRI	89 (21)	4 (7)	0.14 (0.6, 0.21)	3.04 (1.16, 7.97)
ALRI*	69 (19)	4 (7)	0.12 (0.04,0.19)	2.74 (1.04, 7.22)
5-9				
	Endemic HCoVs (%)	SARS-CoV-2 (%)	Risk Difference (95% CI)	Risk Ratio (95% CI)
Measured fever	39 (35)	12 (41)	-0.06 (-0.26, 0.14)	0.86 (0.52, 1.41)
Cough	99 (90)	21 (72)	0.18 (000, 0.35)	1.24 (0.98, 1.57)
Rhinorrhea	87 (79)	25 (86)	-0.07 (-0.22, 0.08)	0.92 (0.77, 1.09)
Congestion	42 (38)	14 (48)	-0.10 (-0.10, 0.30)	0.79 (0.51, 1.23)
Sore throat	52 (47)	12 (41)	0.06 (-0.14, 0.26)	1.14 (0.71, 1.84)
Headache	35 (32)	14 (48)	-0.16 (-0.37, 0.04)	0.66 (0.41, 1.04)
Loss of appetite	19 (17)	7 (24)	-0.07 (-0.24, 0.10)	0.72 (0.33, 1.54)
Diarrhea	4 (4)	3 (10)	-0.07 (-0.18, 0.05)	0.35 (0.08, 1.48)
Hospitalized	5 (5)	2 (7)	-0.02 (-0.12, 0.08)	0.66 (0.13, 3.23)
Abnormal breathing	10 (9)	2 (7)	0.02 (-0.08, 0.12)	1.32 (0.31, 5.69)
ALRI	15 (14)	3 (10)	0.03 (-0.10, 0.16)	1.32 (0.41, 4.25)
ALRI*	12 (13)	3 (10)	0.03 (-0.10, 0.16)	1.26 (0.38, 4.16)
10-14				
	Endemic HCoVs (%)	SARS-CoV-2 (%)	Risk Difference (95% CI)	Risk Ratio (95% CI)
Measured fever	15 (28)	12 (36)	-0.08 (-0.28, 0.12)	0.78 (0.42, 1.45)
Cough	43 (81)	22 (67)	0.14 (-0.05, 0.34)	1.22 (0.93, 1.60)
Rhinorrhea	40 (75)	26 (79)	-0.03 (-0.21, 0.15)	0.96 (0.76, 1.21)
Congestion	20 (38)	10 (30)	0.07 (-0.13, 0.28)	1.25 (0.67, 2.32)
Sore throat	27 (51)	18 (55)	-0.04 (-0.25, 0.18)	0.93 (0.62, 1.41)
Headache	23 (43)	20 (61)	-0.17 (-0.39, 0.04)	0.72 (0.47, 1.08)
Loss of appetite	7 (13)	5 (15)	-0.02 (-0.17, 0.13)	0.87 (0.30, 2.52)
Diarrhea	2 (4)	2 (6)	-0.02 (-0.12, 0.07)	0.62 (0.09, 4.21)
Hospitalized	1 (2)	0	0.02 (-0.02, 0.06)	-

Abnormal breathing	3 (6)	3 (9)	-0.03 (-0.15, 0.08)	0.62 (0.13, 2.90)
ALRI	3 (4)	0	0.06 (-0.01, 0.12)	-
ALRI*	3 (6)	0	0.06 (-0.01, 0.13)	-

*Excluding influenza A, influenza B, RSV, and HMpV coinfections

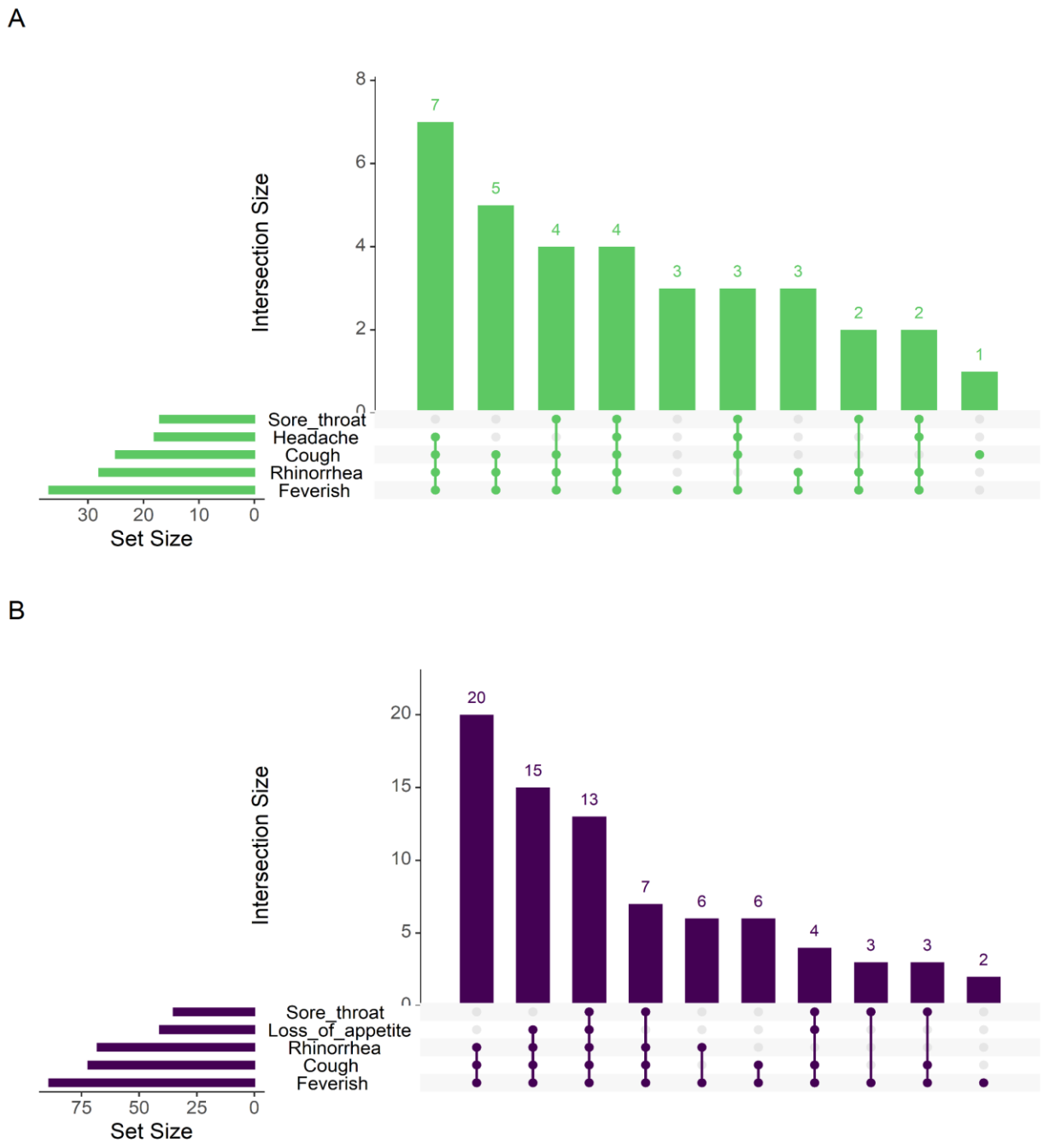

Figure 3.2: Comparison of Common Symptom Groupings between Symptomatic Endemic HCoVs and SARS-CoV-2 Infections

A: Upset plot of symptom groupings for symptomatic SARS-CoV-2 infections. B. Upset plot of symptom groupings for symptomatic endemic HCoV infections.

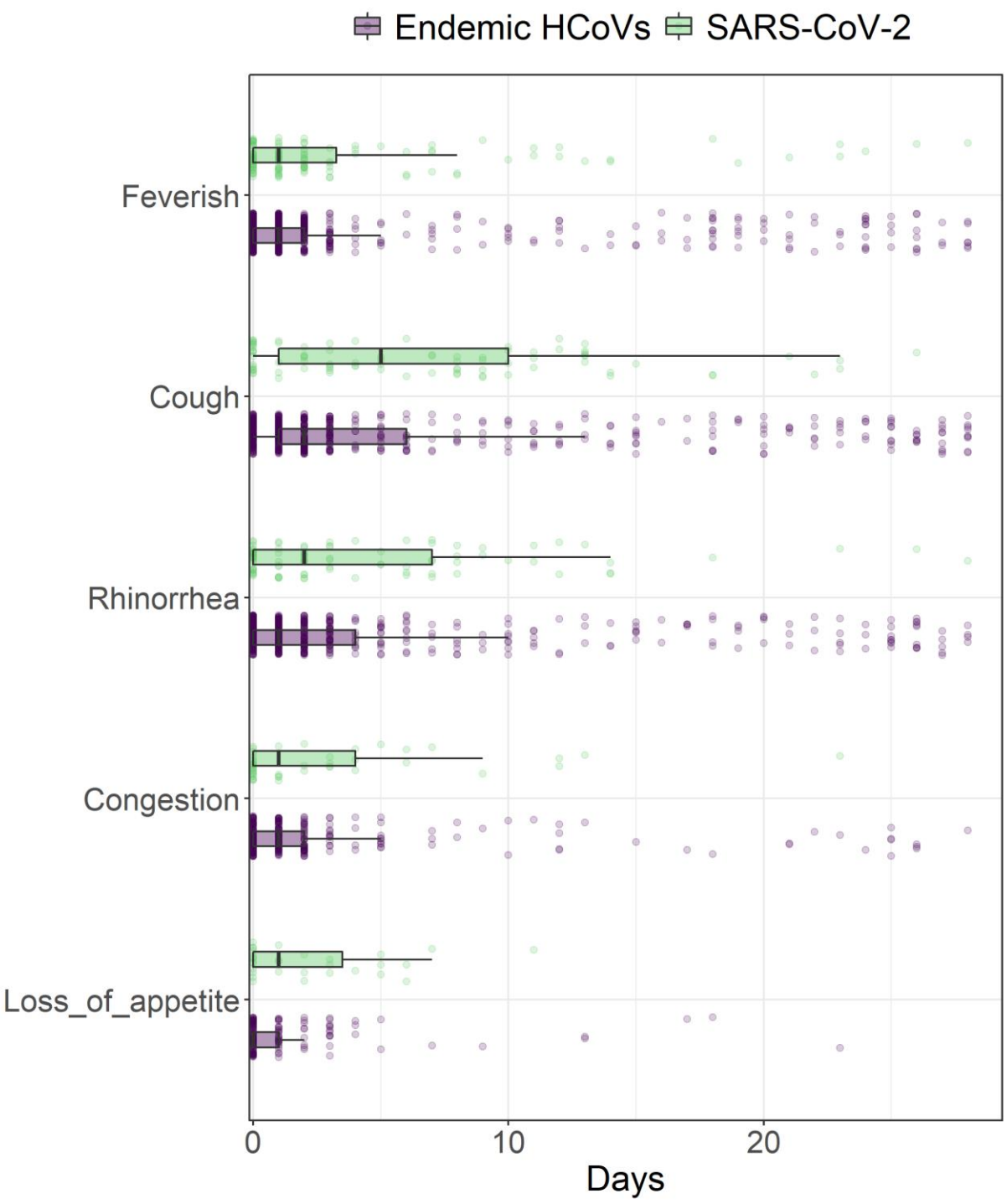

Figure 3.3: Comparison of Symptom Duration between Symptomatic Endemic HCoVs and SARS-CoV-2 Infections

Table 3.4: Symptom Duration Comparison: SARS-CoV-2, Endemic HCoVs

Mean Symptom Duration in Days (SD)

	All		
	SARS-CoV-2	**Endemic HCoVs**	**p-value***
Feverish	3.8 (6.1)	3.8 (7.1)	0.137
Cough	6.7 (6.4)	5.5 (8.0)	0.003
Rhinorrhea	4.4 (5.7)	4.6 (7.3)	0.354
Congestion	2.8 (4.7)	2.8 (5.6)	0.997
Loss of appetite	2.2 (2.8)	1.4 (3.4)	0.032
0-4			
	SARS-CoV-2	**Endemic HCoVs**	**p-value***
Feverish	5.3 (7.3)	4.4 (7.6)	0.069
Cough	6.4 (6.0)	6.4 (8.6)	0.178
Rhinorrhea	5.1 (5.8)	5.4 (7.9)	0.278
Congestion	2.6 (3.8)	3.0 (5.8)	0.875
Loss of appetite	2.5 (3.0)	1.6 (3.7)	0.039
5-9			
	SARS-CoV-2	**Endemic HCoVs**	**p-value***
Feverish	2.0 (4.0)	2.4 (5.4)	0.397
Cough	8.1 (7.5)	3.2 (5.8)	0.002
Rhinorrhea	3.9 (5.5)	2.3 (4.7)	0.125
Congestion	3.4 (6.3)	1.9 (4.4)	0.690
Loss of appetite	2.1 (2.6)	1.0 (1.2)	0.352
10-14			
	SARS-CoV-2	**Endemic HCoVs**	**p-value***
Feverish	2.6 (4.7)	1.7 (3.8)	0.434
Cough	6.0 (6.2)	2.3 (4.3)	0.015
Rhinorrhea	3.5 (6.0)	2.2 (5.0)	0.440
Congestion	2.5 (3.9)	2.6 (5.6)	0.479
Loss of appetite	1.2 (2.2)	0.4 (0.8)	0.698

*From Mann-Whitney U test

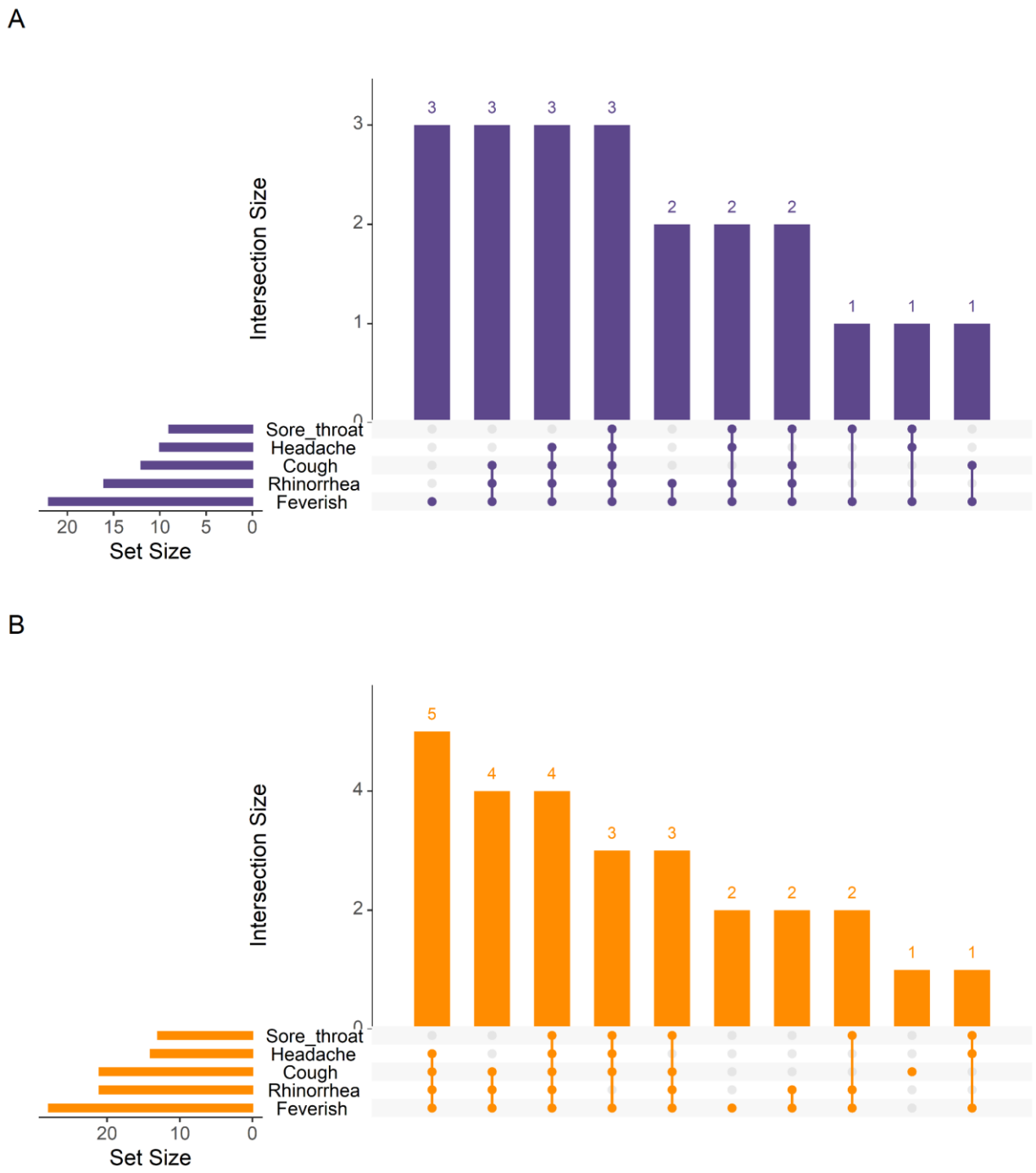

Figure 3.4: Comparison of Common Symptom Groupings between Symptomatic SARS-CoV-2 Infections in 2020 and 2021

A: 2020. B. 2021

Table 3.5: Symptom Duration Comparison: SARS-CoV-2 by Year

	Mean Symptom Duration in Days (SD)		**p-value***
	2020	**2021**	
Feverish	4.1 (7.4)	3.6 (5.0)	0.129
Cough	6.8 (8.0)	6.7 (5.5)	0.382
Rhinorrhea	2.7 (5.0)	5.4 (6.0)	0.004
Congestion	1.6 (2.3)	3.3 (5.2)	0.378
Loss of appetite	3.5 (3.8)	1.6 (1.9)	0.172

*From Mann-Whitney U test

Chapter 4 Infection-induced Immunity is Associated with Protection against SARS-CoV-2 Infection and Decreased Infectivity

4.1 Preface

This chapter of my dissertation was published in *Clinical Infectious Diseases* in 2023 (DOI: 10.1093/cid/ciad074). In addition to myself, the authors include Guillermina Kuan, Roger Lopez, Sergio Ojeda, Abigail Shotwell, Nery Sanchez, Saira Saborio, Miguel Plazaola, Carlos Barilla, Eben Kenah, Angel Balmaseda, and Aubree Gordon.

4.2 Abstract

The impact of infection-induced immunity on SARS-CoV-2 transmission has not been well established. Here we estimate the effects of prior infection induced immunity in adults and children on SARS-CoV-2 transmission in households.

We conducted a household cohort study between March 2020-November 2022 in Managua, Nicaragua where when one household member tests positive for SARS-CoV-2, household members are closely monitored for SARS-CoV-2 infection. Using a pairwise survival model, we estimate the association of time period, age, symptoms, and prior infection with secondary attack risk.

Overall, transmission occurred in 70.2% of households, 40.9% of household contacts were infected, and the secondary attack risk ranged from 8.1%1 to 13.9% depending on the time period. Symptomatic infected individuals were more infectious (RR 21.2, 95% CI: 7.4-60.7) and

participants with a prior infection were half as likely to be infected compared to naïve individuals (RR 0.52, 95% CI:0.38-0.70). In models stratified by age, prior infection was associated with decreased infectivity in adults and adolescents (SAR 12.3, 95% CI: 10.3, 14.8 vs 17.5, 95% CI: 14.8, 20.7). However, while young children were less likely to transmit, neither prior infection nor symptom presentation was associated with infectivity. During the Omicron era, infection-induced immunity remained protective against infection.

