Respiratory Syncytial Virus:
Multimorbidity, Hospitalization Burden, and Genomic Epidemiology

by

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Dedication

For Kyle
We've got the biscuit wheels,
and I know the gravy is on the way.

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And, to coffee, for which without I would be dead.

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RSV	Respiratory Syncytial Virus
ARI	Acute Respiratory Illness
CHF	Congestive Heart Failure
COPD	Chronic Obstructive Pulmonary Disorder
ILI	Influenza-Like Illness
PCR	Polymerase Chain Reaction
LAV	Live-attenuated vaccine
mAb	Monoclonal Antibody
MAARI	Medically Attended Acute Respiratory Illness
MFIVE	Michigan Henry Ford Influenza Vaccine Effectiveness
MWI-ICD10	Multimorbidity-Weighted Index (ICD-10)
CCI	Charlson Comorbidity Index
EM	Elixhauser Method
BMI	Body Mass Index
EHR	Electronic Health Record
qPCR	Quantitative Polymerase Chain Reaction
RT-PCR	Reverse Transcription-PCR
OR	Odds Ratio
OR_{adj}	Adjusted Odds Ratio
CI	Confidence Interval
HAIVEN	Hospitalized Adult Influenza Vaccine Effectiveness Network
LOS	Length of Stay
ICU	Intensive Care Unit
HIVE	Household Influenza Vaccine Effectiveness

Abstract

Globally, acute respiratory infections (ARI) contribute to substantial morbidity and are a leading cause of death, primarily among the very young, elderly, and immunocompromised. Respiratory Syncytial Virus (RSV) accounts for a small yet significant fraction of severe respiratory infections. RSV cases are universally underreported and there is no vaccine available, yet multiple are in development and evaluation. In addition, a prophylactic, palivizumab, is available with limitations. In the absence of a vaccine, additional research on high-risk populations is needed to better inform current surveillance and prevention practices.

This dissertation applies a variety of laboratory and statistical approaches to assess RSV-associated ARI through the utilization of three distinct studies originally developed to measure influenza impact and vaccination effectiveness. These studies provide a robust framework for evaluating RSV in a variety of settings including ambulatory clinics, hospitals, and households. In the first chapter I provide contextual virologic, epidemiologic, and therapeutic background on RSV research.

In the second chapter of this dissertation, I explore the impact of multimorbidity on illness outcomes among adults who sought outpatient medical care for an ARI. While no significant associations between multimorbidity and illness outcomes was found, those with RSV experience higher multimorbidity scores compared to those with influenza or neither RSV nor influenza, highlighting the importance of underlying morbidity as a risk factor for RSV illness. Further, I evaluate relationships between RSV viral characteristics and illness outcomes among

all participants with RSV as well as assess whether RSV viral subtype modifies these associations. This analysis shows higher quantitated viral loads in individuals with RSV-B compared to those with RSV-A, and those with higher viral loads have significantly higher odds of experiencing an extended illness, however, viral subtype did not modify this association.

In the third chapter, using a multi-site, nationally representative network of adults hospitalized with ARI, I evaluate in-hospital outcomes of RSV-associated hospitalization. I compare demographic and comorbidity data as risk factors and assess in-hospital outcomes between patients hospitalized with RSV, influenza, and those negative for both RSV and influenza. The proportion of adults with Congestive Heart Failure (CHF) or Chronic Obstructive Pulmonary Disorder (COPD) is significantly higher in those with RSV compared to those with influenza. Patients with RSV detected have significantly higher odds of experiencing an extended length of hospital stay and need for mechanical ventilation when compared to those with influenza detected.

In chapter four, I provide a detailed characterization of RSV illness epidemiology in a household surveillance study over the past decade. Due to the longitudinal nature of the study, a considerable number of repeat RSV illnesses over time are included for evaluation. Further, sequencing data is included to describe annual RSV circulation at the genotype level. This data demonstrates that the median age of individuals with RSV-B detected was lower than the median age of individuals with RSV-A detected. Our data revealed an average interval of one and a half years between first-detected and repeat RSV infections. Sixty-five percent of reinfection pairs were heterologous with respect to viral subtype. Per our phylogenetic analysis, RSV-A genotype ON-1 and RSV-B genotype BA-11 are the predominate strains circulating among southeastern Michigan households within the past ten years. Findings from this dissertation provide evidence

supporting the need to improve ongoing and future RSV surveillance and vaccine-development approaches.

CHAPTER 1

Introduction

RSV-associated ARI have been documented to cause severe illness, particularly in individuals with underlying morbidity, the elderly, and young children. Each group has a greater susceptibility to illness due to the decreased functionality of their immune systems resulting from either their underlying condition(s), age, or both. In the first two chapters of this dissertation, I will evaluate demographic and morbidity data as risk factors and assess their impact on illness outcomes. Moreover, varied evidence is available on the relationships between RSV subtype, viral load, and illness outcomes, and in the first aim I contribute findings to further develop our knowledge of those relationships. In the final aim, I provide an in-depth description of RSV illness epidemiology and evaluate repeat infections using a decade's worth of data from a household-based surveillance study. I complement this analysis with genomic data to characterize local strain circulation and expand publicly available complete RSV sequences. The laboratory and analytic methods used in this dissertation, combined with respective study design strengths, contribute to the expansion of our understanding of RSV epidemiology. I conclude this dissertation with suggestions for upcoming targeted RSV vaccine campaigns as well as expansions for future research with this data.

1.1 Specific Aims and Hypothesis

Aim 1: Assess the impact of multimorbidity on illness outcomes among adults with ARI and depict population characteristics among adults and children with ARI. Describe RSV viral

characteristics among adults and children as well as associations between quantitative viral load and illness outcomes and whether RSV subtype modifies this association.

Hypothesis 1: We hypothesize that multimorbidity and RSV viral load will be associated with at least one illness outcome and that viral subtype will be an effect modifier. Further, we expect RSV viral load will differ by viral subtype.

Aim 2: Using data from a multi-site hospital network, we aim to evaluate and compare epidemiologic characteristics and clinical outcomes of hospitalized adults with RSV or influenza detected as well as those who tested negative for both RSV and influenza.

Hypothesis 2: We hypothesize that adults with RSV detected will have more underlying comorbidities and be older on average as well as experience higher odds of at least one in-hospital illness outcome.

Aim 3: Our objective is to describe RSV illness epidemiology as well as repeat infections between 2010 and 2020 using data from a longitudinal household ARI surveillance study in southeast Michigan. We aim to implement whole genome sequencing to describe strain circulation in this population during this period.