Infection-induced immunity is associated with decreased infectivity for adults and adolescents. While young children are less infectious, prior infection and asymptomatic presentation did not reduce their infectivity as was seen in adults. As SARS-CoV-2 transitions to endemicity, children may become more important in transmission dynamics.

4.3 Introduction

Prior studies show that vaccination reduces the likelihood of SARS-CoV-2 transmission,[72,73] and infection-induced immunity is associated with shorter shedding duration and lower viral load;[74] however, the effect of infection-induced immunity on SARS-CoV-2 transmission has not been well established.[75] Given the high infectivity of SARS-CoV-2 and its emerging variants, most of the population including many children have already been infected worldwide.[26,76,77] Further, as of November 2022, SARS-CoV-2 vaccine availability and uptake has been limited for children globally.[78]

Questions persist about the contribution of children to SARS-CoV-2 transmission. Evidence on the contribution of children to transmission generally shows that children have a lower risk of SARS-CoV-2 transmission when infected compared to adults [79-81] while other work, particularly after the emergence of SARS-CoV-2 variants, finds that children have similar or increased risk of transmission. [82,83]

In Nicaragua, as with much of the rest of the world, SARS-CoV-2 transmission picked up in March/April of 2020 with a large wave of the pre-variant virus that ended by August 2020. In our cohort, ~60% of adults were infected in that initial wave [65] . A second large wave, primarily of delta and gamma, occurred in 2021 from April-November [84]. SARS-CoV-2 vaccination did not become widely available until September-November of 2021. Omicron, and it's subvariants, was introduced in 2022 quickly becoming the dominant virus.[85] However, by that time a majority of the population had been previously infected and most subsequently vaccinated.[84]

We present results from an ongoing, community-based, household transmission study located in Managua, Nicaragua from March 2020-November 2022. We evaluate the effect of prior infection-induced immunity on transmission as well as the contribution of children to SARS-CoV-2 household transmission.

4.4 Methods

This study was approved by institutional review boards at the Nicaraguan Ministry of Health and the University of Michigan. Adults and parents/guardians of children provided written informed consent and children six years or older provided verbal assent.

Participants included in this analysis are members of the ongoing Household Influenza Cohort Study (HICS) which began in 2017. HICS is a community-based prospective household cohort study located in District II of Managua, Nicaragua. In June 2020, the study was expanded to include a transmission sub-study of SARS-CoV-2. Participants attended the Health Center Sócrates Flores Vivas at the first signs of a fever or respiratory illness. A respiratory sample was collected and tested for influenza and SARS-CoV-2 via reverse-transcription polymerase chain reaction (PCR).

Household activation occurred when a cohort participant tested positive for SARS-CoV-2 and they and their household members agreed to be monitored intensively for SARS-CoV-2 transmission. Study staff visited the home up to six times to collect respiratory samples (days 0, 3, 7, 14, 21, and 30) and conducted a final follow-up visit at day 45-60. Daily symptom data was collected by staff during each visit.[65] The primary case was identified as the household member with earliest symptom onset date.

Each year, blood samples were collected twice from March-April and again from October-December. Serum samples collected from 2019-2022 were paired (current vs baseline) and were tested for SARS-CoV-2 IgG antibodies to the spike receptor binding domain (RBD) via an enzyme-linked immunosorbent assay (ELISA) following a protocol adapted from the Krammer laboratory at Mt. Sinai.[37] Blood samples from participants that were previously vaccinated against SARS-CoV-2 were tested for SARS-CoV-2 IgG antibodies to the nucleocapsid (N) via ELISA.

Prior SARS-CoV-2 infection-induced immunity included both PCR and serologically confirmed infections (RBD+ before SARS-CoV-2 vaccination or N+ after). We categorized SARS-CoV-2 infections into three periods: March 2020-February 2021 (pre-variant era), March 2021-December 2021 (pre-Omicron variants, predominantly gamma and delta), and January 2022-November 2022 (Omicron variant).[84,85] To determine the date of prior infection for serologically confirmed infections, we estimated the infection date as a randomly selected day during the epidemic wave prior to the blood sample collection.[74]

SARS-CoV-2 vaccinations in the cohort began in January 2021. Most vaccinated participants received their first vaccine beginning in September of 2021. A variety of vaccines have been used, with AstraZeneca (2 dose, second dose between days 56-128), Abdala (3 dose,

second dose on day 14 and third on day 28), and the Soberana 02 (2 dose, second dose on day 28) being the three most common vaccines administered. Participants are considered fully vaccinated 14 days after the final dose.

We compared age at enrollment, sex, SARS CoV-2 vaccination, and presence of SARS-CoV-2 antibodies before January 1, 2022, between participants who did and did not participate in intensive monitoring using a chi-square and Fisher-exact tests. Using these tests, we also compared time period, sex, age, bedroom- and bed-sharing, prior infections, vaccination, and primary case symptoms between households that did and did not have transmission (an observed SAR-CoV-2 infection among household members) and (except for symptoms) between PCR- and PCR+ household contacts.

To estimate the household secondary attack risk (SAR) and rate ratios (RR), we used pairwise survival models. Pairwise survival models are statistical models of disease transmission that overcome weaknesses of binomial models in estimating the household SAR by accounting for multiple generations of transmission. These models can use the entire household observation period to estimate the SAR, not just the infectious period of the primary case, even when who-infects-who is not observed.[86,87] Additionally, these models account simultaneously for within-household transmission and the risk of infection from outside the household.[88] The SAR from these models can be interpreted as the probability of transmission from one infected household member to one susceptible during the infectious period.[86,87]

We assumed an incubation period of six days, a latency period of three, and a 10-day duration of infectiousness;[89-91] therefore, participants were considered infectious three days before to seven days following symptom onset or their first PCR+ test, whichever occurred first. All primary cases were symptomatic and PCR+ household members were considered

symptomatic during their infectious period if symptoms were reported (loss of taste or smell, fever, cough, rhinorrhea, nasal congestion, headache, sore or itchy throat, joint or muscle pain, diarrhea, vomiting, fatigue, rash, conjunctivitis, loss of appetite, difficulty breathing, rapid breathing, shortness of breath, and chest pain) within seven days following the infection date.

SAS version 9.4 (SAS Institute Inc.) and R version 4.1.1 with the transtat package were used to conduct the analysis. [86,92] The models included time period, characteristics of the susceptible household member (sex, age, prior infection, and vaccination) and characteristics of the infected household member (sex, age, presence of symptoms, cough, rhinorrhea, prior infection, vaccination, number of household members, and bed- and bedroom- sharing). We also ran separate models that included age and an interaction term for age with infector characteristics (symptoms, cough, rhinorrhea, and prior infection) and for prior infection status of the susceptible household member.

To evaluate if the household SARs were different when considering only households infected with the Omicron variant, we reran the univariate models for household activation for 2020/2021 and 2022 separately. For sensitivity analyses, we adjusted the incubation (4-7 days), latency (2-4 days), and infectious periods (8-15 days). We also reran the univariate models including only households where all household members consented to participate in the household activation and serial swabbing. Finally, we ran a univariate model with time since last infection instead of prior infection (yes/no). To assess the impact of our assumption about the estimated infection date for serologically confirmed infections, we adjusted that date; we shifted all estimates to a random day within the first 15 days of the wave and the last 15 days of the wave prior to the blood sample collection.

4.5 Results

From March 2020-November 2022, there were 2,399 active participants in the cohort with 87 new/re- enrollees, 394 withdrawn, and 27 deaths (Figure C.1). Within the SARS-CoV-2 transmission sub-study, a total of 228 households (51.9% of all cohort houses) were activated (some multiple times) with 349 total activations. Of the 1,661 individuals in those households, 1,353 (81.5%) household contacts consented to intensive monitoring, 308 (18.5%) declined participation/were not present. Participants in activated households that did not participate in intensive monitoring were generally working-age adults and male (Table 4.1). They also had lower cohort participation, were more likely to have missed cohort blood collections since the start of the pandemic and were less likely to have reported vaccination or have documented SARS-CoV-2 antibodies. In addition to the 349 primary cases, 553 household contacts (40.8%) were infected.

Close to half of household activations (n=164, 47.0%) occurred from March 2021-December 2021, a period when multiple variants circulated, and delta predominated. Additionally, there were 29 (8.3%) participating households in March 2020-Febuary 2021 and 156 (44.7%) households in January 2022- November 2022. 79.9% of household activations began within 6 days of primary case symptom onset. Overall, transmission occurred in 70.2% of households.

4.5.1 Primary cases and household members

Next, we looked for differences in primary cases in households where transmission did and did not occur as well as differences in PCR+ and PCR- household members. There were a greater proportion of primary cases aged 20-64 years old in households that had transmission

compared to those where no transmission occurred (49.0% vs 34.6%) and the overall age group distribution was significantly different (p-value: 0.0134) (Table C.1). PCR- household members overall had a greater number of prior SARS-CoV-2 infections (p-value: 0.0029) (Table C.2). Around half of all young children (aged 0-4), children (aged 5-10) and adults and adolescents (aged 11+) had been previously infected at the start of intensive monitoring (Table 4.2).

4.5.2 SAR and susceptibility

Next, we evaluated the household SAR and variables associated with susceptibility. The overall estimated household SAR was 12.5% and ranged from 8.1-13.9% depending on the study period (Figure 4.1). Compared to those with no prior SARS-CoV-2 infection, participants with a prior infection had half the risk of infection within the household (RR 0.52, 95% CI:0.38, 0.70).

4.5.3 SAR and infectivity

We also evaluated factors associated with infectivity. The household SAR was smaller for larger households (8.0% compared to 16.4% for households with 10+ and 2-5 members, respectively). Children, and adults and adolescents were much more likely to infect others compared to young children (RR 3.6, 95% CI 1.4, 9.4 and 6.1, 95% CI: 2.5, 15.0 respectively). In absolute terms, the difference in the secondary attack risk between young children, and adults and adolescents was 11.2% (SAR 3.4% vs 14.6%). Symptomatic infected individuals were 21.2 times (95% CI: 7.4, 60.7) more likely to transmit the virus compared to asymptomatic individuals, with an absolute difference in the probability of transmission of 14.6% (SAR 15.9% vs 1.3%). Overall, prior infection was not associated with decreased infectivity.

4.5.4 SAR stratified by age

We also compared the age-specific associations between symptom presentation and prior infection with infectivity. The probability of transmission was lower for asymptomatic compared to symptomatic children, and adults and adolescent (Figure 4.2; 9.8% vs 13.5% and 0.8% vs 18.2%, respectively). For infected young children, we observed no difference by symptom status in the risk of transmitting the virus. Of note, prior infection was associated with decreased infectivity in adults and adolescents (SAR 12.3%, 95% CI: 10.3, 14.8 and 17.5% 95% CI: 14.8, 20.7) but not children. When evaluating susceptibility stratified by age, prior infection was associated with decreased SAR in all age groups, but the difference was not significant in young children.

4.5.5 SAR and Omicron

Next we evaluated susceptibility and infectivity during the Omicron era. Consistent with the pre-Omicron era results, prior infection was associated with protection against infection. Likewise, susceptibility did not vary by age; (Figure C.2, C.3) however, the estimated SAR for infected young children in the Omicron era was almost 3 times that of the pre-Omicron era (2.7% vs 7.5%) while there was little difference seen for adults and adolescents (14.7% vs 15.9%). Infectivity was still associated with symptomatic presentation (RR= 9.0, 95% CI: 2.7, 29.9).

4.5.6 SAR and time since last infection

Since not only prior infection status, but how recently someone was infected might affect susceptibility and infectivity, we next ran models examining the effects of time since last infection. When examining the association of time since last infection and susceptibility, the results were similar to the association between prior infection (yes/no) and susceptibility (Figure

C.4). At each time point (up to 6 months, 6-12 months, and 12+ months since), those with a prior infection, were less likely to be infected compared to those who never had a prior infection. Not surprisingly, the SAR was lowest for household members who had a prior infection within 6 months (9.5%, 95% CI: 6.3, 14.1). However, it was still similar to the SAR of those with any prior infection (10.3%, 95% CI: 8.8, 12.0). For an infected household member, there was no association between time since last infection and infectivity.

4.5.7 Sensitivity Analyses

To examine the effect of our assumptions on our estimates, we varied the incubation, latency, and infectious parameters (Figure C.5). Overall, there were minor differences in the estimated SARs; however, our main findings held. To examine the effect of non-participation, we reran models limiting to households where all members participated. The overall SAR was slightly higher, but there were no differences in the direction of the association age, infection-induced immunity, or any other variable (Figure C.6). The associations between SAR and time since last infection for both susceptible and infected household members had little variation when we adjusted the estimated infection date for serologically detected infections.

4.6 Discussion

We found that prior infection impacted both susceptibility and infectivity of SARS-CoV-2 in a household setting. Overall, and as expected, prior infection reduced susceptibility. However, the decrease in susceptibility was less marked in young children. We found that the effect of prior infection on infectivity was age dependent. Previously infected adults and adolescents were less infectious compared to those who did not have a prior SARS-CoV-2

infection. But in children aged 10 and under, we did not observe any reduction in infectivity associated with prior infection.

Our finding of decreased risk of transmission for previously infected adults and adolescents is consistent with decreased shedding duration and viral load among those previously infected individuals aged ten years and older.[74] Our results also concur with the finding that prior infection was also associated with decreased infectivity during the Omicron wave.[75] Similarly, SARS-CoV-2 vaccination has been associated with decreased infectivity.[72,73,75] We note that these results are from a population, like many in the world, where most were infected prior to the availability of SARS-CoV-2 vaccines.[84] However, both infection then vaccination and vaccination then infection produces broad, hybrid immunity to SARS-CoV-2 with no observed differences by sequence.[93,94] Thus, we expect that as robust immunity develops globally through expanded vaccination efforts and repeat and breakthrough infections occur there will be a decrease in infectivity and lower rates of SARS-CoV-2 infections.

In children prior infection was not associated with decreased infectivity. Additionally, infectiousness was similar between symptomatic and asymptomatic young children (aged 0-4); the increased likelihood of asymptomatic presentation for pediatric SARS-CoV-2 infections does not account for the differences in infectiousness between adults and children.[95] These results suggest distinct immune responses to natural SARS-CoV-2 infection between younger and older individuals that may impact transmission dynamics.[27,96]

Consistent with recent work, prior infection in the Omicron era was still associated with protection against infection.[97] While we observed increased infectivity for each age for during the Omicron era, infectivity was proportionally higher for young children compared to adults.

Although children are generally less infectious,[79-81] the changes in infectivity by age during the Omicron era may suggest changing SARS-CoV-2 dynamics.[83,98]

As expected and consistent with the findings of a recent meta-analysis, more recent prior SARS-CoV-2 infections are associated with lower risk of infection.[97] However, this protection may be attenuated in our study due to the mixing of effects of increased infectivity of variants with time and the antigenic differences between the prior and current infecting strains. Future work should expand on the current research by comparing susceptibility by SARS-CoV-2 infection histories that include information about the specific strains of prior infection in addition to the timing.

Our study has several strengths and limitations. Strengths include close monitoring of participants inside of an ongoing cohort, which allows us to know infection histories prior to SARS-CoV-2 entering the household as well as detect mild and asymptomatic infections. Our study is also large and spans both pre-variant and variant eras. Because of our use of a statistical transmission model that also accounts for risk of external infection, each of the SAR estimates in this study can be properly interpreted as the probability of transmission. One limitation of our study is that although PCR testing occurred frequently during monitoring, it is possible that SARS-CoV-2 infections were missed and thus we may underestimate the household SAR. As prior infection was in part determined using serological testing, it is possible that we miscategorized some participants as non-previously infected because they did not seroconvert to their first infection, or their antibodies waned rapidly. In addition, household members that declined or were not available for intensive monitoring were different from those that did participate. The exclusion of these participants likely leads to an underestimation of the household SAR; however, when analyzing only households where the associations between prior

infection and infectivity and susceptibility did not change significantly. Lastly, sequencing results were not available for all household infections which limits our ability to evaluate strain-specific infection induced immunity effects.

Our study highlights that infection-induced immunity is associated with decreased infectivity for adults and adolescents. Even with the emergence of the Omicron variant, infection-induced immunity remained associated with protection against infection. However, for young children, neither infection-induced immunity nor symptom presentation was associated with infectivity. At the beginning of the SARS-CoV-2 pandemic, it was established that the contribution of children to SARS-CoV-2 transmission was minor.[81] The absence of decreased infectivity from infection-induced immunity among children and the changing transmission dynamics from emerging SARS-CoV-2 variants suggests that children may already have more meaningful contributions to SARS-CoV-2 transmission; this contribution may further increase as new children are born without immunity to SARS-CoV-2 and increasingly represent the greatest proportion of primary cases.[8]

Table 4.1: Demographics of participants eligible for SARS-CoV-2 intensive monitoring in Managua Nicaragua, March 2020-November 2022

		Participants (n=975)	Declined/not present for activation enrollment (n=308)	p-value*
Age at enrollment (%)				0.0001
	0-4	233 (23.9)	44 (14.3)	
	5-10	197 (20.2)	46 (14.9)	
	11-19	136 (13.9)	71 (23.1)	
	20-64	379 (38.9)	142 (46.1)	
	65+	30 (3.1)	5 (1.6)	
Female (%)		614 (63.0)	161 (52.3)	0.0008
SARS-CoV-2 vaccination (%)†				0.0039
	Full	299 (30.1)	77 (25.0)	
	Partial	369 (37.9)	105 (34.1)	
	Unvaccinated	50 (5.1)	12 (3.9)	
	No reported vaccination	257 (26.4)	114 (37.0)	
SARS-CoV-2 antibodies (%)†				<.0001
	Yes	882 (90.5)	244 (79.2)	
	No	88 (9.0)	59 (19.2)	
	Missing	5 (0.5)	5 (1.6)	
Blood samples collected				<.0001
	0	5 (0.5)	5 (1.6)	
	1	15 (1.5)	10 (3.3)	
	2	21 (2.2)	38 (12.3)	
	3	130 (13.3)	87 (28.3)	
	4	804 (82.5)	168 (54.6)	

*from chi-square or Fisher's exact test

†before Jan 1, 2022

A chi-square test was used to compared demographics between those that participated in and declined/were not present for household activation.

Table 4.2: Prior infection by case status and age

Prior Infection		Overall (%)	Age (%*) 0-4	5-10	11+
All		1702	142	348	1212
	Yes	1017 (59.8)	70 (49.3)	184 (52.9)	648 (53.5)
Primary cases		349	28	66	255
	Yes	177 (32.0)	12 (28.6)	32 (27.1)	133 (33.8)
PCR+ household members		553	42	118	393
	Yes	311 (56.2)	21 (50.0)	54 (45.8)	236 (60.1)
PCR- household members		800	72	164	564
	Yes	529 (66.1)	37 (51.4)	102 (62.2)	390 (69.1)

Data are grouped by primary cases, PCR+ household members, and PCR- household members.
*%s are of the corresponding age within each case status group.

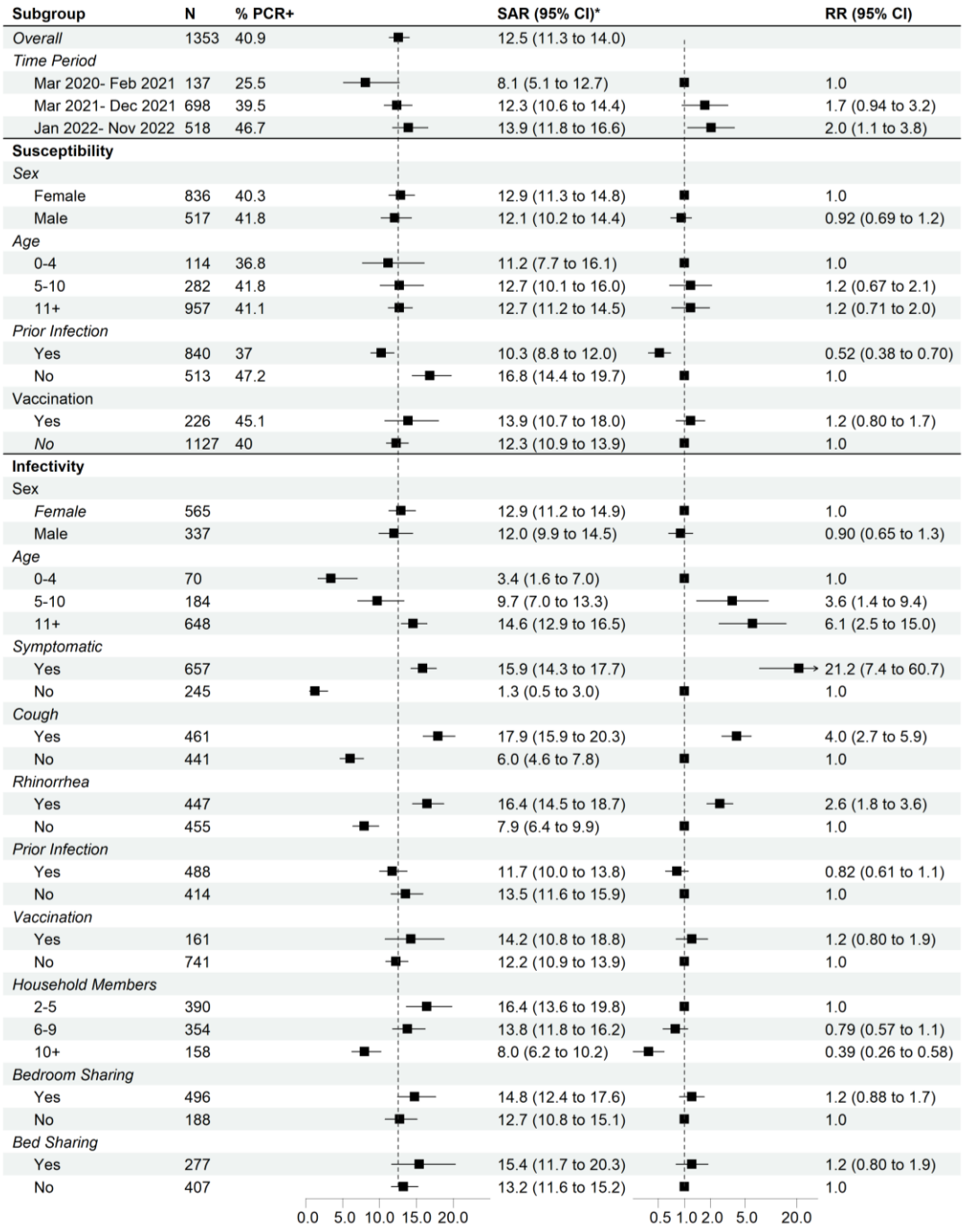

Figure 4.1: Estimated secondary attack risk and rate ratios.

The models are univariate and only include the intercepts, and log-shape parameters in additional to the single variable of interest. Variables are grouped by susceptible variables (characteristics of the susceptible individual in the paired data) and infector variables (characteristics of the infectious individual in the paired data).

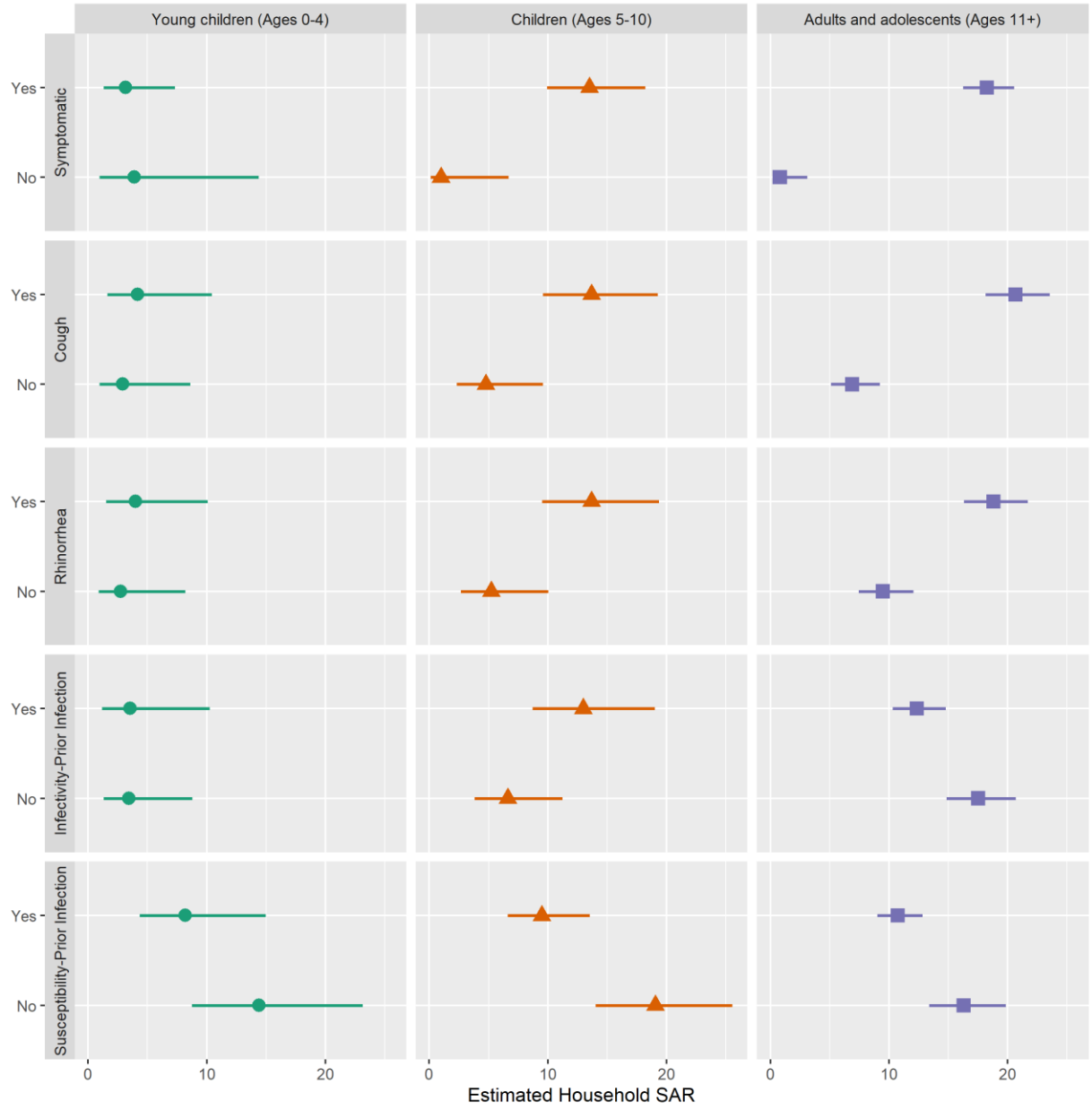

Figure 4.2: Secondary attack risk stratified by age

The presented models include age and an interaction term of age and the infectivity (symptomatic, cough, rhinorrhea, and prior infection) or susceptibility variable (prior infection). The results are stratified by age group: young children (ages 0-4), children (ages 5-10), and adults and adolescents (ages 11+).

Chapter 5 Knowledge Added and Future Directions

This dissertation examined both ccCoV and SARS-CoV-2 infection and immunity with a particular focus on children. We characterized the burden and seasonality of ccCoV infections in children in Aim 1. Further, we investigated the impact of infection-induced immunity on symptom presentation by comparing primary and secondary ccCoV infections. In Aim 2, we directly compared symptom presentation and duration of ccCoV infections and SARS-CoV-2 infections in children. In Aim 3, we investigated factors associated with SARS-CoV-2 infection susceptibility and infectivity in children and adults; we also determined the contribution of infection-induced immunity to SARS-CoV-2 susceptibility and infectivity. Together these aims address gaps in our current understanding of HCoV epidemiology, especially in children, and provide insight into the transition of SARS-CoV-2 from a pandemic to an endemic virus.

5.1 Aim 1

In Aim 1 we found that the burden of symptomatic ccCoV infections and ccCoV-associated LRI was greatest among those aged 0-1. Incidence of symptomatic ccCoV infections rapidly decreased with increasing age until about age 6. This suggests that while ccCoV reinfections are frequent throughout life,[21] infection-induced immunity may convey protection against symptomatic infection. This is supported by our finding that, after adjusting for age, secondary compared to primary ccCoV infections were less likely to be associated with breathing problems or LRI. This protection wanes more quickly early in life,[23] but with repeated exposure may last longer.

Examining ccCoV seasonality in Managua, Nicaragua, a location where ccCoV infections are not limited to one season, we found that two alpha ccCoVs did not have peaks that co-occurred; this may suggest sub-group specific cross-reactive immunity among children.[47-53] While cross-reactive antibodies within and between groups have been discovered,[27] their association with infection and symptom presentation is not well understood.

5.2 Aim 2

In Aim 2, we found that SARS-COV-2 and ccCoV infections in children were very similar, both presenting typically with symptoms of the common cold. In this community-based study, the risk of SARS-CoV-2-associated severe illness was roughly equivalent to the risk from ccCoVs. This suggests that, for children, SARS-CoV-2 is similar to a ccCoV; this is relevant information as parents and others make decisions regarding SARS-CoV-2. SARS-CoV-2 remains relevant for children because of its potential, albeit small, for severe illness and the contributions of children to SARS-CoV-2 transmission. Additionally, emerging variants may change the severity of SARS-CoV-2 infections, although the currently spreading Omicron variant is less severe than prior variants.[99]

The similarity of disease presentation of SARS-CoV-2 and ccCoV for children is important to consider as SARS-CoV-2 transitions to endemicity. Repeated ccCoV exposure during early life may lead to limited ccCoV pathogenicity when those children become adults. The effects of SARS-CoV-2 vaccination early in life should be modeled to avoid unintended adverse outcomes later in life. Despite the increased transmissibility and immune escape capabilities of the Omicron variant, SARS-CoV-2 vaccination is associated with decreased severity among immunocompromised adults;[100] this suggests that current vaccine development

may be in line with the suggestion to allow for transmission while protecting against severe illness.[8]

5.3 Aim 3

Aim 3 showed that adults and adolescents had decreased infectivity when infected with SARS-CoV-2 and decreased susceptibility to infection when previously infected. This is consistent with research that showed decreased infectivity following vaccination[72,73,75] and decreased shedding among those who had a prior infection.[74] This is an encouraging finding as most of the world was infected prior to being vaccinated.[84] Even during the Omicron variant era, prior infection was associated with decreased risk of infection.

Children, however, had similar infectivity regardless of history of prior infection or symptom presentation. This difference between adults and children may be a manifestation of the distinct immune responses to HCoV infections.[27,96] It is encouraging, however, that despite the lack of decreased infectivity associated with prior infection or asymptomatic presentation, that risk of SARS-CoV-2 transmission from children remained low.

5.4 Research Implications

If SARS-CoV-2 follows the hypothesized transition to endemicity,[8] we expect that for children, the burden of SARS-CoV-2 will approximate that of ccCoVs over time. We expect that SARS-CoV-2 infections will likely occur within the first years of life (Aims 1 and 2). Unless there is a major genetic shift in SARS-CoV-2, we expect that symptomatic SARS-CoV-2 infections will present similarly to ccCoVs (Aim 2) and may further decrease in severity as has been observed with Omicron and its subvariants.[99] In children, infection-induced immunity against SARS-CoV-2 will likely continue to protect against infection and severe illness; thus, in

children, the burden of illness caused by SARS-CoV-2 should shift to the youngest children (Aims 1 and 3).

Further, we anticipate transmission to regularly occur among children as occurs with the ccCoVs (Aims 1 and 3). However, the exact timing of annual SARS-CoV-2 transmission is unknown, especially in areas with year-round ccCoV spread (Aim 1). Each year, a large proportion of children will have asymptomatic or very mild SARS-CoV-2 infections (Aims 1 and 2). These asymptomatic infections, however, will not likely impact the infectivity of SARS-CoV-2 among children (Aim 3). Overall infectivity for SARS-CoV-2 may decrease as adults are repeatedly infected and vaccinated (Aim 3).

To guide control efforts during this transition to endemicity, SARS-CoV-2 transmission dynamics should be continually monitored. While we expect the burden of illness to eventually be similar to that of ccCoVs in people that have exposure to the virus as children, it is not clear what the level of severity will eventually be in adults. Importantly, the risk of severe illness from SARS-CoV-2 infections for vulnerable groups, especially the elderly and immunocompromised, remains elevated.[101] Understanding current transmission dynamics, including SARS-CoV-2 seasonality, will allow us to better develop and adapt strategies to protect high-risk groups. Current SARS-CoV-2 vaccines do not provide sterilizing immunity; that is, they allow for infection and, over time, protection against symptomatic infection and severe infection wanes. This means that without the development of improved vaccines, periodic revaccination will be necessary to maintain protection against more severe manifestations. Like current influenza vaccination efforts, SARS-CoV-2 vaccines should be administered annually before SARS-CoV-2 spread to provide higher levels of protection during periods of high transmission.

While the risk of severe illness for children is comparatively low relative to adults, we must continue monitoring for more rare and severe manifestations of SARS-CoV-2 illness. These manifestations include MIS-C and post-acute sequelae SARS-CoV-2 infection (PASC), commonly known as long COVID.

5.5 Future Directions

Future research should focus on the development of HCoV immunity in early childhood. Like the response to influenza, subsequent immune responses to HCoVs may be impacted by our first HCoV exposures.[102,103] Global cocirculation of ccCoVs and SARS-CoV-2 may influence susceptibility to HCoV infections. As children are born during a time of global SARS-CoV-2 circulation, we should investigate differences in the immune response and incidence, duration, and severity of HCoV illness among children with different infection histories. These investigations should focus on and compare those first exposed to ccCoVs to those first exposed to SARS-CoV-2, and should compare those whose first SARS-CoV-2 exposure was vaccination to those first infected. Additionally, examining the changes in immune response to HCoVs with repeated exposure may provide insights into how HCoV immunity develops over time. This early-life framework may provide insights as to why adults and children have distinct immune responses to ccCoVs.[27]

To better understand SARS-CoV-2 transmission, immune correlates of decreased infectivity for SARS-CoV-2 infection should also be examined. Identifying immune correlates of decreased infectivity could provide insight into the mechanism for our observed age-dependent association between prior infection and infectivity.

Further, the impact of HCoV immune profiles should be investigated relative to changing SARS-CoV-2 transmission dynamics. Currently, except for infants and some young children,

those infected with SARS-CoV-2 were first infected with a ccCoV. This will shift as SARS-CoV-2 continues to circulate and more immunologically naïve children are born. It is unclear whether differences in immune imprinting from HCoV exposure would impact SARS-CoV-2 transmission.

5.6 Conclusions

This work contributes to our limited knowledge of HCoV epidemiology. Throughout, we have identified important characteristics of pediatric HCoV infection and immunity that are important first steps to understanding early life exposure to HCoVs. This understanding is of particular importance during a global pandemic as we work to better understand SARS-CoV-2 and prevent associated morbidity and mortality. As SARS-CoV-2 transitions to endemicity, better understanding of pediatric HCoV immunity will help us to understand and prepare for this transition.

Appendices

Appendix A: Supplemental Material for Chapter 2

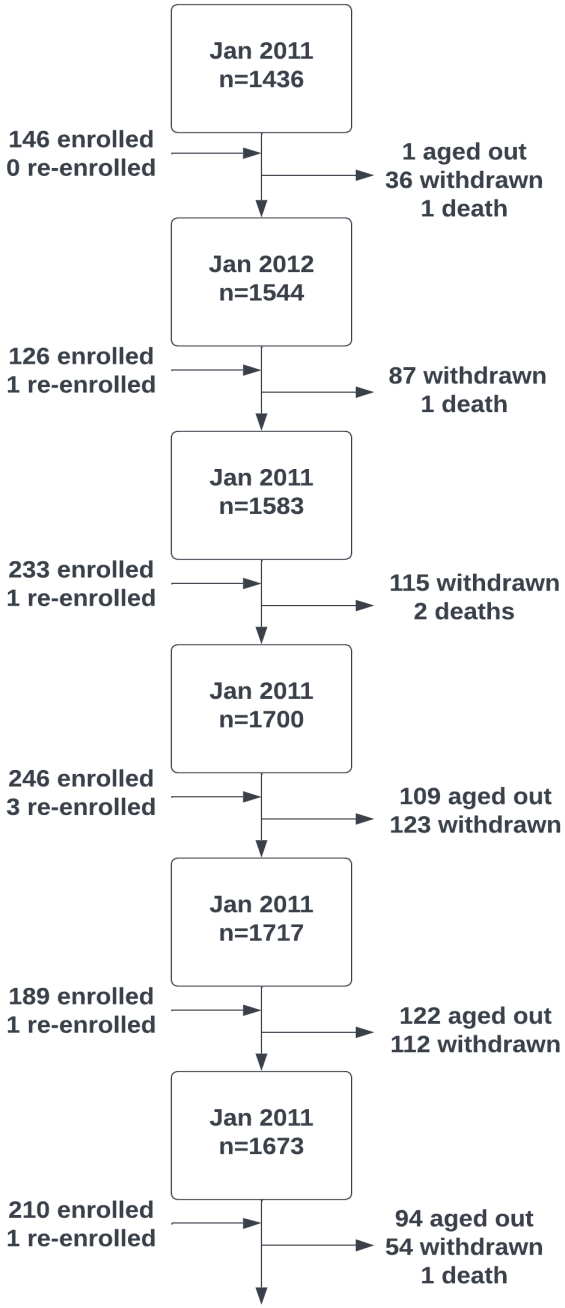

Figure A.1: Participant Enter-Exit by Year

Flow chart represent total active participants in January for each year from 2011-2016 with total number of participants entering (enrolled, re-enrolled) and exiting (aged out, withdrawn, or deaths) the cohort.