Hypothesis 3: We hypothesize individuals with RSV-associated illness will differ from the RSV-negative ARI control group, particularly with respect to age. We expect to quantify an interval between first detected infection and repeat infections, and we predict subtype distribution will vary across reinfection. We anticipate subtype predominance to vary by season and genotype predominance to be relatively consistent within this period.

1.2 Background and Significance

Once a safe and effective vaccine for RSV is available, results from this dissertation can aid in developing more refined recommendations for targeted vaccine and non-vaccine

prevention strategies. Until then, this research should inform healthcare providers, caregivers, and surveillance networks on who is most susceptible as well as which groups of people an emphasis on preventative measures should be placed.

1.2.1 Virology

RSV is a lipid-enveloped, non-segmented, negative-sense RNA orthopneumovirus from the *Paramyxoviridae* family (Collins et al., 2013). RSV is comprised of a ~15,000 base pair genome containing ten genes that encode for eleven distinct proteins (Figure 1.1) (Collins et al., 2013; Rebuffo-Scheer et al., 2011). The viral envelope houses four of the most functionally important proteins: glycoprotein (G), fusion protein (F), matrix protein (M), and the small hydrophobic protein (SH) (Collins et al., 2013).

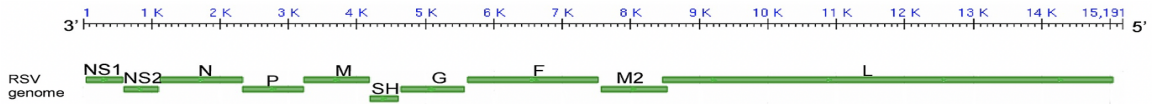

Figure 1.1 RSV Genome and Corresponding Proteins (Rebuffo-Scheer et al., 2011)

The G glycoprotein plays a crucial role in viral attachment (Levine et al., 1987) and a lesser known role in evading host defenses by imitating an immune-activating, cell-signaling protein (Tripp et al., 2001). The hypervariable region of the G gene is often sequenced for genomic surveillance efforts and determines RSV genotype (Mufson et al., 1985; Sande et al., 2013; Zlateva et al., 2004). In early seroreactivity studies, the G, F, M, and NP genes have been targets for differentiating RSV subtype strains through targeting viral surface antigenic variation with the use of monoclonal antibodies (Anderson et al., 1985; Mufson et al., 1985).

The F protein assists with virus-host cell fusion through viral penetration and the formation of syncytia. The F protein also plays an import role in initiating signal transduction and triggering host innate immunity through binding specific receptors (Haynes et al., 2001). The only prophylactic currently licensed for use against RSV specifically targets the F glycoprotein

of RSV (Johnson et al., 1997). Both the F and G RSV glycoproteins indiscriminately bind specific respiratory-tract cell receptors in humans; thus, RSV is readily recovered from nasal secretions, nasopharyngeal swabs, lung washes, and sinus samples (Collins et al., 2013).

Early in the course of infection, the M protein is thought to be responsible for inhibition of host transcription and later is associated with cytoplasmic inclusion bodies where viral RNA synthesis occurs. The SH protein also promotes infectivity through modification of host cell membrane permeability, which can lead to a reduction in apoptosis in addition to inhibition of the host antiviral cytokine, TNF-alpha (Fuentes et al., 2007).

1.2.2 Epidemiology & Pathophysiology

Two subtypes, RSV-A and RSV-B, are found in humans and can be distinguished using either serologic (antigen-antibody) or molecular (viral RNA) methods. The two subtypes circulate annually, typically with one subtype predominating in an illness season (Hall et al., 1990; Waris, 1991) although some co-circulation of types is often seen. RSV seasonality is like that of other respiratory viral illnesses in the United States, typically spanning from October to May with a peak identified between November and January. Globally, seasonality varies by geographic region and climate, currently understood to be influenced by factors such as temperature, humidity, and sunlight and their effect on the virus' environmental stability.

RSV surveillance efforts do not uniformly include testing for determining subtype, making it difficult to accurately assess subtype circulation across seasons worldwide (Griffiths et al., 2017). In 2005, a modelling group in the UK suggested that the dominant subtype patterns observed are likely explained by reduced susceptibility to and infectiousness of repeat infections (White et al., 2005). In general, longitudinal studies have noted that RSV-A tends to be more prevalent than RSV-B (Esposito et al., 2015; Jafri et al., 2013). Previously, higher viral loads of

RSV-A have been isolated from nasopharynx samples compared to RSV-B, which may indicate a potential for increased transmissibility between RSV-A-positive individuals due to higher viral shedding (Buchman et al., 2002; Hall et al., 1981; Y.-I. Kim et al., 2015).

Whole genome sequencing is a valuable tool for closely monitoring RSV molecular epidemiology and genomic evolution, and sequencing data can be evaluated for developing novel therapeutics and vaccines (Houldcroft et al., 2017). Multiple circulating clades of RSV have been identifiable since the discovery of RSV in 1956, with RSV-A and RSV-B each having their respective set of unique genotypes; however, there is no consensus on how genotypes are defined (Schobel et al., 2016; Trento et al., 2015). For RSV-A, nine distinct genotypes have been identified (Cui et al., 2013; Eshaghi et al., 2012; Hirano et al., 2014; Muñoz-Escalante et al., 2019; T. C. Peret et al., 1998; T. C. T. Peret et al., 2000; Shobugawa et al., 2009; Venter et al., 2001), and at least 37 genotypes for subtype B have been described in the literature (Blanc et al., 2005; Dapat et al., 2010; Muñoz-Escalante et al., 2021; T. C. Peret et al., 1998; Trento et al., 2006; Venter et al., 2001). The emergence of the ON-1 RSV-A genotype has been genomically characterized as recently as 2010 and is circulating globally (Comas-García et al., 2018; Duvvuri et al., 2015; Schobel et al., 2016; Thongpan et al., 2017).

As with most RNA viruses, RSV has a rapid rate of mutation; however, these nucleotide substitutions – mostly concentrated in the G protein gene region – manifest slowly over time in circulating strains (Collins et al., 2013). Relatively few RSV sequences have been constructed worldwide, particularly from North America in the past decade. With proper allocation of resources and implementation of feasible sequencing techniques, we can begin to move towards improved global RSV surveillance equipped with a better understanding of transmission, evolution, and therapeutic targets.

RSV is the leading cause of lower respiratory tract illness in young children (Borchers et al., 2013; Hall et al., 2009; Meng et al., 2014), as well as a significant contributor to morbidity and mortality in older adults (Ackerson et al., 2019; Falsey et al., 2005; Malosh et al., 2017a; Prill et al., 2021). Epidemiologically and seasonally, the start of community RSV transmission is often indicated by an uptick in pneumonia and bronchitis cases as well as hospitalization of young children, particularly infants with lower respiratory tract infections (Boyce et al., 2000; Hall et al., 2009; Iwane et al., 2004).

Primary RSV infection in otherwise healthy children, often occurring in the first year of life, is associated with severe lower respiratory illness. Almost all children are infected with RSV by the age of two, and reinfection throughout life is common (Agoti et al., 2012; Falsey et al., 2005; Glezen et al., 1986; Hall et al., 1976; Monto et al., 1974). Among infants, RSV is the most frequent cause of bronchiolitis, and the virus is estimated to be responsible for 40-90% of bronchiolitis-associated hospitalizations (Shay et al., 1999). RSV is estimated to account for 50% of pneumonia hospitalizations involving infants (Iwane et al., 2004). Annually, estimates report up to as many as 120,000 children are hospitalized with infection due to RSV (Pelletier et al., 2006; Shay et al., 1999). Even when presented as estimates, these statistics highlight RSV as a significant viral pathogen of childhood ARI.

Early surveillance efforts focused on children as the primary demographic at risk for infection with RSV, thus, for decades adult RSV cases were undercounted and underreported. A systematic review and meta-analysis of RSV-associated ARI in older adults, including 44 studies, estimates that in 2015 there were 1.5 million cases, ~214,000 of which resulted in hospitalization (Falsey et al., 2005; Glezen et al., 1986; Hall et al., 1976). In one analysis of adults aged 65 years and older, it was estimated that 2-9% of all U.S. lower respiratory tract-

associated hospitalizations were due to RSV-associated ARI, ranging in healthcare costs between $150-$680 million per year (Han et al., 1999).

Due to a lack in robust testing for and reporting of RSV, many epidemiologic estimates of burden, morbidity, and mortality are incomplete or outdated. ARI surveillance networks often perform molecular panel testing which typically includes detecting RSV. In contrast, clinicians in healthcare settings may not indicate the need for testing for RSV – especially in adults – and are not required to report positive findings. Improved surveillance, reporting, and global RSV sequence characterization would advance our understanding of RSV epidemiology. RSV surveillance has the potential for improvement solely by expanding subtype testing as well as consistently testing for RSV in adults presenting with influenza-like illness (ILI). This dissertation will directly address some of these shortcomings. For example, whole genome sequencing RSV isolates from a household cohort in chapter four of this dissertation will contribute to global strain surveillance and provide a detailed snapshot of community RSV circulation in southeast Michigan between 2010 and 2020.

1.2.3 Infection, Transmission, Manifestations, & Natural Immunity

RSV infection begins in the nasopharyngeal cavity where the virus attaches to airway epithelium. Viral replication occurs in the nasopharynx and spreads to the bronchiolar epithelial lining in small airways. Once in the lungs, RSV can cause edema or tissue swelling, increased mucus production, and tissue death. RSV gets its name from the hallmark syncytia-forming cytopathic cell effect it produces in respiratory epithelial cells — an essential viral trait that enables RSV to maintain cell-to-cell transmission within the host (Domachowske & Rosenberg, 1999). Syncytia, or large, webbed cells with intracytoplasmic inclusions, form when adjacent host cells fuse together via viral fusion proteins that were initially used for host cell entry and are

now functionally present on the host cells surface. Syncytia formation is facilitated by interactions between the F protein and the small protein RhoA of the GTPase enzyme, and this interaction could be a target in vaccine and prophylactic development (McLellan et al., 2013).

Infections with RSV can present variably in terms of disease produced and severity experienced. RSV is known for causing acute upper and lower respiratory tract infections, namely bronchiolitis – inflammation of small airways in the lungs – and pneumonia – the infection of lung tissues. Childhood airway hyperreactivity can occur and may result in predisposition to recurrent wheezing or asthma throughout childhood (Driscoll et al., 2020). A majority of RSV infections are self-limiting and typically treated with supportive care — such as fluids and fever-reducing agents. However, as severity increases – particularly in at-risk populations – hospitalization, ICU admission, need for mechanical ventilation, and death are potential outcomes as well (Malosh et al., 2017a; Prill et al., 2021). In the third chapter, I compare characteristics and in-hospital outcomes of hospitalized adults with RSV detected to hospitalized adults with influenza or neither RSV nor influenza detected using data from a large, multi-site network. Adults hospitalized with influenza provide a strong benchmark for comparison, because influenza is commonly clinically tested for in hospitalized populations and is well-researched.

The modes of transmission for RSV are similar to that of many other ARIs. RSV is spread via droplet nuclei directly from an infected person via coughing, sneezing, or kissing. RSV can also be transferred indirectly through contact with contaminated fomites such as doorknobs, tables, and other shared household items (*CDC*, 2019). The spread of respiratory viruses is exacerbated in environments like childcare centers and households — particularly if either setting is prone to crowding (Chu et al., 2013). Children are often regarded as the origin of

household infections, and this theory should be further corroborated with the application of highly granular molecular techniques – such as whole genome sequencing – in a variety of household study settings worldwide (Agoti et al., 2019; Scott et al., 2019).

Possible symptoms of infection with RSV include a runny nose, cough, sneezing, fever, wheezing, and a decreased appetite. These signs and symptoms may not appear together if at all, and adults and young children often have varying presentations of illness. For infants and young children, who often experience lower respiratory tract infections with RSV, the only evident signs may be difficulty breathing, irritability, and reduced activity (*CDC*, 2019). On the contrary, in adults, RSV tends to appear as an upper respiratory tract illness and may exacerbate underlying acute asthmatic bronchitis or other chronic lung conditions (Pickering et al., 2006). Not surprisingly, these signs and symptoms have some overlap with other common ARI's, making it necessary for clinicians to consider all possible respiratory viral pathogens to make an accurate diagnosis; however, unlike other ARI's, host-derived immunity to RSV is not well-understood.

Protection against repeat RSV infection(s) is not well-established, and repeated infections are common, particularly in young children but throughout life as well (Agoti et al., 2012; Glezen et al., 1986; Ohuma et al., 2012; Wong et al., 2021). Cytotoxic killer T cells and antibody-mediated responses are known factors in host-derived immunity against RSV. Research has shown that deficiencies in these specific T cells have been associated with RSV deaths in both immunocompromised and pediatric populations (Hall et al., 1986; Welliver et al., 2008). Immunity developed in response to natural infection with RSV is typically described as partial and short-lived (Agoti et al., 2012; Bont et al., 2002; Meng et al., 2014).