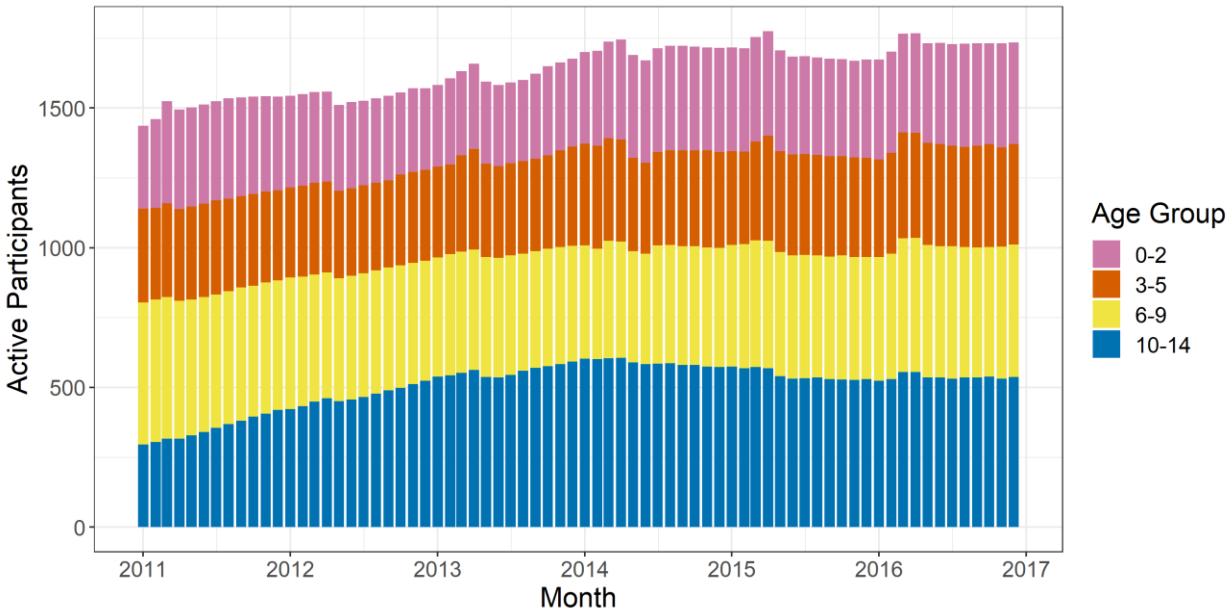

Figure A.2: Participation by Age, Month

Total number of monthly active participants in the cohort over the study period by age groups.

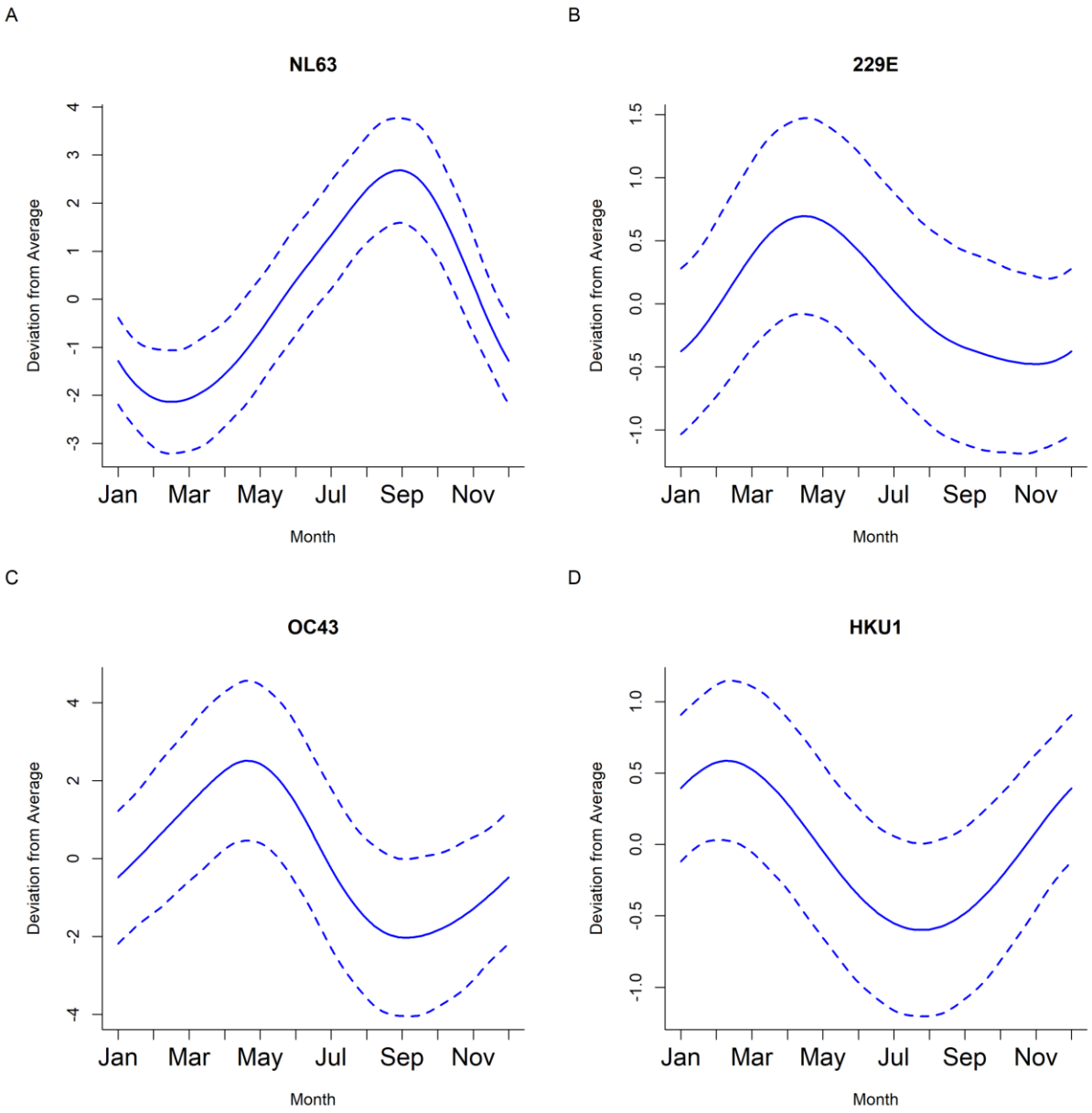

Figure A.3 Generalized Additive Model Analysis for Peak Month by ccCoV Type

Analysis uses month as the predictive variable for time series data for each ccCoV type. Dotted lines represent 95% confidence intervals. If confidence intervals at the peaks overlap with confidence intervals of the trough, there is no significant peak month.

A: NL63, B: 229E, C: OC43, D: HKU1

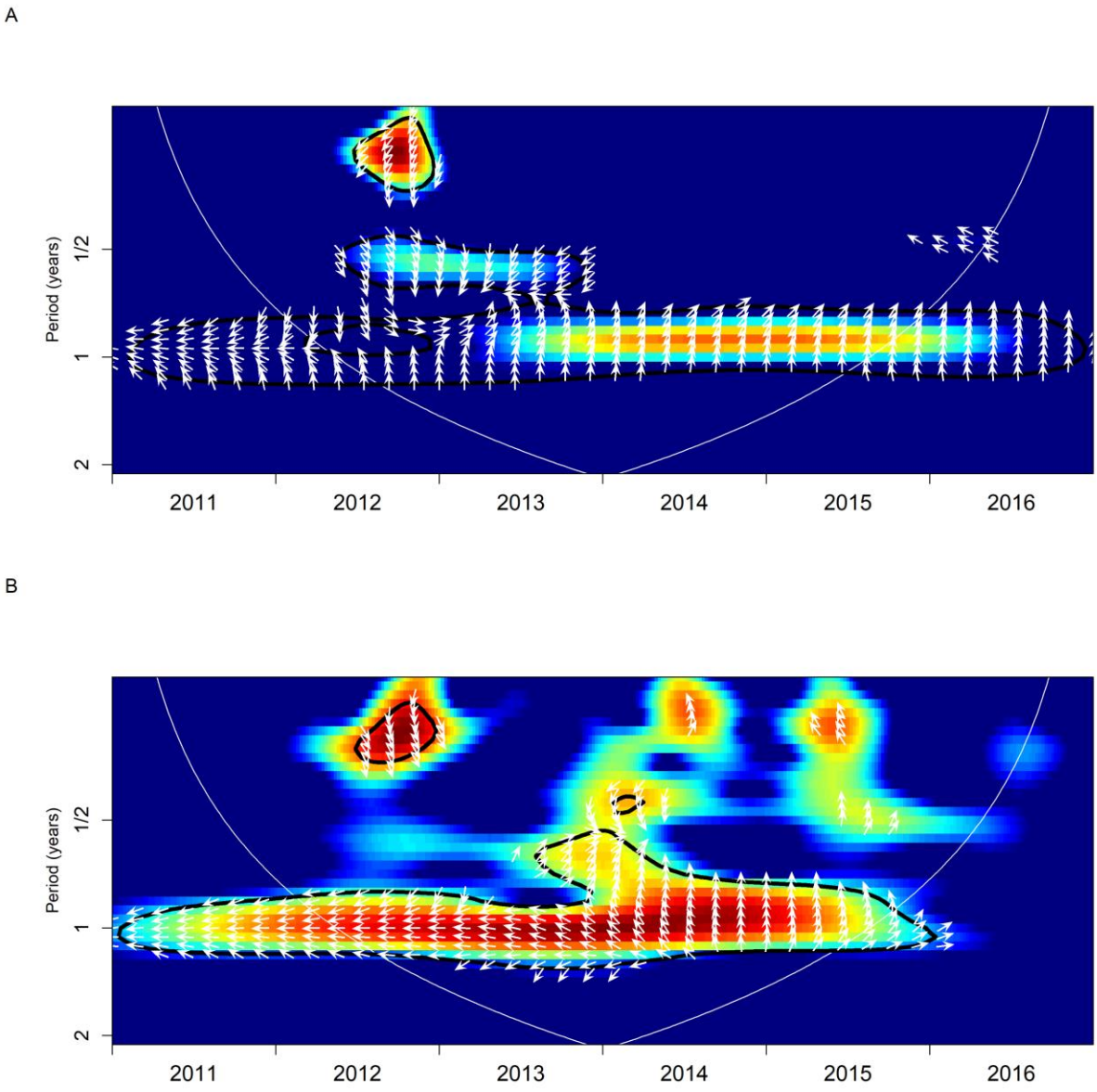

Figure A.4: Cross-wavelet Analysis

Cross-wavelet analysis of two ccCoV types to evaluate temporal relationship. White arrows pointing up at a period of 1 year represent a three-month lag between the first list ccCoV type and the second. Arrows pointing to the left at a period of 1 year represent a six-month lag between types.

A: 229E-NL63, B: OC43-NL63

Table A.1: Incidence Rates by Sex

	Symptomatic ccCoV Incidence Rate per 1, 000 Person Years (95% CI)				
	All	**NL63**	**229E**	**OC43**	**HKU1**
Overall	61.1 (56.3, 66.2)	16.8 (14.4, 19.5)	8.6 (7.0, 10.7)	32.0 (28.6, 35.8)	6.9 (5.4, 8.7)
Sex					
Female	63.4 (56.8, 70.9)	18.6 (15.2, 22.9)	7.8 (5.6, 10.7)	32.0 (27.3, 37.4)	7.0 (5.0, 9.7)
Male	58.6 (52.2, 65.9)	14.9 (11.8, 18.7)	9.5 (7.1, 12.7)	32.1 (27.4, 37.5)	6.8 (4.8, 9.6)
	ccCoV-Associated LRI Incidence Rate per 1, 000 Person Years (95% CI)				
	All	**NL63**	**229E**	**OC43**	**HKU1**
Overall	11.0 (9.1, 13.3)	2.8 (1.9, 4.0)	2.0 (1.2, 3.1)	5.2 (4.0, 6.9)	1.5 (0.9, 2.6)
Sex					
Female	9.9 (7.4, 13.1)	2.0 (1.1, 3.8)	1.2 (0.6, 2.7)	4.5 (3.0, 6.8)	2.0 (1.1, 3.8)
Male	12.2 (9.5, 15.8)	3.5 (2.2, 5.6)	2.7 (1.6, 4.6)	6.0 (4.2, 8.6)	1.0 (0.4, 2.5)

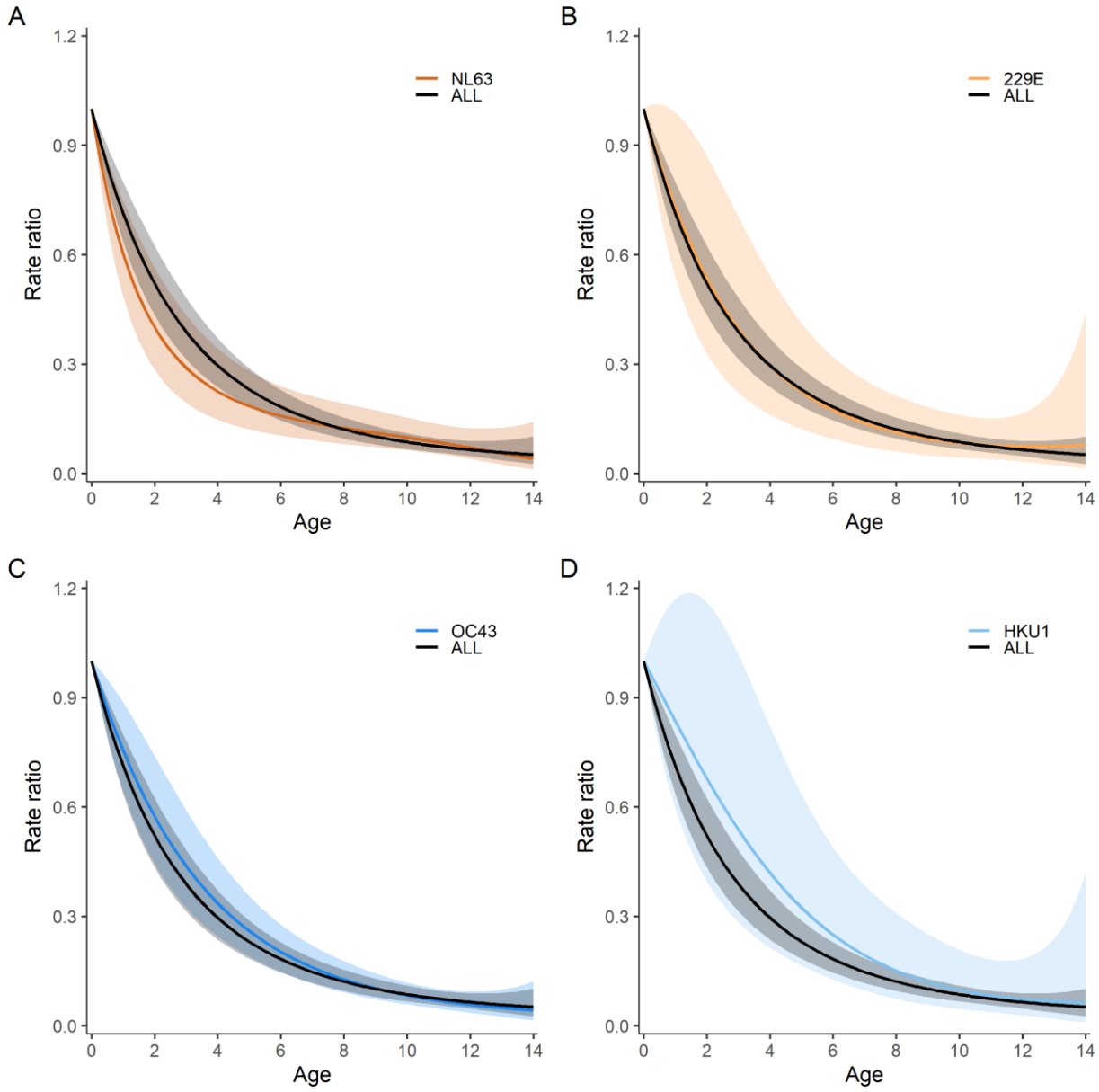

Figure A.5: Age-period Incidence Model- Age Effects

Predicted rate ratios by age from age-period model by ccCoV type. Black line represents predicted rate ratios for all ccCoV infections for comparison.

A: NL63, B: 229E, C: OC43, D: HKU1

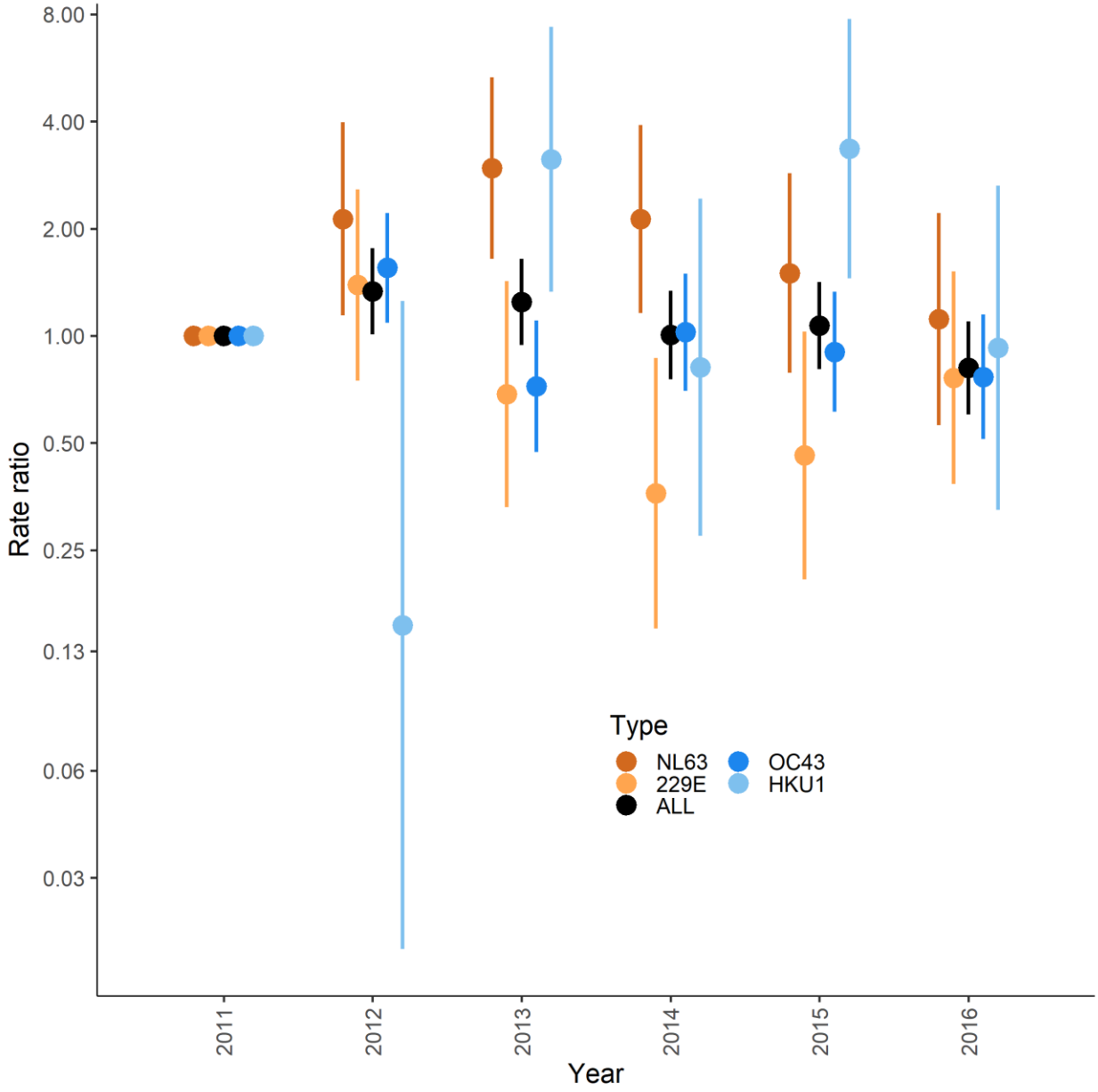

Figure A.6: Age-period Incidence Model- Period Effects

Predicted rate ratios by year from age-period model by ccCoV type. 2011 is the reference category. Black points and confidence intervals represent predicted rate ratios for all ccCoV infections for comparison.

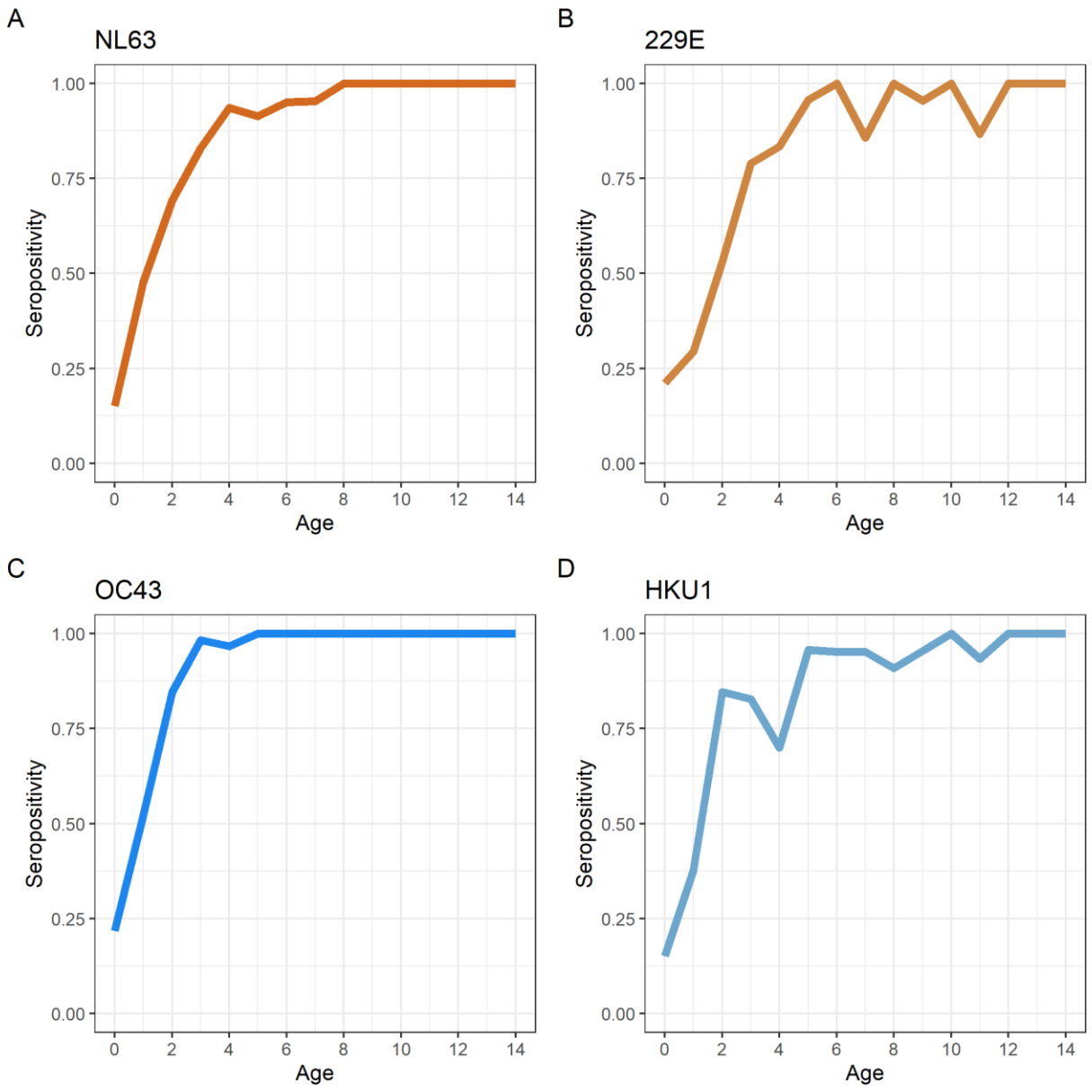

Figure A.7: ccCoV Seropositivity by Age, Type

Proportion of participants with ccCoV-antibodies before ccCoV PCR+ infection by one year age groups and type.

A: NL63, B: 229E, C: OC43, D: HKU1

Appendix B: Supplemental Material for Chapter 3

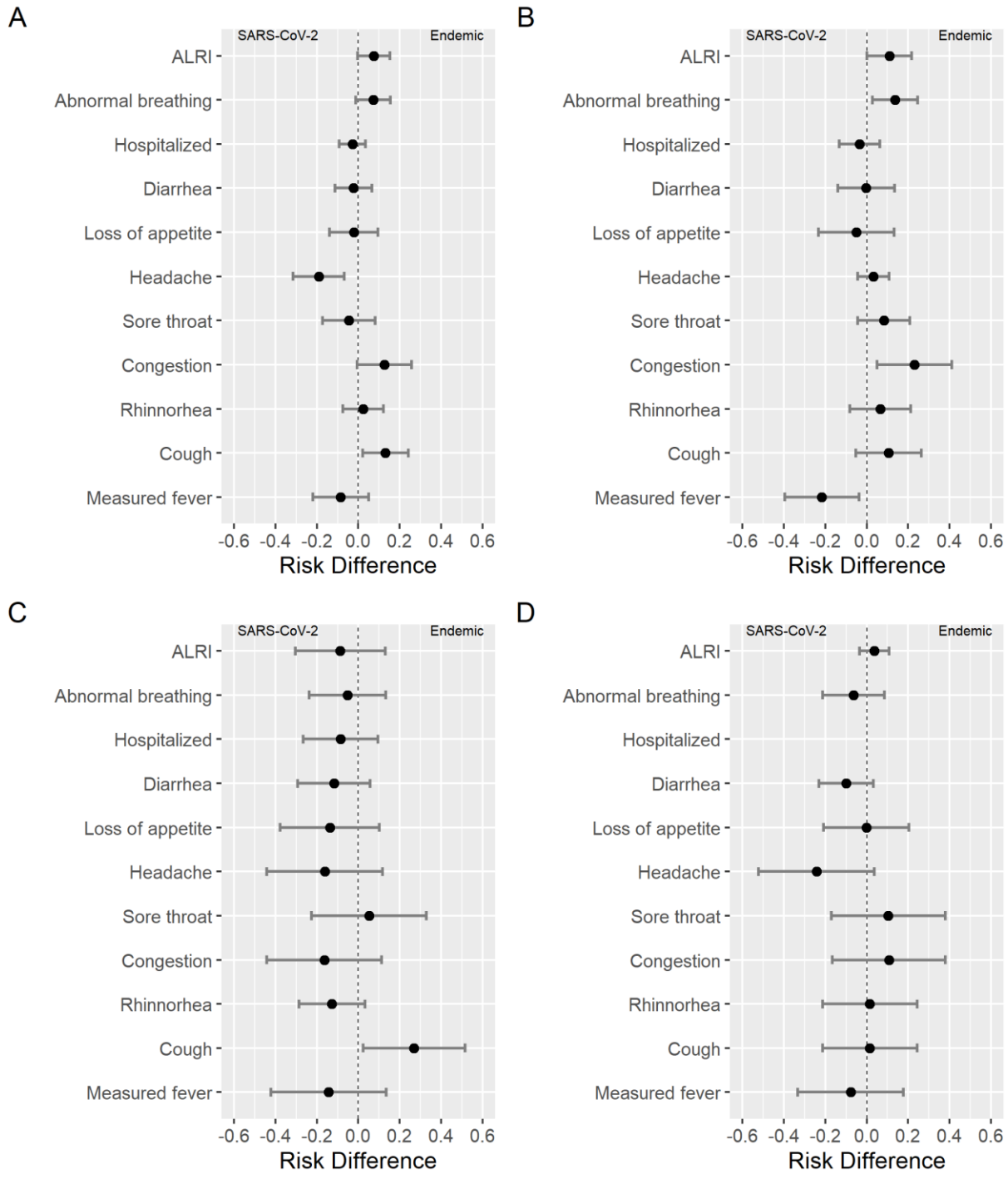

Figure B.1: Symptom Risk Difference between Endemic HCoVs and SARS-CoV-2 for Females

A: All participants. B: Ages 0-4. C: Ages 5-9. D Ages: 10-14.

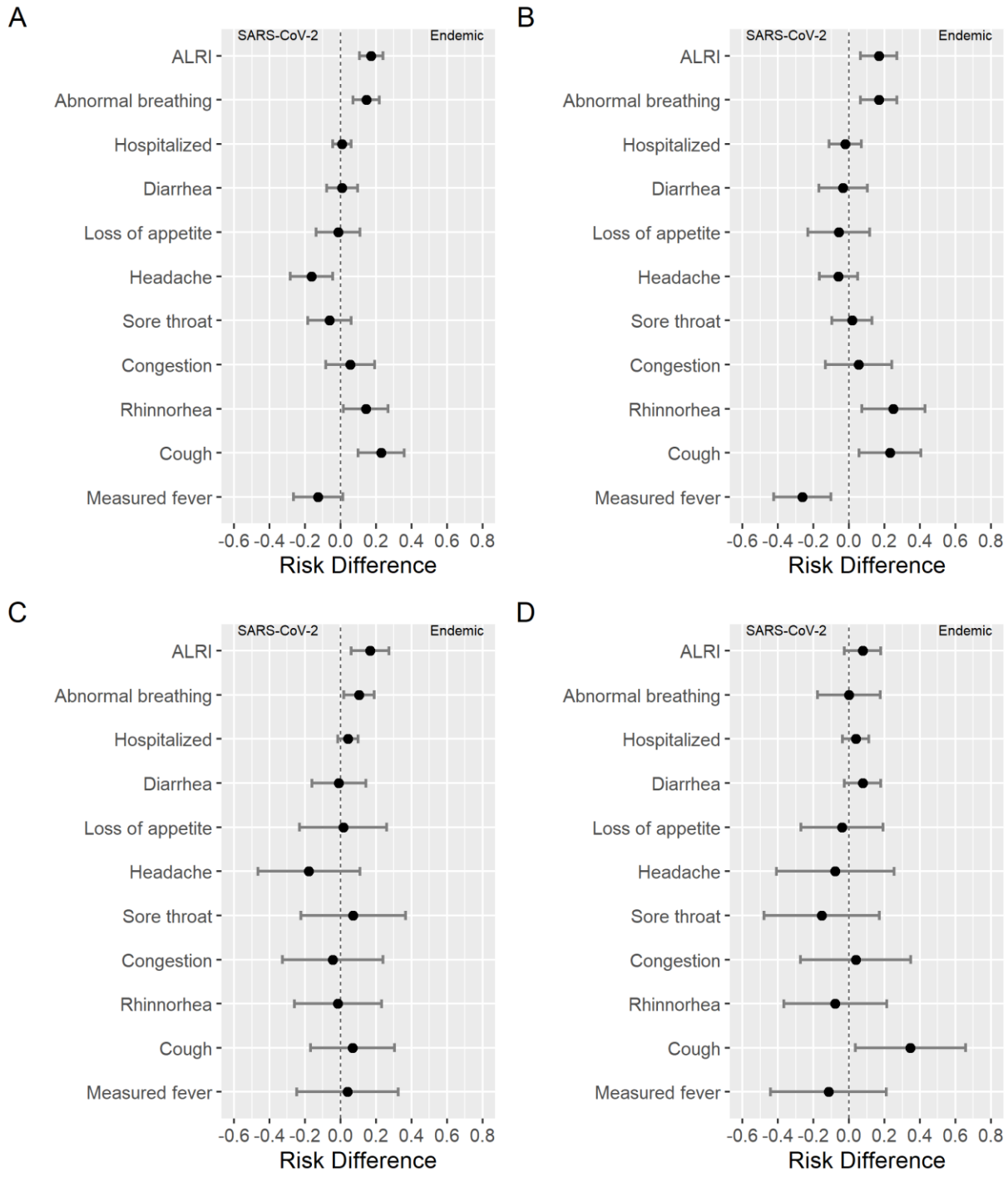

Figure B.2: Symptom Risk Difference between Endemic HCoVs and SARS-CoV-2 for Males

A: All participants. B: Ages 0-4. C: Ages 5-9. D Ages: 10-14.

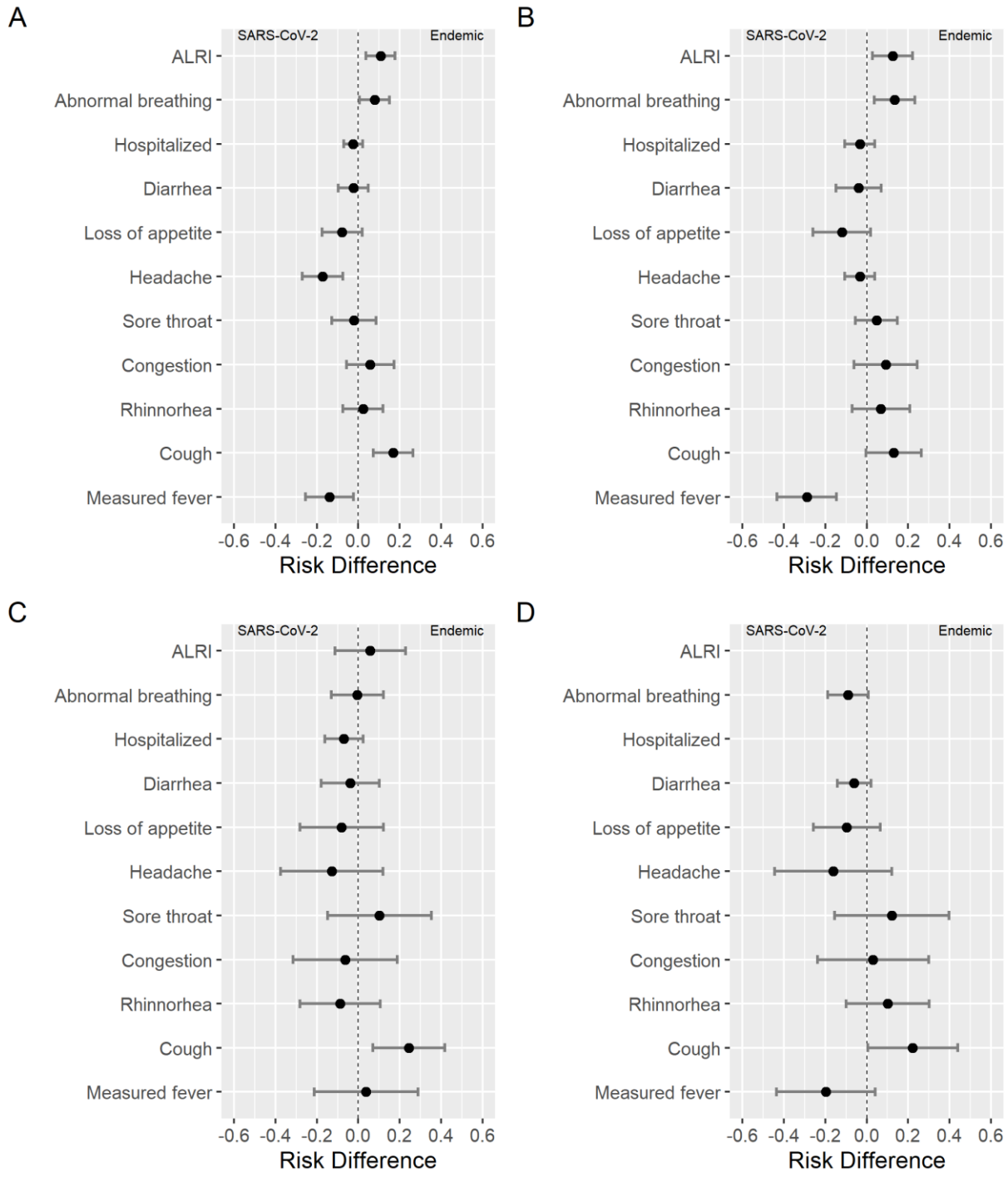

Figure B.3: Symptom Risk Difference NL63 and SARS-CoV-2

A: All participants. B: Ages 0-4. C: Ages 5-9. D Ages: 10-14.

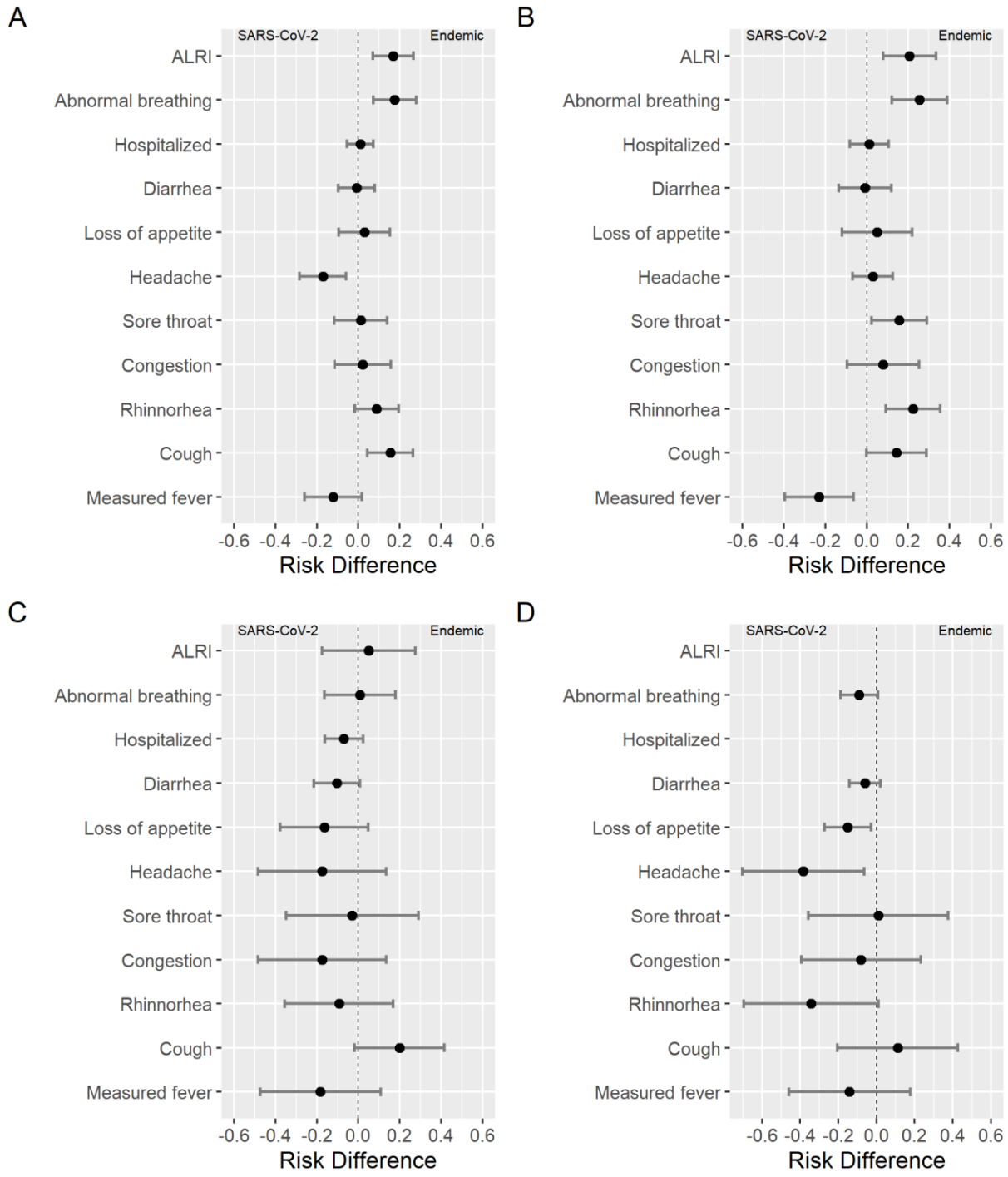

Figure B.4: Symptom Risk Difference between 229E and SARS-CoV-2

A: All participants. B: Ages 0-4. C: Ages 5-9. D Ages: 10-14.