The subtype circulation model from White et al. also demonstrated that immunity is temporary and lasts two years on average (White et al., 2005). These findings partially explain how a person can become reinfected with the same strain of RSV across their life course. On the other hand, in a birth cohort study in Kenya, repeat infections were associated with a decrease in risk for lower respiratory tract infections, even if the repeat occurred within the first year of life (Ohuma et al., 2012). This knowledge gap with respect to RSV immunity will be addressed in the fourth chapter, where I characterize the subtype distribution and interval between reinfections detected in a household cohort study.

1.3 Risk Factors & High-Risk Populations

The following are substantial risk factors for infection with RSV: age, underlying comorbidities, immunosuppression, narrow or reactive airways, and, potentially, RSV strain heterogeneity. This research will use three distinct studies that will support focusing surveillance efforts on subgroups at higher risk for RSV infection: the immunocompromised, older adults, and households with young children.

1.3.1 Adults vs. Children

Infants and young children are the primary subpopulation experiencing severe RSV infection, often leading to hospitalization (Hall et al., 2009). Susceptibility to infection among young children is enhanced by their naïve immune systems and age-specific contact patterns paired with less-than-ideal hygiene behaviors. Adults, particularly the elderly, are increasingly being recognized as a susceptible group to severe RSV illness as well (Falsey et al., 2005). An additional risk factor – consequently, a downstream effect of age – is that physicians do not often consider RSV when diagnosing ARI in adults. This can lead to prolonged diagnoses, delayed or improper treatment (e.g., use of antibiotics), increased length of illness, and subsequent seeking

of care. Aging is associated with decreased immunocompetence and increased prevalence of morbid conditions, another risk factor for RSV (Divo et al., 2014). This may explain why some younger adults – those with comorbid conditions – can also experience severe RSV illness, similar to infants and the elderly.

1.3.2 Impact of Multimorbidity

It is well-established that individuals requiring hospitalization for infections have more comorbidities compared to individuals who do not require hospitalization. There is good data to suggest elderly individuals infected with influenza are at a higher risk for hospitalization due to their infection compared to younger individuals (*CDC*, 2019; Reed et al., 2015; Zhou et al., 2012). Over the past few decades, elderly individuals have been recognized as a growing population susceptible to infection with RSV requiring hospitalization, compared to the characteristic demographic of young children. The impact of multimorbidity on RSV illness among adults will be explored in the second chapter of this dissertation.

Epidemiologic characteristics as well as illness and in-hospital outcomes of adults hospitalized with influenza are well understood; therefore, adults hospitalized with influenza are a valuable comparison group for addressing gaps in understanding how older individuals are impacted by hospitalization with RSV. Differences in outcomes could be in part due to the presence of an effective vaccine, readily available and cost-effective antivirals, and reliable rapid detection assays — indicating the potential for more efficient care regarding influenza patients. The third chapter of this dissertation will compare characteristics and in-hospital outcomes of hospitalized adults with either influenza, RSV, or neither detected — highlighting the importance of recognizing differences between these distinct populations upon hospitalization with ARI among adults.

1.3.3 Subtype as a Risk Factor

When considering viral subtype and strain as risk factors for infection or varying severity, multiple mixed findings have been reported (Fodha et al., 2007; Hornsleth et al., 1998; Kneyber et al., 1996; McConnochie et al., 1990; Papadopoulos et al., 2004; Walsh et al., 1997). Few studies have since been conducted assessing associations between viral subtype, viral load, and illness outcomes. The previous studies had multiple limitations including small sample sizes and the inclusion of only one illness season. Most of the studies were limited by a lack of access to polymerase chain reaction (PCR)-based laboratory methods, which is considered more reliable than serologic-based testing. More recently, using a large, prospective cohort study (Mansbach et al., 2012), researchers concluded that infection with RSV-A was significantly associated with a higher odds of requiring intensive care compared to those infected with RSV-B; however, in a separate study, overall severity of presenting bronchiolitis did not differ by subtype (Laham et al., 2017).

As mentioned, the two subtypes are identifiable through genetic and antigenic differences, but there are few distinguishable illness characteristics when comparing infection with RSV-A vs. RSV-B. Infection with RSV can differ from other ARIs – whether or not the two subtypes differ from each other; however, it is essential to characterize differential effects between the subtypes, if any, for developing an effective vaccine. In chapter two, we report data on differences between RSV-A and RSV-B with respect to illness outcomes and viral load, and in chapter four we report differences in age of infected individuals by viral subtype.

1.4 Interventions for RSV

There is currently no vaccine available and prophylaxis is only utilized in limited situations. This section will provide context on past RSV intervention development that led

researchers to current vaccines in production and will continue to shape future efforts. This dissertation is not directly related to vaccine or prophylactic development; however, our aims focus on providing data to improve clinical and surveillance guidance for mitigating RSV infections in susceptible populations.

1.4.1 Historical Context

In the mid-to-late 1960's, roughly a decade after RSV was first isolated from humans, an ultracentrifuged, precipitate-concentrated formalin-inactivated, aluminum-adjuvanted RSV vaccine was developed from whole-virus grown in monkey kidney cell cultures (Figure 1.2). The vaccine stimulated a moderately high production of antibodies; however, the vaccine ultimately failed to induce protection against infection with RSV (Chin et al., 1969; Fulginiti et al., 1969).

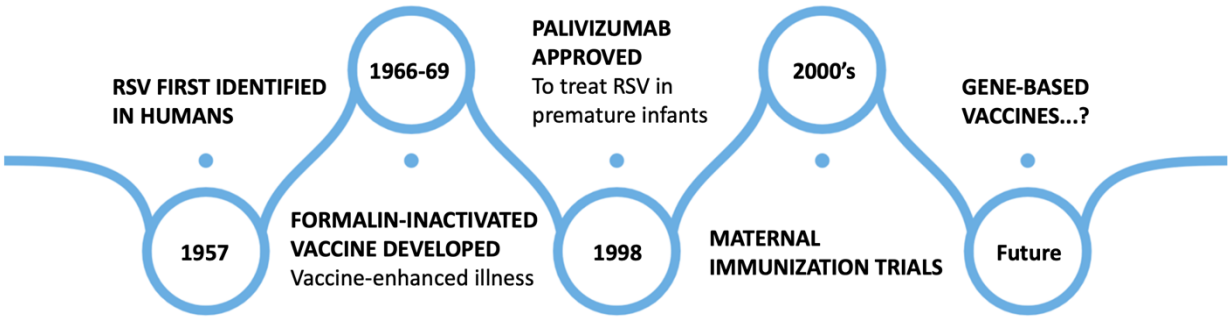

Figure 1.2 Condensed RSV Vaccine Timeline, Adapted from Novavax

Tragically, infants who received the vaccine and were later naturally infected with RSV experienced what is called 'vaccine-enhanced illness' (H. W. Kim et al., 1969). This was hypothesized to have been an immunologic phenomenon due to interactions between viral and host serum antibodies (H. W. Kim et al., 1969). Infants under the age of six months, who still have maternal antibodies present, had the highest incidence of severe vaccine-enhanced illness and two infants died as a result (H. W. Kim et al., 1969). The notoriety of this event, and

consequent valid apprehensions, has shadowed RSV vaccine development efforts over the past five decades; however, multiple attempts at producing a vaccine have been made.