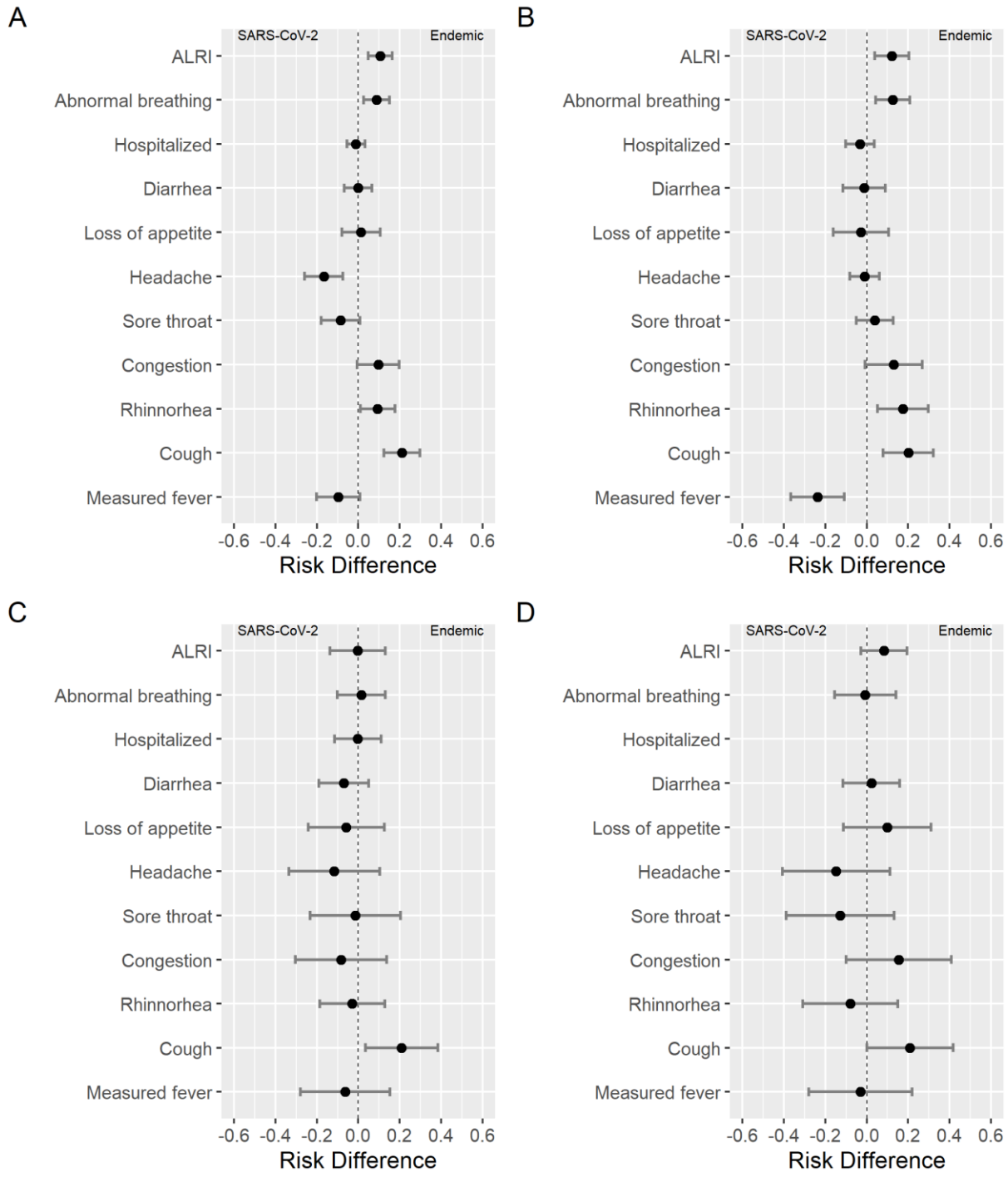

Figure B.5: Symptom Risk Difference between OC43 and SARS-CoV-2

A: All participants. B: Ages 0-4. C: Ages 5-9. D Ages: 10-14.

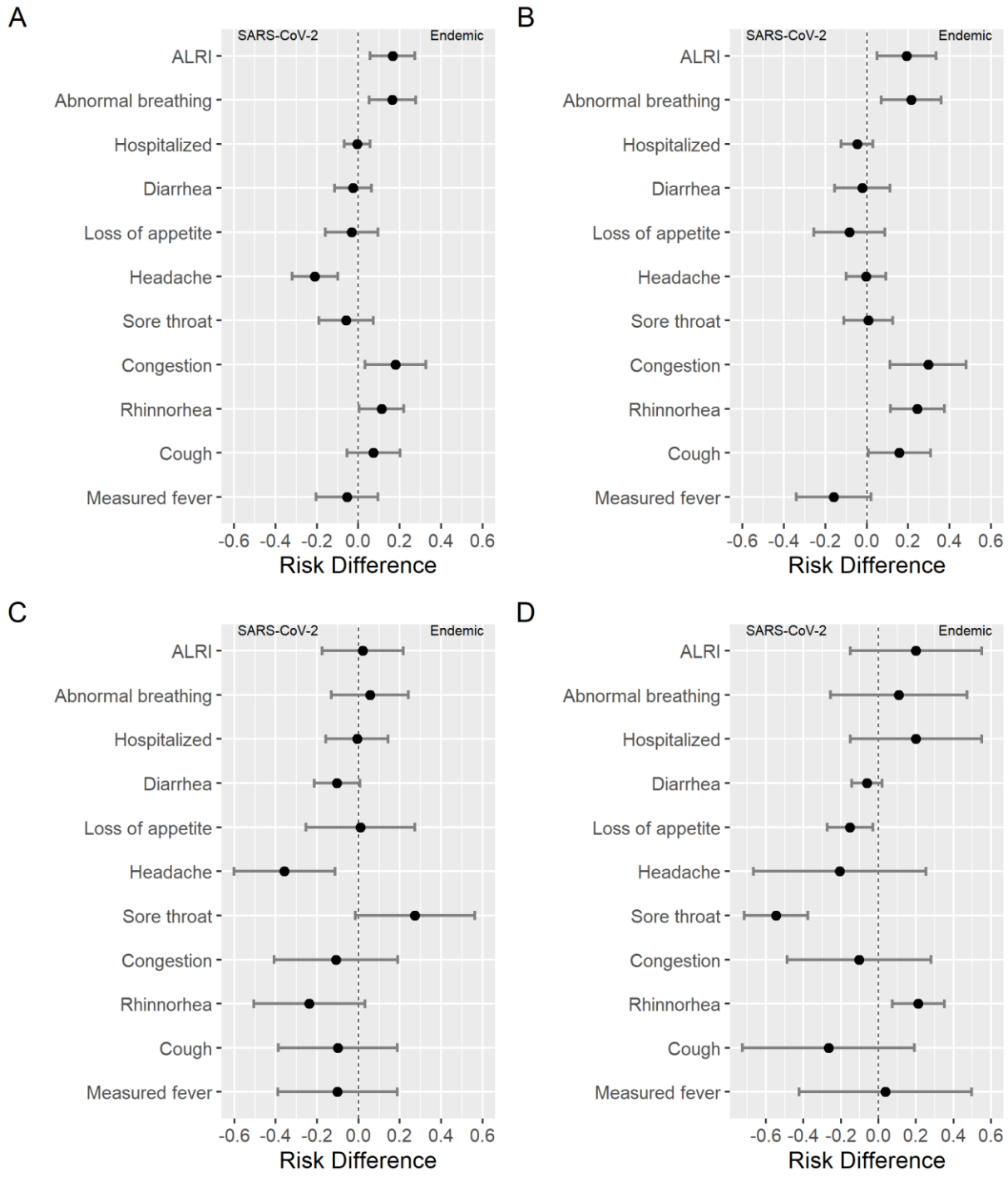

Figure B.6: Symptom Risk Difference between HKU1 and SARS-CoV-2

A: All participants. B: Ages 0-4. C: Ages 5-9. D Ages: 10-14.

Appendix C: Supplemental Material for Chapter 4

Supplementary Methods

Risk factor surveys were collected annually from March-April included surveys information on height and weight, household demographics, bed- and room-sharing, individual and household smoking, household assets, and education. Bedroom- and bed-sharing information was available from household enrollment surveys. 684/902 (75.8%) infectious household members had complete information about bedroom- and bed-sharing.

A separate consent was collected for the sub-study; day of enrollment into the sub-study was considered day 0. Households were eligible for household activation again after completing the final follow-up visit. Day of onset for each symptom was also recorded, including if symptoms began before sub-study enrollment.

Screening for IgG antibodies was conducted with RBD proteins because it is more specific than spike antigen. [3] RBD and N proteins for ELISA were produced in single batches at the Life Sciences Institute at the University of Michigan. As this was an ongoing cohort, we were able to use blood samples collected in March-April 2019, March-April 2020, October-December 2020, March-April 2021, October-December 2021, and March-April 2022 to detect serologically confirmed infections.

Example of estimated infection date for serologically confirmed infections: if a 2020 midyear blood showed a serologically confirmed infection, then a random day was selected during the wave that occurred between May 2020 and August 2020.

Pairwise survival models estimate the contact interval distributions in all pairs consisting of an infectious household member and a susceptible member of the same household. The contact interval in each pair is the time from the onset of infectiousness in the infected individual to the transmission of infection within the pair. If the infected individual infects the susceptible individual, this is an observed contact interval. The contact interval is right-censored if the susceptible is infected from another source, the infected individual recovers without making infectious contact, or observation ends while the pair is still at risk of transmission. The data on within-household transmission is set up with a row for each pair, a start time and end time for risk of transmission, an outcome indicator, and covariates.

To account for external risk of transmission, an external rate parameter was estimated by including all HICS participants (even if they were never activated) in the model. These data were set-up as if for a traditional survival analysis with a row for each participant, a start time and end time for risk of infection, an outcome indicator, and covariates. An additional indicator variable is included which distinguishes between external and internal rows.

For both the external and internal models, we used a log-logistic contact interval distribution which generally produced better model fit (lower AIC) than the exponential or Weibull distributions.

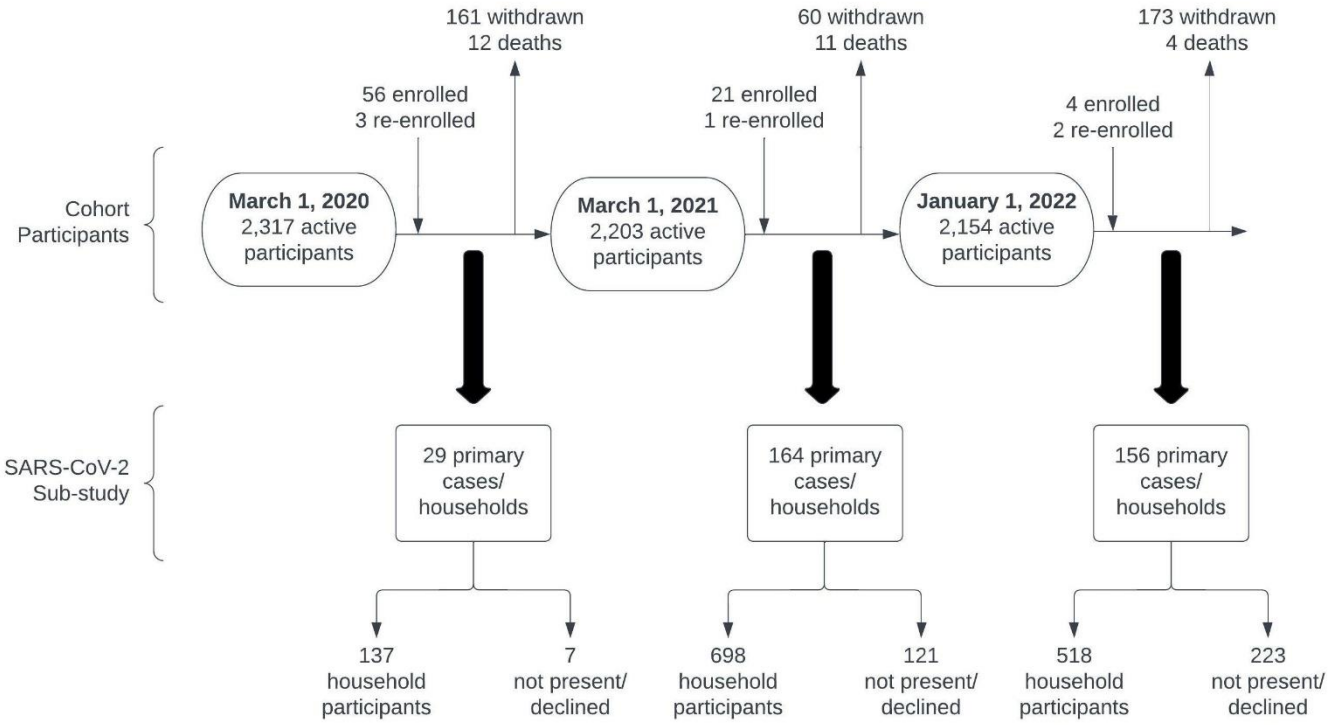

Figure C.8:Flowchart of participants by year

The top section includes all household cohort participants regardless of SARS-CoV-2 sub-study participation. The lower section shows household activations and subsequent participation as part of the sub-study.

Table C.1: Primary case characteristics by presence of household transmission

	≥ 1 Household Contact PCR+		
	No (n=104)	Yes (n=245)	p-value*
Pandemic Period (%)			0.0566
Mar 2020 - Feb 2021	14 (13.5)	15 (6.1)	
Mar 2021 - Dec 2021	43 (41.4)	121 (49.4)	
Jan 2022 - May 2022	47 (45.2)	109 (44.5)	
Female (%)	71 (68.3)	157 (64.1)	0.4522
Age Group (%)			0.0134
0-4	15 (14.2)	13 (5.3)	
5-10	18 (17.3)	48 (19.6)	
11-19	29 (27.9)	53 (21.6)	
20-64	36 (34.6)	120 (49.0)	
65+	6 (5.8)	11 (4.5)	
Share bedroom	85 (81.7)	201 (82.0)	0.9451
Share bed	53 (51.0)	146 (59.6)	0.1363
Prior SARS-CoV-2 Infections (%)			0.8315
0	49 (47.1)	123 (50.2)	
1	48 (46.2)	107 (43.7)	
2	6 (5.8)	14 (5.7)	
3	1 (1.0)	1 (0.4)	
Completed SARS-CoV-2 Vaccinations (%)	17 (16.4)	42 (17.1)	0.8559
Symptoms			
Cough	78 (75.0)	190 (77.6)	0.6056
Rhinorrhea	71 (68.3)	179 (73.1)	0.3637

*from chi-square or Fisher's exact test, uncorrected

A chi-square test was used to compared demographics of primary cases in households with transmission (at least one household member PCR+) and without transmission (no household member PCR-).

Table C.2: Household contact characteristics by SARS-CoV-2 infection

	PCR- Contacts (n=800)	PCR+ Contacts (n=553)	p-value*
Female	499 (62.4)	337 (60.9)	0.5934
Age Group (%)			0.6603
0-4	72 (9.0)	42 (7.6)	
5-10	164 (20.5)	118 (21.3)	
11-19	185 (23.1)	140 (25.3)	
20-64	342 (42.8)	223 (40.3)	
65+	37 (4.6)	30 (5.4)	
Share bedroom	649 (81.1)	467 (84.5)	0.1139
Share bed	480 (60.0)	325 (58.8)	0.6506
Prior SARS-CoV-2 Infections (%)			0.0029
0	271 (33.9)	242 (43.8)	
1	470 (58.8)	276 (49.9)	
2	52 (6.5)	33 (5.0)	
3	6 (0.8)	2 (0.4)	
4	1 (0.1)	0	
Completed SARS-CoV-2 Vaccination (%)	124 (15.5)	102 (18.4)	0.1534

*from chi-square or Fisher's exact test, uncorrected

A chi-square test was used to compared demographics of household members (excluding primary cases) that were PCR+ and PCR- during intensive monitoring.