1.4.2 Current Practices & Challenge

Synagis – the trade name for a monoclonal antibody (mAb) product, Palivizumab – is the only prophylactic agent currently available. Palivizumab has limited indications for use and is cost-prohibitive in many instances. In 1998, the FDA approved the prophylactic for use in premature infants. Applicability has since been expanded to infants with congenital heart or lung diseases, neuromuscular disorders affecting airway secretion, chemo-induced immunocompromising conditions, and cystic fibrosis. Doses are delivered through intramuscular injection, recommended to be administered monthly, and cost $6,000 (USD) per dose, on average (Shahabi et al., 2018).

Monoclonal antibodies have also been considered for treatment as opposed to prophylaxis of RSV infections. Unlike vaccines, mAb efficacy does not rely on stimulation of the host's immune system, which is an important consideration when developing a therapeutic that should be effective in a high-risk, immunocompromised population. However, similar to Palivizumab, the protection mAb's provide only lasts for the duration the antibodies remain in circulation in a person. The average half-life of Palivizumab is approximately 20 days, and the dosing schedule recommends five monthly doses to maintain a protective level of neutralizing antibody (Griffin et al., 2017),

Antivirals (e.g., Ribavirin), once used extensively for treatment, as well as corticosteroids or bronchodilator therapy (e.g., beta-adrenergic agents) are no longer recommended for treating RSV due to various reasons including high cost, toxicity, or ineffectiveness (Pickering et al., 2006). As previously mentioned, treatment for RSV infections is often supportive and includes

fever management, hydration, and rest. More severe illness requiring healthcare intervention or hospitalization may necessitate oxygen therapy or ventilation.

Currently, multiple RSV vaccine products – manufactured by various companies in a race to develop a safe and effective vaccine – are in production or clinical trials. Examples of vaccine products in development include live-attenuated, vector-based, subunit-based, particle-based, monoclonal antibody, and mRNA vaccines. Live-attenuated vaccine (LAV) candidates – which incorporate modified versions of RSV that can replicate but are weakened to avoid severe disease – possess unique challenges. LAVs have yet to demonstrate sufficient immunogenicity to protect against wild-type RSV, and instability of the virus may complicate production and storage (Karron et al., 2013). With LAVs, there is potential of a partial reversion to a wild-type variation of the virus upon infection; however, some recent LAV candidates have incorporated "reversion-resistant" genetic components to stabilize mutations in an attempt to prevent this phenomenon (Luongo et al., 2012). Vector-based vaccines are developed by inserting non-replicating RSV components into a carrier vector – often a different, non-replicating virus – and there is no risk for severe disease due to wild-type reversion (Killikelly et al., 2020).

The next series of options move away from using variants of the virus itself. Subunit-based vaccines are comprised of purified RSV proteins – administered alone or with an adjuvant – and primarily function by inducing host CD4+ T-cell activation (Rossey & Saelens, 2019). However, other studies have demonstrated the importance and necessity of CD8+ T-cell stimulation in response to suppressing RSV infections (Rossey et al., 2014). Particle-based vaccines are similar in desired effect – boosting a strong immunological response – however, these vaccines are made of synthetic select antigenic particles (Killikelly et al., 2020).

1.4.3 Future Directions

The challenges above are specific to their respective protective mechanisms; however, RSV itself has characteristics that pose further challenges for developing a safe and effective vaccine. To do so, additional immunology studies need to be conducted to better understand RSV antigenic diversity, its ability to inhibit host immune responses, and correlates of protection. Identifying correlates of protection for RSV is especially important for those with weakened immune systems, such as the immunocompromised, young infants, and the elderly (Killikelly et al., 2020).

For some vaccine products, phase 3 clinical trials targeting special populations such as pregnant women, elderly, and pediatric populations have begun (Killikelly et al., 2020). Different product strategies require having detailed understanding of the virus' infectivity, transmissibility and gene functions, as well as host immunity. Gene-based vaccines have been proposed in the past; however, they have cost as well as functionality restraints. As we near a finished vaccine that is safe for use at a population-level, it is important to keep in mind that those at high-risk of severe infection with RSV should be given priority as recipients of the vaccine. All chapters of this dissertation provide data that indicate the prioritization of high-risk groups in upcoming vaccination campaigns.

1.5 Household Studies for RSV Research

1.5.1 Pioneer Household ARI Surveillance Studies

Household studies have been a valuable epidemiologic tool for conducting community-based surveillance of diseases since the early 20th century. In the 1960s and 1970s, the Tecumseh Study of Respiratory Illness (Tecumseh, MI) and the Seattle Virus Watch (Seattle, WA) studies were two of the largest ARI surveillance studies to be conducted of their time — both of which were formative for developing current household studies, such as the Household Influenza

Vaccine Evaluation (HIVE) study utilized in chapter four of this dissertation (Monto et al., 2019). Early studies collected fundamental epidemiologic data on ARIs, including case frequency and illness symptoms and relied on serology-based testing or self-reported illnesses (Fox et al., 1972; Monto et al., 1971).

These earlier studies were limited by multiple factors, often by what was available at the time. It is not difficult to imagine the labor-intensiveness of conducting a household-based study with crude phone lines and lack of internet. Precursory ARI surveillance methods were used, such as serologic sampling of participants only twice annually to look at antibody titers — which greatly reduces the ability to adequately evaluate transmission and depict ARI frequency across the full respiratory illness season (Monto et al., 1971). Most notably was the absence of highly sensitive molecular laboratory methods. When possible, these studies could use viral cell culture – a labor intensive, low-sensitivity method with processing time constraints – to identify the etiologic agent of ARIs (Templeton et al., 2004).

1.5.2 Current Studies

Decades later, many ARI household surveillance studies hold characteristics of and carry forward lessons-learned from their predecessors, with added improvements in study design and laboratory methods. For example, the HIVE study conducts annual enrollments of households, yet offers more opportunities for participation and sample collection, compared to twice annually. HIVE laboratory procedures include highly sensitive and specific molecular-based methods – such as PCR – to detect multiple viruses in samples. Like all prospective studies, household-based cohorts are still labor and cost intensive, and implementation can vary widely based on available resources. For instance, some studies only conduct surveillance during a respiratory illness season, whereas others include asymptomatic sampling which can generate

more detailed transmission data. Regardless of study design variation, one can appreciate the broad improvements to this method of surveillance over time.

1.5.3 Challenges of Household Studies

Even though progress has been made, challenges still exist that must be addressed for certain goals of these studies to be accomplished. Most notably, we cannot identify where infections within households originate from without the implementation of sequencing-based analyses. Estimates of household infection origin can be approximated with self-reported date of illness onset data; however, this becomes less useful for asymptomatic infection or if time of onset is similar for household members or recall bias impacts an individual's ability to accurately remember when an illness began. In a sense, we are still conducting fundamental respiratory illness surveillance and generating data that only provides a glimpse into household RSV epidemiology, transmission, and seasonality.

1.5.4 Proposed Solutions

One option for improving these studies is through the implementation of whole genome sequencing. Agoti et al. has recently demonstrated the potential of evaluating household transmission through sequence-based analyses (Agoti et al., 2015, 2017, 2019). Worldwide, there is limited RSV genomic data, and this solution would contribute more sequence characterizations, helping expand available RSV sequence profiles. However, whole genome sequencing methods are not without their limitations. Sequencing is a laborious process from start to finish with multiple, time-consuming steps and expensive reagents and assay platforms.

A portion of this dissertation aims to characterize strain circulation in households through performing whole genome sequencing on identified RSV isolates collected from the HIVE study. Another aim of this research – using the same HIVE data – is to characterize repeat RSV

infections to further inform our understanding of the interval between infections and characteristics of individuals who experience reinfection. The integration of molecular and epidemiologic data in this thesis will contribute to improving global RSV surveillance and ultimately our understanding of a complex virus.

RSV is a significant cause of acute respiratory infections among vulnerable populations that can result in severe illness or death. Each aim of this dissertation focuses on three populations – those with underlying chronic illness, older adults, and households with young children – drawing from three distinct yet geographically related study settings. Each study utilized captures RSV-associated illnesses at a unique level of severity: mild and asymptomatic illness from a household surveillance study, moderate illness from an ambulatory care clinic, and severe illness from a hospitalized adult ARI network (Figure 1.3) (Troeger et al., 2019).

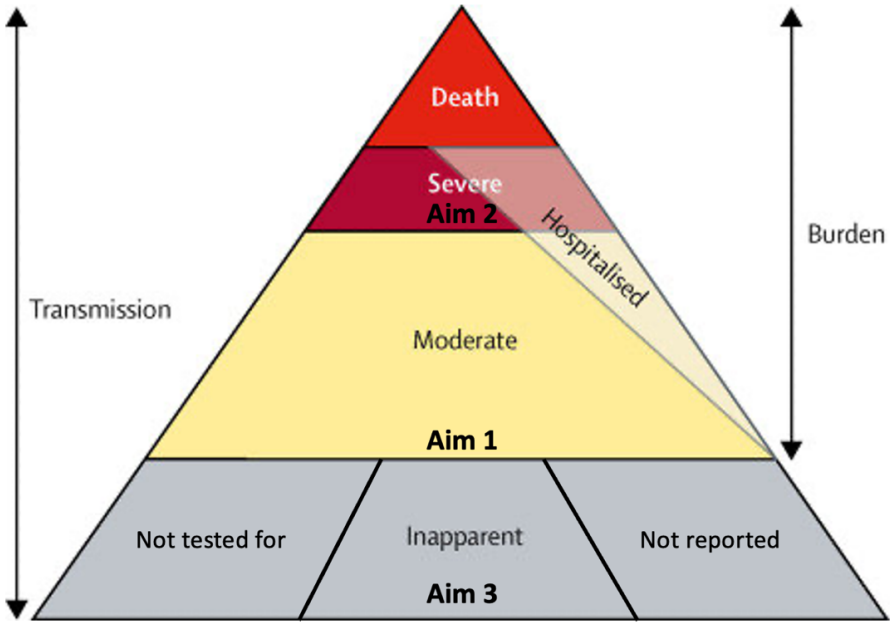

Figure 1.3 Acute Respiratory Illness Burden Pyramid Adapted from Troeger et al.

Evaluating RSV across the spectrum of severity is a major strength of this dissertation, allowing us to provide a comprehensive, in-depth analysis of RSV epidemiology across multiple years and settings.

CHAPTER 2

Assessing the Impact of Multimorbidity and Viral Characteristics on RSV Illness in an Ambulatory Clinic Cohort

2.1 Author Summary

In this aim, I explore the impact of multimorbidity on ARI illness outcomes among adults using a patient-centric, validated measure of multimorbidity. Participants in this study were enrolled from a prospective, ambulatory, clinic-based study (MFIVE), and those with RSV-associated ARI had significantly higher multimorbidity scores, although multimorbidity was not associated with measured illness outcomes. Further, I describe viral characteristics and the relationship between viral load and illness outcomes among all eligible RSV-positive participants. RSV-B samples had significantly higher viral loads detected, and higher viral loads were significantly associated with extended length of illness.

2.2 Abstract

Older adults are at greater risk of experiencing medically attended RSV infections as well as severe RSV-associated illness outcomes when compared to younger adults, and the prevalence of multimorbidity increases significantly with age. Additionally, there is conflicting evidence regarding the dynamics between RSV viral load, viral subtype, and subsequent illness outcomes. The goal of this project was to evaluate the relationship between multimorbidity and illness outcomes among adults with acute respiratory illness (ARI). Further, among all RSV-positive

participants we assessed associations between quantitative viral load and illness outcomes as well as whether viral subtype was an effect modifier.

Among 4,490 participants enrolled in the MFIVE ambulatory vaccine effectiveness study between 2017-2020, 4,442 (n=441 cases of RSV, n=1,341 cases of influenza, and n=2,660 negative for both RSV and influenza) individuals were included in our analysis with 12.8% (n=568) of participants ≥ 65 years of age. To measure multimorbidity in adults, we used diagnostic codes extracted from participant health records and implemented the Multimorbidity-weighted index-ICD10 (MWI-ICD10) macro, a validated, patient-centric measure of multimorbidity. RSV sample viral subtype and quantitative viral load were determined by quantitative PCR (qPCR) using subtype-specific primers and probes. Demographic and illness outcome data were collected during enrollment interviews and follow-up surveys. Multimorbidity characteristics and RSV viral characteristics were compared using non-parametric tests where appropriate. Multivariable, Firth-adjusted logistic regression models were used to generate odds ratios where indicated.