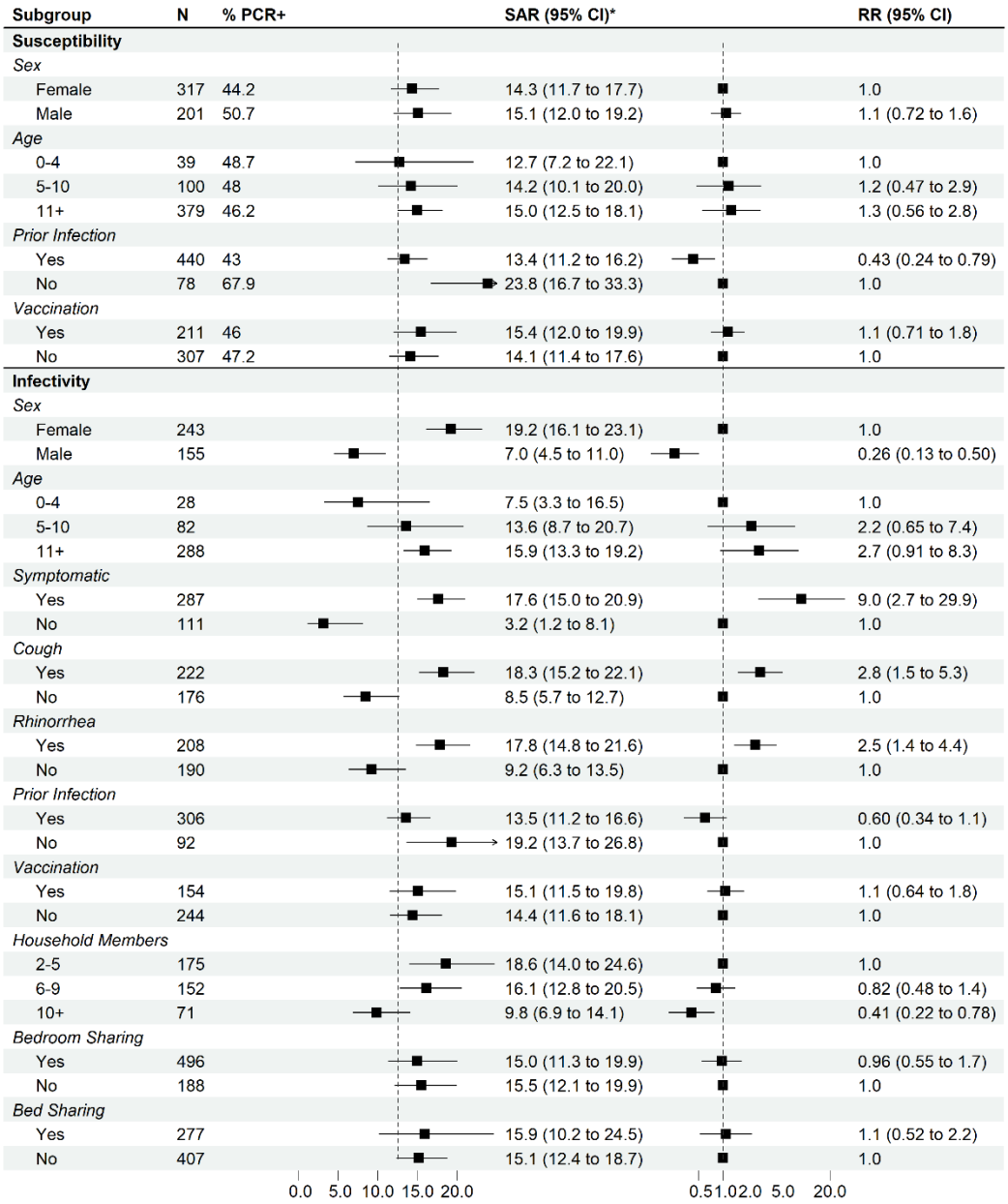

Figure C.9: Omicron era estimated secondary attack risk and rate ratios

Results are from the period of Omicron spread included in this analysis (January 2022-November 2022) The models are univariate and only include the intercept, and log-shape parameters in additional to the single variable of interest. Variables are grouped by susceptible variables (characteristics of the susceptible individual in the paired data) and infector variables (characteristics of the infectious individual in the paired data).

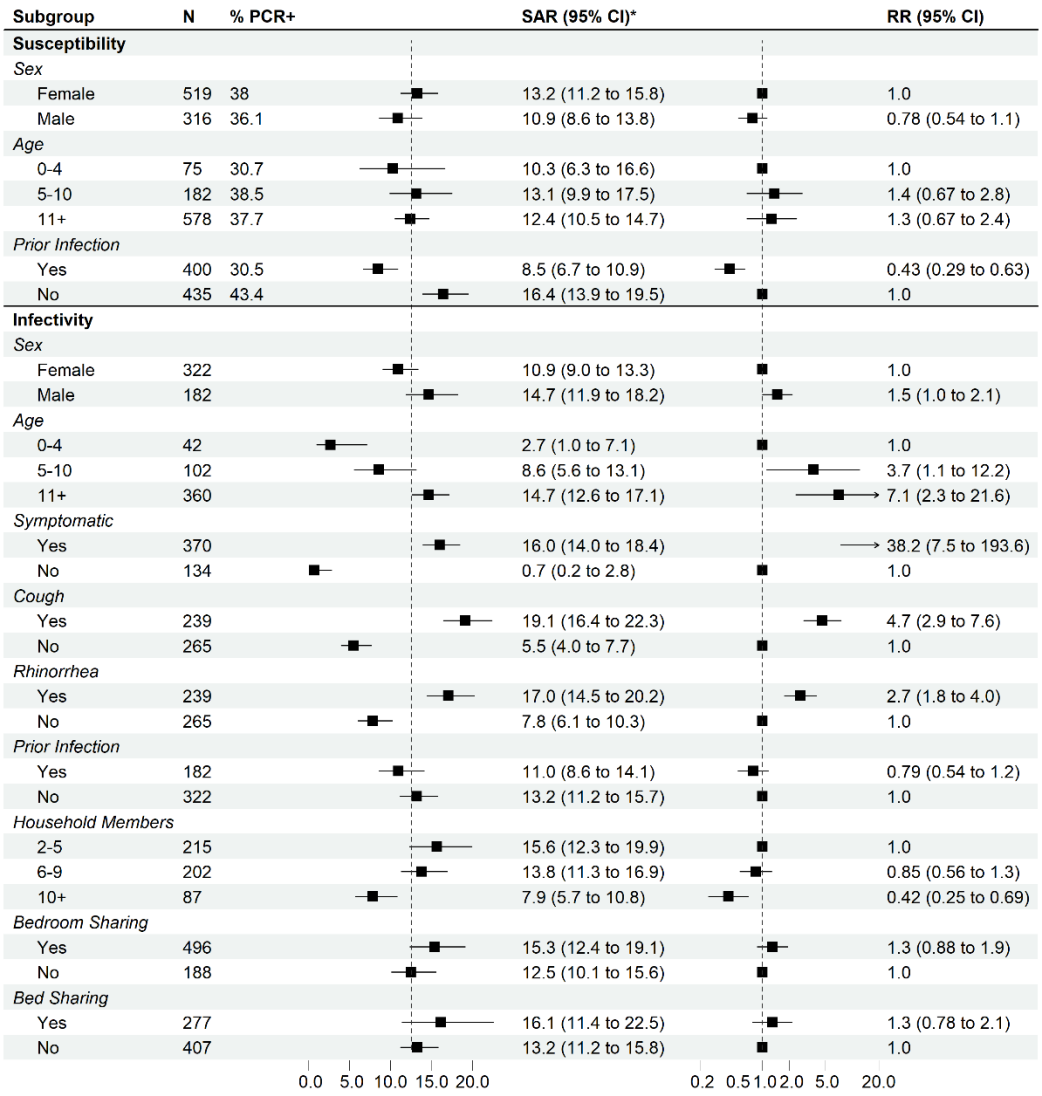

Figure C.3: Pre-Omicron era estimated secondary attack risk and rate ratios

Results are from the period of pre-Omicron spread included in this analysis (March 2020-Dec 2021) The models are univariate and only include the intercept, and log-shape parameters in additional to the single variable of interest. Variables are grouped by susceptible variables (characteristics of the susceptible individual in the paired data) and infector variables (characteristics of the infectious individual in the paired data).

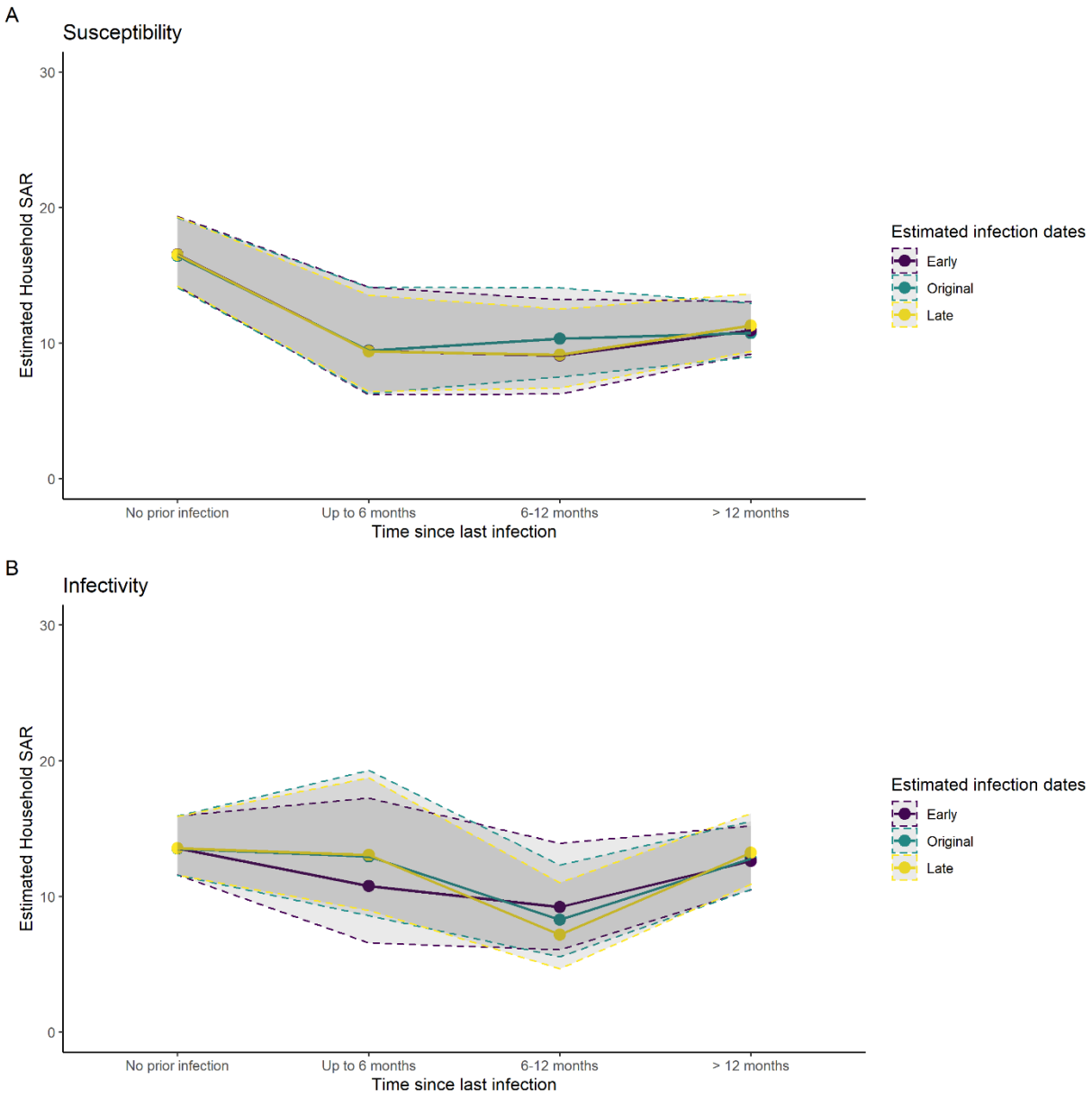

Figure C.4: SAR and time since last infection after adjusting serologically detected infection dates

Results of the sensitivity analysis adjusting the estimated infection dates for serologically detected infections are also included. The original method was a randomly selected day during the epidemic wave prior to the blood sample collection. Early refers to estimates that all serologically detected infections occurred within the first 15 days of the epidemic wave prior to blood sample collection. Late refers to estimates that all serologically detected infections occurred within the last 15 days of the epidemic wave prior to blood sample collection. Date for infections detected via PCR did not change. A- Time since last infection of the susceptible individual. B- Time since last infection for the infectious individual.

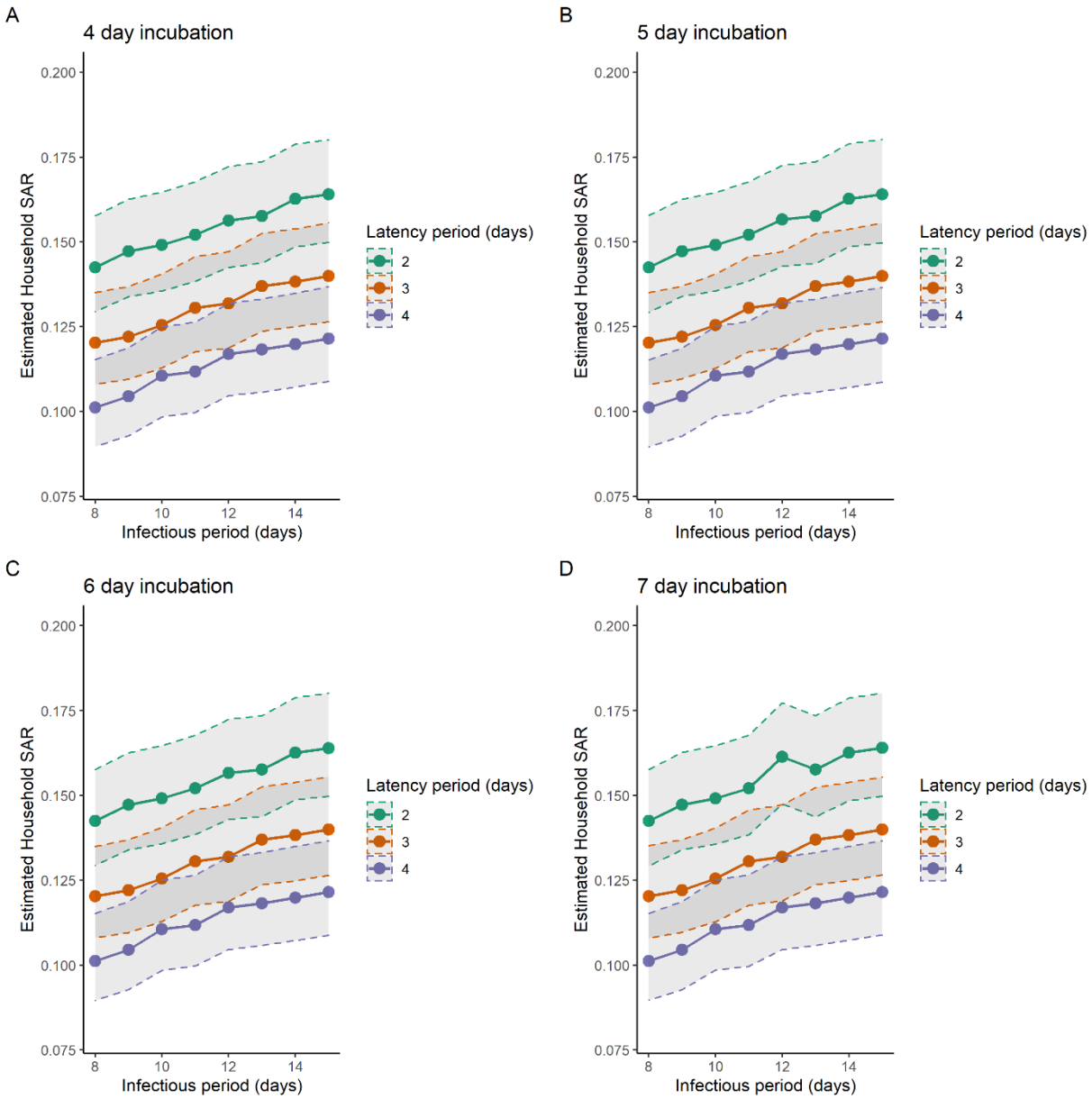

Figure C.5: Secondary attack risk by changes in the latency, incubation, and infectivity periods

Results of the sensitivity analysis adjusting the contact intervals by altering the latency, incubation, and infectivity periods of infection. A- 4-dayday incubation period. B- 5-day incubation period. C- 6-day incubation period. D- 7-day incubation period.

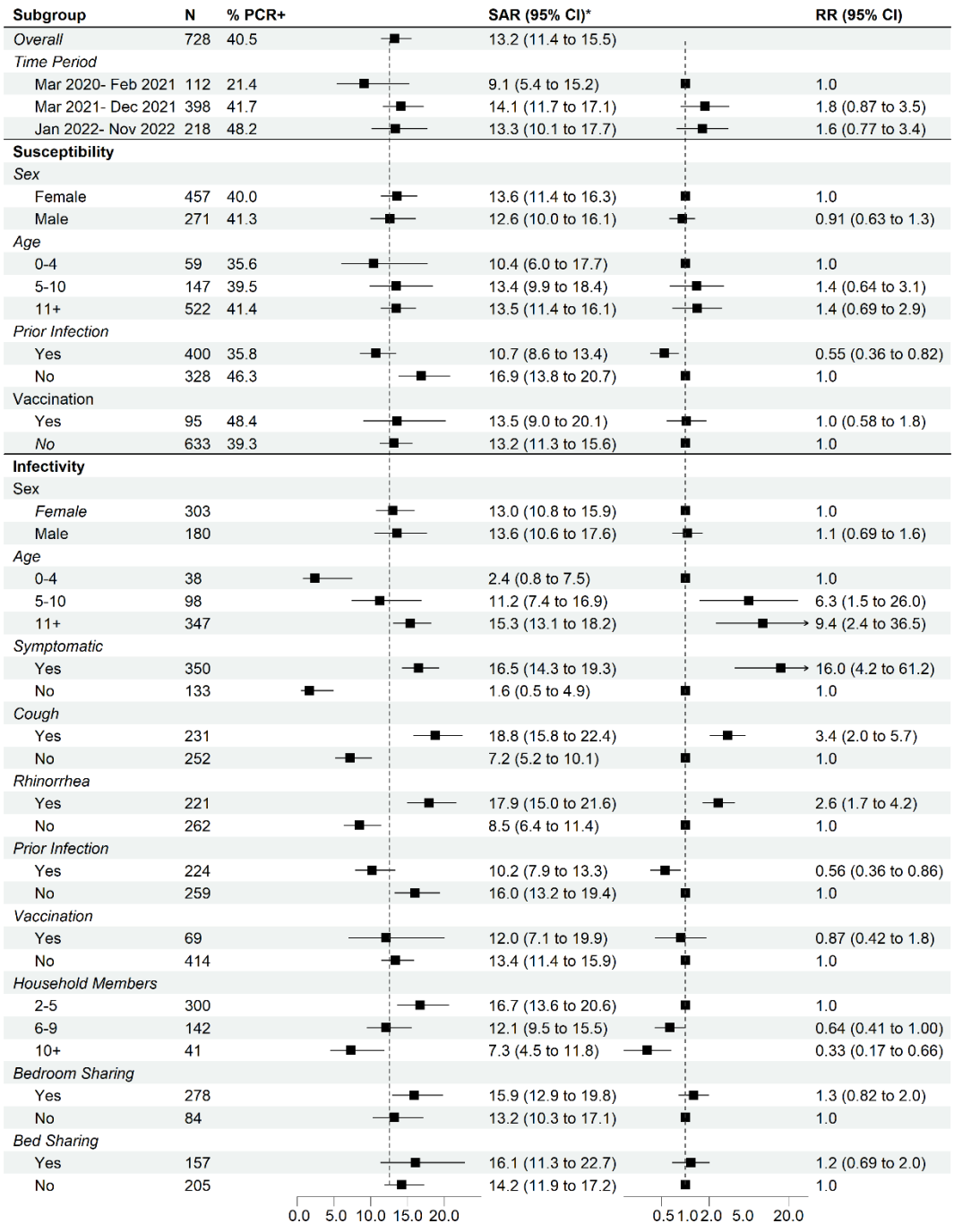

Figure C.6: Households with complete participation, estimated secondary attack risk and rate ratios

Results of the sensitivity analysis limiting to only households where all member consented to participation. The models are univariate and only include the intercept, and log-shape parameters in additional to the single variable of interest. Variables are grouped by susceptible variables (characteristics of the susceptible individual in the paired data) and infector variables (characteristics of the infectious individual in the paired data).