Adults with RSV had significantly higher median multimorbidity scores (Median: 1.62) when compared to adults with influenza (Median: 0.40) or neither RSV nor influenza (Median: 0.64) (p-value <0.0001). RSV-B specimens had significantly higher quantitated viral loads (Median: 3.32×10^4 copies/mL) detected when compared to RSV-A samples (Median: 1.35×10^4 copies/mL) (p-value <0.0001). RSV-positive participants with a viral load ≥ 2.20×10^4 copies/mL – measured as viral RNA copies per milliliter of collection media containing a nasal swab – had over twice the odds of experiencing an extended illness (≥ 7 days) when compared to participants with a viral load < 2.20×10^4 copies/mL [OR_{adj}=2.39 (95% CI: 1.03-5.51) p-

value=0.04], and effect modification by viral subtype was not present. No findings with respect to the impact of multimorbidity on illness outcomes were significant.

This is an investigative study using the MWI-ICD10 to assess the impact of multimorbidity on ARI in an outpatient clinic setting, and additional research will provide insight regarding the clinical value of implementing the MWI-ICD10 in medically attended acute respiratory illness (MAARI) patients. Shifting our focus to regularly include older adults and those with multimorbidity in the identification and management of RSV illness may reduce disease burden as well as subsequent care utilization. Consistent differentiation of RSV subtype will improve ongoing ARI surveillance and may also inform future vaccination strategies.

2.3 Introduction

Respiratory Syncytial Virus (RSV) is one of the leading global causes of acute respiratory illness (ARI) among children and is known to be a significant cause of ARI among high-risk adults, particularly those with underlying cardiopulmonary conditions (Falsey et al., 2005, 2006; Malosh et al., 2017a; Sundaram et al., 2014; Walsh et al., 2004). Current research supports including RSV in routine ARI surveillance–much like monitoring annual influenza–as well as clinically ruling out RSV in adults (Shi et al., 2019, 2021). To fully understand the implications of RSV detection in the outpatient setting, research assessing the impact of underlying conditions on RSV illness outcomes among adults is needed.

The prevalence of multimorbidity–the coexistence of multiple chronic conditions–increases significantly with age, and individuals with multimorbidity are more likely to experience adverse health outcomes including physical and cognitive decline, hospital readmission, and death (Barnett et al., 2012; Koroukian, 2015; Lai et al., 2019; Salisbury et al., 2011; Wei et al., 2019). A majority of medically-attended RSV infections among adults are

classified as moderate in severity, and treatment for such cases can extend to antibiotic prescription, bronchodilator use, or systemic corticosteroids (Belongia et al., 2018). However, older adults experience a higher incidence of medically attended RSV infections as well as severe RSV-associated illness outcomes, including hospitalization, when compared to younger adults (Belongia et al., 2018; McClure et al., 2014; Zhou et al., 2012).

While RSV infection has been linked to specific chronic conditions, less is known about RSV ARI occurrence across the spectrum of multimorbidity (Falsey et al., 2006; Walsh et al., 2004). Moreover, there is no conclusive evidence regarding host and viral factors associated with illness outcomes and severity, and many prior studies were limited to hospitalized infants. Host factors such as age and multimorbidity – especially among adults – and viral factors such as subtype and viral load are key factors that may be associated with illness outcomes and severity. In an ambulatory care setting, we aimed to assess the relationship between multimorbidity and ARI outcomes among adults as well as to evaluate the relationship between quantitative viral load and illness outcomes and whether viral subtype modifies this association among all RSV-positive participants.

2.4 Methods

2.4.1 Source Population

Data for this study comes from three recent seasons–2017/18, 2018/19, and 2019/20–of the Michigan Henry Ford Influenza Vaccine Effectiveness (MFIVE) study. Data collection for this aim concluded on March 13, 2020, in response to nationwide COVID-19 shutdowns. MFIVE is an ongoing, prospective, ambulatory-care study that enrolls over 1,000 people from southeast Michigan, annually. MFIVE is an arm of a nationwide respiratory illness surveillance network, the US Influenza Vaccine Effectiveness Network, which assesses influenza vaccine

effectiveness annually (Chung et al., 2020; Dawood, 2020; Flannery et al., 2018; Jackson et al., 2017). Patients presenting with medically attended acute respiratory illness (MAARI) lasting ≤ seven days with a cough and who have not taken antiviral treatment for their current illness are eligible for participation.

2.4.2 Data collection

After a participant provides consent, MFIVE research staff conduct an enrollment interview to collect demographic and household characteristic data, information on their current illness, vaccination status, perceived general health prior to the current illness (Excellent, Very Good, Good, Fair, or Poor), and whether children under the age of twelve reside in the household — either self-reported by participants or a proxy, such as a parent, when applicable. Staff collect throat and nasal swab specimens and deliver them to the Michigan Center for Respiratory Virus Research and Response for processing and storage. Seven days post-enrollment, participants receive an online follow-up questionnaire to complete at home. This survey asks participants to self-report illness duration and recovery metrics including subsequent care-seeking behavior. Additional personal health data such as BMI were determined using the most recent recorded height and weight in a participant's EHR, for adults only.

2.4.3 Multimorbidity (MWI-ICD10)

To determine adult multimorbidity status, the main exposure for multimorbidity analyses, we applied the MWI-ICD10, a validated, patient-centric measure of multimorbidity (Wei et al., 2018; Wei & Mukamal, 2018). Compared to the Charlson Comorbidity Index (CCI) and Elixhauser Method (EM), which calculate mortality risk scores, the MWI-ICD10 measures the impact of underlying conditions on patient physical functioning. Ninety-five conditions are represented in the MWI-ICD10 across the following categories: cardiovascular, endocrine,

gastrointestinal, hematologic, immunologic, integumentary, musculoskeletal, nervous, oncologic, ophthalmologic, oral, psychiatric, pulmonary, renal, and reproductive (Wei et al., 2018). Condition-specific multimorbidity weightings are predictive of a condition's impact on patient physical functioning. Conditions such as congestive heart failure (CHF) and chronic obstructive pulmonary disease (COPD) are given higher weights (4.77 and 4.32, respectively), whereas elevated cholesterol and depression/anxiety are assigned lower weights (0.343 and 1.29, respectively).

Electronic Health Records (EHR) were reviewed to gather ICD-10 diagnosis codes indicating high-risk conditions up to one year prior to enrollment and all ICD-10 codes recorded by the healthcare provider seen at enrollment. All ICD-10 diagnosis codes extracted from participant EHR were included to construct individual MWI-ICD10 scores using available macros on SAS software version 9.4 (SAS Institute, Cary NC). We classified adults with a non-zero MWI-ICD10 score as those with multimorbidity and adults with an MWI-ICD10 equal to zero to be without multimorbidity. Given the distribution of multimorbidity scores in adults from this sample, for our primary analysis we evaluated multimorbidity continuously and dichotomously (multimorbidity, yes/no), where indicated.