Bibliography

1. Tyrrell DA, Bynoe ML. Cultivation of a Novel Type of Common-Cold Virus in Organ Cultures. Br Med J 1965;1:1467-70.
2. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. Proc Soc Exp Biol Med 1966;121:190-3.
3. Ogimi C, Kim YJ, Martin ET, Huh HJ, Chiu CH, Englund JA. What's New With the Old Coronaviruses? J Pediatric Infect Dis Soc 2020;9:210-7.
4. Ogimi C, Englund JA, Bradford MC, Qin X, Boeckh M, Waghmare A. Characteristics and Outcomes of Coronavirus Infection in Children: The Role of Viral Factors and an Immunocompromised State. J Pediatric Infect Dis Soc 2019;8:21-8.
5. Nickbakhsh S, Ho A, Marques DFP, McMenamin J, Gunson RN, Murcia PR. Epidemiology of seasonal coronaviruses: Establishing the context for COVID-19 emergence. J Infect Dis 2020.
6. Gaunt ER, Hardie A, Claas EC, Simmonds P, Templeton KE. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. J Clin Microbiol 2010;48:2940-7.
7. McIntosh K, Perlman S, Monto A, Englund JA. PERSPECTIVE: A Proposal To Name Four Coronaviruses of Limited Virulence "Common Cold Coronaviruses". J Infect Dis 2022.
8. Lavine JS, Bjornstad ON, Antia R. Immunological characteristics govern the transition of COVID-19 to endemicity. Science 2021;371:741-5.
9. Uddin SMI, Englund JA, Kuypers JY, et al. Burden and Risk Factors for Coronavirus Infections in Infants in Rural Nepal. Clin Infect Dis 2018;67:1507-14.
10. Heimdal I, Moe N, Krokstad S, et al. Human Coronavirus in Hospitalized Children With Respiratory Tract Infections: A 9-Year Population-Based Study From Norway. J Infect Dis 2019;219:1198-206.
11. Zhou W, Wang W, Wang H, Lu R, Tan W. First infection by all four non-severe acute respiratory syndrome human coronaviruses takes place during childhood. BMC Infect Dis 2013;13:433.
12. Monto AS, DeJonge P, Callear AP, et al. Coronavirus occurrence and transmission over 8 years in the HIVE cohort of households in Michigan. J Infect Dis 2020.
13. Kumar P, Mukherjee A, Randev S, et al. Epidemiology of Coronavirus Infection in Children and Their Impact on Lung Health: Finding from a Birth Cohort Study. Pediatr Infect Dis J 2020.
14. Otieno GP, Murunga N, Agoti CN, Gallagher KE, Awori JO, Nokes DJ. Surveillance of endemic human coronaviruses (HCoV-NL63, OC43 and 229E) associated with childhood pneumonia in Kilifi, Kenya. Wellcome Open Res 2020;5:150.
15. Zeng ZQ, Chen DH, Tan WP, et al. Epidemiology and clinical characteristics of human coronaviruses OC43, 229E, NL63, and HKU1: a study of hospitalized children with acute respiratory tract infection in Guangzhou, China. Eur J Clin Microbiol Infect Dis 2018;37:363-9.
16. van der Hoek L, Sure K, Ihorst G, et al. Croup is associated with the novel coronavirus NL63. PLoS Med 2005;2:e240.

17. Transmission of SARS-CoV-2 in K-12 schools. COVID-19 Science and Research: National Center for Immunization and Respiratory Diseases; 2021.
18. Song X, Delaney M, Shah RK, Campos JM, Wessel DL, DeBiasi RL. Comparison of Clinical Features of COVID-19 vs Seasonal Influenza A and B in US Children. JAMA Netw Open 2020;3:e2020495.
19. Siddiqui M, Gultekingil A, Bakirci O, Uslu N, Baskin E. Comparison of clinical features and laboratory findings of coronavirus disease 2019 and influenza A and B infections in children: a single-center study. Clin Exp Pediatr 2021.
20. Li Y, Wang H, Wang F, et al. Comparison of hospitalized patients with pneumonia caused by COVID-19 and influenza A in children under 5 years. Int J Infect Dis 2020;98:80-3.
21. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. Nat Med 2020.
22. Petrie JG, Bazzi LA, McDermott AB, et al. Coronavirus Occurrence in the Household Influenza Vaccine Evaluation (HIVE) Cohort of Michigan Households: Reinfection Frequency and Serologic Responses to Seasonal and Severe Acute Respiratory Syndrome Coronaviruses. J Infect Dis 2021;224:49-59.
23. Kolehmainen P, Heroum J, Jalkanen P, et al. Serological Follow-Up Study Indicates High Seasonal Coronavirus Infection and Reinfection Rates in Early Childhood. Microbiol Spectr 2022;10:e0196721.
24. Pulliam JRC, van Schalkwyk C, Govender N, et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of Omicron in South Africa. Science 2022;376:eabn4947.
25. Powell AA, Kirsebom F, Stowe J, et al. Protection against symptomatic infection with delta (B.1.617.2) and omicron (B.1.1.529) BA.1 and BA.2 SARS-CoV-2 variants after previous infection and vaccination in adolescents in England, August, 2021-March, 2022: a national, observational, test-negative, case-control study. Lancet Infect Dis 2022.
26. Kubale J, Balmaseda A, Frutos AM, et al. Association of SARS-CoV-2 Seropositivity and Symptomatic Reinfection in Children in Nicaragua. JAMA Netw Open 2022;5:e2218794.
27. Khan T, Rahman M, Ali FA, et al. Distinct antibody repertoires against endemic human coronaviruses in children and adults. JCI Insight 2021;6.
28. Kim KY, Han SY, Kim HS, Cheong HM, Kim SS, Kim DS. Human Coronavirus in the 2014 Winter Season as a Cause of Lower Respiratory Tract Infection. Yonsei Med J 2017;58:174-9.
29. Varghese L, Zachariah P, Vargas C, et al. Epidemiology and Clinical Features of Human Coronaviruses in the Pediatric Population. J Pediatric Infect Dis Soc 2018;7:151-8.
30. Masse S, Capai L, Villechenaud N, Blanchon T, Charrel R, Falchi A. Epidemiology and Clinical Symptoms Related to Seasonal Coronavirus Identified in Patients with Acute Respiratory Infections Consulting in Primary Care over Six Influenza Seasons (2014-2020) in France. Viruses 2020;12.
31. Killerby ME, Biggs HM, Haynes A, et al. Human coronavirus circulation in the United States 2014-2017. J Clin Virol 2018;101:52-6.
32. Razuri H, Malecki M, Tinoco Y, et al. Human Coronavirus-Associated Influenza-Like Illness in the Community Setting in Peru. Am J Trop Med Hyg 2015;93:1038-40.
33. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. J Clin Virol 2010;49:16-20.

34. Gordon A, Kuan G, Aviles W, et al. The Nicaraguan pediatric influenza cohort study: design, methods, use of technology, and compliance. BMC Infect Dis 2015;15:504.
35. Real-Time RT-PCR Assays for Non-Influenza Respiratory Viruses. In: Diseases NCfIaR, ed.: Centers for Disease Control and Prevention; 2015:1-16.
36. Frutos AM, Kubale J, Kuan G, et al. SARS-CoV-2 and endemic coronaviruses: Comparing symptom presentation and severity of symptomatic illness among Nicaraguan children. PLOS Glob Public Health 2022;2.
37. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. Current Protocols in Microbiology 2020;57:e100.
38. Torrence C, Compo GP. A practical guide to wavelet analysis. Bulletin of the American Meteorological society 1998;79:61-78.
39. Bakker KM, Eisenberg MC, Woods R, Martinez ME. Exploring the Seasonal Drivers of Varicella Zoster Virus Transmission and Reactivation. Am J Epidemiol 2021;190:1814-20.
40. Issartel J, Bardainne T, Gaillot P, Marin L. The relevance of the cross-wavelet transform in the analysis of human interaction - a tutorial. Front Psychol 2014;5:1566.
41. Jalal H, Burke DS. Hexamaps for Age-Period-Cohort Data Visualization and Implementation in R. Epidemiology 2020;31:e47-e9.
42. Kenmoe S, Tchendjou P, Vernet MA, et al. Viral etiology of severe acute respiratory infections in hospitalized children in Cameroon, 2011-2013. Influenza Other Respir Viruses 2016;10:386-93.
43. Goes LGB, Zerbinati RM, Tateno AF, et al. Typical epidemiology of respiratory virus infections in a Brazilian slum. J Med Virol 2019.
44. Wong-Chew RM, Garcia-Leon ML, Noyola DE, et al. Respiratory viruses detected in Mexican children younger than 5 years old with community-acquired pneumonia: a national multicenter study. Int J Infect Dis 2017;62:32-8.
45. Cabeca TK, Granato C, Bellei N. Epidemiological and clinical features of human coronavirus infections among different subsets of patients. Influenza Other Respir Viruses 2013;7:1040-7.
46. Shah MM, Winn A, Dahl RM, Kniss KL, Silk BJ, Killerby ME. Seasonality of Common Human Coronaviruses in the United States, 2014-2021. medRxiv 2022:2022.03.22.22272640.
47. Huang AT, Garcia-Carreras B, Hitchings MDT, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. Nat Commun 2020;11:4704.
48. Bradburne AF. Antigenic relationships amongst coronaviruses. Arch Gesamte Virusforsch 1970;31:352-64.
49. Agnihothram S, Gopal R, Yount BL, Jr., et al. Evaluation of serologic and antigenic relationships between middle eastern respiratory syndrome coronavirus and other coronaviruses to develop vaccine platforms for the rapid response to emerging coronaviruses. J Infect Dis 2014;209:995-1006.
50. Dijkman R, Jebbink MF, Gaunt E, et al. The dominance of human coronavirus OC43 and NL63 infections in infants. J Clin Virol 2012;53:135-9.
51. Gao X, Zhou H, Wu C, et al. Antibody against nucleocapsid protein predicts susceptibility to human coronavirus infection. J Infect 2015;71:599-602.
52. Macnaughton MR, Madge MH, Reed SE. Two antigenic groups of human coronaviruses detected by using enzyme-linked immunosorbent assay. Infect Immun 1981;33:734-7.

53. Trivedi SU, Miao C, Sanchez JE, et al. Development and Evaluation of a Multiplexed Immunoassay for Simultaneous Detection of Serum IgG Antibodies to Six Human Coronaviruses. Sci Rep 2019;9:1390.
54. Kim JM, Jung HD, Cheong HM, et al. Nation-wide surveillance of human acute respiratory virus infections between 2013 and 2015 in Korea. J Med Virol 2018;90:1177-83.
55. Frutos AM, Kuan G, Lopez R, et al. Infection-induced immunity is associated with protection against SARS-CoV-2 infection, but not decreased infectivity during household transmission. medRxiv 2022:2022.10.10.22280915.
56. Liu P, Shi L, Zhang W, et al. Prevalence and genetic diversity analysis of human coronaviruses among cross-border children. Virol J 2017;14:230.
57. Dong Y, Mo X, Hu Y, et al. Epidemiology of COVID-19 Among Children in China. Pediatrics 2020;145.
58. Do LAH, Anderson J, Mulholland EK, Licciardi PV. Can data from paediatric cohorts solve the COVID-19 puzzle? PLoS Pathog 2020;16:e1008798.
59. Dhochak N, Singhal T, Kabra SK, Lodha R. Pathophysiology of COVID-19: Why Children Fare Better than Adults? Indian J Pediatr 2020;87:537-46.
60. Pokorska-Spiewak M, Talarek E, Popielska J, et al. Comparison of clinical severity and epidemiological spectrum between coronavirus disease 2019 and influenza in children. Sci Rep 2021;11:5760.
61. COVID-19 advice for the public: Getting vaccinated. World Health Organization, 2022. (Accessed 1/24/2022, 2022, at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice.)
62. Nicaragua to Immunize the Child Population With Cuban Vaccines. teleSUR, 2021. (Accessed 1/24/2022, at https://www.telesurenglish.net/news/Nicaragua-to-Immunize-the-Child-Population-With-Cuban-Vaccines-20211003-0002.html.)
63. Reardon S. Cuba's bet on home-grown COVID vaccines is paying off. Nature 2021;600:15-6.
64. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020;57:e100.
65. Maier HE, Kuan G, Saborio S, et al. Clinical spectrum of SARS-CoV-2 infection and protection from symptomatic re-infection. Clin Infect Dis 2021.
66. Chu DKW, Pan Y, Cheng SMS, et al. Molecular Diagnosis of a Novel Coronavirus (2019-nCoV) Causing an Outbreak of Pneumonia. Clin Chem 2020;66:549-55.
67. WHO Regional Office for Europe guidance for sentinel influenza surveillance in humans: May 2011 Edition. Copenhagen, Denmark: The Regional Office for Europe of the World Health Organization; 2011.
68. de Koff EM, van Houten MA, Sanders EAM, Bogaert D. Severity of Respiratory Infections With Seasonal Coronavirus Is Associated With Viral and Bacterial Coinfections. Pediatr Infect Dis J 2020.
69. Molteni E, Sudre CH, Canas LS, et al. Illness duration and symptom profile in symptomatic UK school-aged children tested for SARS-CoV-2. Lancet Child Adolesc Health 2021.
70. Children and COVID-19: State-Level Data Report: American Academy of Pediatrics

2021 August 12, 2021.

71. Payne AB, Gilani Z, Godfred-Cato S, et al. Incidence of Multisystem Inflammatory Syndrome in Children Among US Persons Infected With SARS-CoV-2. JAMA Netw Open 2021;4:e2116420.
72. Madewell ZJ, Yang Y, Longini IM, Jr., Halloran ME, Dean NE. Household Secondary Attack Rates of SARS-CoV-2 by Variant and Vaccination Status: An Updated Systematic Review and Meta-analysis. JAMA Netw Open 2022;5:e229317.
73. Jung J, Kim JY, Park H, et al. Transmission and Infectious SARS-CoV-2 Shedding Kinetics in Vaccinated and Unvaccinated Individuals. JAMA Netw Open 2022;5:e2213606.
74. Maier HE, Plazaola M, Lopez R, et al. SARS-CoV-2 infection-induced immunity and the duration of viral shedding: Results from a Nicaraguan household cohort study. Influenza Other Respir Viruses 2022.
75. Tan ST, Kwan AT, Rodriguez-Barraquer I, et al. Infectiousness of SARS-CoV-2 breakthrough infections and reinfections during the Omicron wave. Nat Med 2023.
76. Madhi SA, Kwatra G, Myers JE, et al. Population Immunity and Covid-19 Severity with Omicron Variant in South Africa. N Engl J Med 2022;386:1314-26.
77. Almudarra S, Kamel S, Saleh E, et al. High seroprevalence of SARS-CoV-2 among high-density communities in Saudi Arabia. Infection 2022;50:643-9.
78. Interim statement on COVID-19 vaccination for children and adolescents. World Health Organization; 2021.
79. Dattner I, Goldberg Y, Katriel G, et al. The role of children in the spread of COVID-19: Using household data from Bnei Brak, Israel, to estimate the relative susceptibility and infectivity of children. PLoS Comput Biol 2021;17:e1008559.
80. Kim J, Choe YJ, Lee J, et al. Role of children in household transmission of COVID-19. Arch Dis Child 2021;106:709-11.
81. Zhu Y, Bloxham CJ, Hulme KD, et al. A Meta-analysis on the Role of Children in Severe Acute Respiratory Syndrome Coronavirus 2 in Household Transmission Clusters. Clin Infect Dis 2021;72:e1146-e53.
82. Li F, Li YY, Liu MJ, et al. Household transmission of SARS-CoV-2 and risk factors for susceptibility and infectivity in Wuhan: a retrospective observational study. Lancet Infect Dis 2021;21:617-28.
83. Zhu Y, Xia Y, Pickering J, Bowen AC, Short KR. The Role of Children in SARS-CoV-2 Variant of Concerns Transmission within Households: A Meta-analysis. medRxiv 2022:2022.07.21.22277914.
84. Maier HE, Balmaseda A, Saborio S, et al. Protection Associated with Previous SARS-CoV-2 Infection in Nicaragua. N Engl J Med 2022;387:568-70.
85. Kubale JT, Frutos AM, Balmaseda A, et al. High Co-circulation of Influenza and Severe Acute Respiratory Syndrome Coronavirus 2. Open Forum Infect Dis 2022;9:ofac642.
86. Sharker Y, Kenah E. Estimating and interpreting secondary attack risk: Binomial considered biased. PLoS Comput Biol 2021;17:e1008601.
87. Kenah E. Handbook of infectious disease data analysis. In: Held Lee, Hens Nee, O'Neill PDee, Wallinga Jee, eds. Chapman & Hall/CRC handbooks of modern statistical methods: CRC Press; 2020:221-42.
88. Sharker Y, Kenah E. Pairwise Accelerated Failure Time Models for Infectious Disease Transmission with External Sources of Infection. arXiv 2019.

89. Ending Isolation and Precautions for People with COVID-19: Interim Guidance. U.S. Department of Health & Human Services, 2022. (Accessed 5/6/2022, 2022, at https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html.)
90. Xin H, Li Y, Wu P, et al. Estimating the Latent Period of Coronavirus Disease 2019 (COVID-19). Clin Infect Dis 2022;74:1678-81.
91. Zhao S, Tang B, Musa SS, et al. Estimating the generation interval and inferring the latent period of COVID-19 from the contact tracing data. Epidemics 2021;36:100482.
92. transtat: Statistical Methods for Infectious Disease Transmission. 2021. at https://github.com/ekenah/transtat.)
93. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature 2021;595:426-31.
94. Goldberg Y, Mandel M, Bar-On YM, et al. Protection and Waning of Natural and Hybrid Immunity to SARS-CoV-2. N Engl J Med 2022;386:2201-12.
95. Silverberg SL, Zhang BY, Li SNJ, et al. Child transmission of SARS-CoV-2: a systematic review and meta-analysis. BMC Pediatr 2022;22:172.
96. Di Chiara C, Cantarutti A, Costenaro P, et al. Long-term Immune Response to SARS-CoV-2 Infection Among Children and Adults After Mild Infection. JAMA Netw Open 2022;5:e2221616.
97. Bobrovitz N, Ware H, Ma X, et al. Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. The Lancet Infectious Diseases 2023.
98. Chen F, Tian Y, Zhang L, Shi Y. The role of children in household transmission of COVID-19: a systematic review and meta-analysis. Int J Infect Dis 2022;122:266-75.
99. Lewnard JA, Hong VX, Patel MM, Kahn R, Lipsitch M, Tartof SY. Clinical outcomes associated with SARS-CoV-2 Omicron (B.1.1.529) variant and BA.1/BA.1.1 or BA.2 subvariant infection in Southern California. Nat Med 2022;28:1933-43.
100. Britton A, Embi PJ, Levy ME, et al. Effectiveness of COVID-19 mRNA Vaccines Against COVID-19-Associated Hospitalizations Among Immunocompromised Adults During SARS-CoV-2 Omicron Predominance - VISION Network, 10 States, December 2021-August 2022. MMWR Morb Mortal Wkly Rep 2022;71:1335-42.
101. Risk for COVID-19 Infection, Hospitalization, and Death By Age Group. Centers for Disease Control and Prevention

2022. 2023,
102. Chemaitelly H, Ayoub HH, Tang P, et al. Immune Imprinting and Protection against Repeat Reinfection with SARS-CoV-2. N Engl J Med 2022;387:1716-8.
103. Aydillo T, Rombauts A, Stadlbauer D, et al. Immunological imprinting of the antibody response in COVID-19 patients. Nat Commun 2021;12:3781.