2.4.4 Illness Outcomes

The primary outcomes of interest – self-reported illness outcomes – were defined using data collected from the enrollment interview and follow-up survey. Symptoms reported–fever, sore throat, congestion–were operationalized dichotomously for assessing symptom burden (low 0-1 symptoms vs. high 2-3 symptoms), using any combination of symptoms reported. Length of illness was determined using self-reported illness onset date recorded at enrollment and recovery date from the follow-up survey and then dichotomized as extended illness (≥ 7 days) or not (< 7

days). Variables for subsequent seeking of medical care or treatment were combined and dichotomized (sought subsequent treatment, yes/no) and included visiting a doctor's office, urgent care clinic, retail pharmacy clinic, or emergency department/hospital.

2.4.5 Viral Characteristics

For the secondary analysis, quantitative viral load was the exposure of interest, and we evaluated the illness outcomes described above as outcomes of interest. Additionally, we explored whether RSV viral subtype modified the relationship between viral load and illness outcomes. The Michigan Influenza Center laboratory at the University of Michigan's School of Public Health (Ann Arbor, MI) performed testing for initial detection of RSV and other respiratory pathogens using Fast Track Diagnostics real-time multiplex PCR respiratory panel (A Siemens Healthineer Company, Luxembourg). Specimens were frozen and stored at -70°C upon completion of testing. Viral RNA was extracted with Qiagen QiaAmp Viral RNA mini kits, following manufacturer's instructions. Sample viral subtype and quantitative viral load were determined by Real-Time PCR (RT-PCR) on an ABI 7500 instrument (Thermo Fisher Scientific).

To determine viral subtype, all available RSV-positive samples from included study years were analyzed with a multiplex RT-PCR assay, protocol described elsewhere (Kuypers et al., 2004; Martin et al., 2008). Forward and reverse primers specific to RSV as well as fluorescent probes specific to RSV-A and RSV-B were included in the RT-PCR reaction master mix. A negative control, nuclease-free water, and two positive controls – conserved RSV plasmids for both RSV-A and RSV-B – were included on each assay plate for quality control.

We analyzed all subtyped samples with a qPCR assay that measures copies of RSV RNA per mL of sample, protocol described elsewhere (Kuypers et al., 2004; Martin et al., 2008). This

reaction included three TaqMan primers (Thermo Fisher Scientific) – RSV forward, RSV reverse A, and RSV reverse B – as well as an RSV probe designed to target the consensus region of the matrix protein gene. All test plates included nuclease-free water as a negative control as well as six RNA transcript standards, ranging from $1 \times 10^4 - 1 \times 10^9$ copies of viral RNA. Viral load was determined through comparing unknown samples to transcript standards using curves generated by qPCR. For statistical analyses, viral load is log10-transformed for analysis as a continuous variable as well as dichotomized using median viral load as the cut point. Using median sample viral load as a threshold, samples with a quantitative viral load $\geq 2.2 \times 10^4$ copies/mL (log10-transformed ≥ 4.3) were compared to samples with a quantitative viral load $< 2.2 \times 10^4$ copies/mL (log10-transformed < 4.3).

2.4.6 Statistical Analysis

For statistical analyses, participants from all seasons were pooled together to increase statistical power, and we adjusted for seasonality indicated by study year in all regression models. Participants positive for influenza or negative for both RSV and influenza were included for baseline comparisons. For multimorbidity analyses, only adults with data available to calculate an MWI-ICD10 score were included. Participants under the age of 18 were excluded from multimorbidity analyses as the MWI-ICD10 is not validated for use in children. We defined age continuously and categorically using epidemiologically meaningful cut points: young children, older children, young adults, older adults, and the elderly (age groups 0-4, 5-17, 18-49, 50-64, and ≥ 65, respectively). Race (White, Black, and Other) and education status (Less than High School, Graduated High School/GED, Some College, Bachelor's Degree, and Advanced Degree) were defined categorically, and a BMI cut point ≥ 30 indicated obesity. Overall descriptive statistics were calculated for all eligible study participants (Table 2.1) as well as

separately for adults included in multimorbidity analyses as a supplemental table (Table 2.2). For RSV viral characteristic analyses, time between illness onset and specimen collection (days) was categorized (0-2 and 3+).

For multimorbidity-specific analyses, Firth-adjusted multivariate logistic regression models were used to determine whether there was an association between multimorbidity and ARI outcomes, and we provide overall estimates adjusted for age, sex, race, and season. For viral characteristic analyses, we included all RSV-positive participants who had samples that were able to have subtype and viral load determined (n=353). We used Pearson's *r* correlation coefficient to assess the association between log viral load and viral subtype. The Mann-Whitney U Test was used to detect differences in viral load (copies/mL) between RSV-A and RSV-B samples.

We provide odds ratios from univariate and multivariate Firth logistic regression models – adjusting for age, sex, race, season, and time between illness onset and specimen collection – to test the association between quantitated viral load and RSV-associated illness outcomes. Firth-adjusted regression models were used to compensate for potential bias from smaller counts in stratified analyses (Firth, 1993). We provide overall and age-stratified estimates, where indicated, as well as estimates stratified by viral subtype to assess effect modification. To test for effect modification more precisely, a statistical interaction term between viral load and viral subtype was included in all overall illness outcome models. Statistical analyses were conducted on SAS software version 9.4 (SAS Institute, Cary NC).

2.5 Results

2.5.1 Epidemiology

From 2017-2020, MFIVE enrolled 4,490 people and collected 6,491 samples suitable for laboratory processing (Figure 2.1). Sixteen participants were missing infection result data and 32 participants with an RSV-influenza coinfection were excluded from analyses. Overall, 441 study-eligible cases of RSV, 1,341 cases of influenza, and 2,660 participants negative for both RSV and influenza were included, making the total analytic sample size equal to 4,442.

The annual prevalence of symptomatic RSV among participants under 18 years of age with MARRI across 2017/18, 2018/19, and 2019/20 was 16.6%, 11.7%, and 10.2%, respectively. The annual prevalence of symptomatic RSV among adults with MAARI across included study years was 9.4%, 6.8%, and 6.8%, respectively. These estimates were comparatively lower than the annual prevalence of symptomatic influenza among participants with MAARI (34.2%, 24.4%, and 34.1%, respectively). Of all eligible samples with RSV detected, 13.6% (n=60) were unable to have viral subtype determined and were excluded from appropriate analyses (Figure 2.1). Fewer than 7.5% (n=28) of subtyped samples were unable to have quantitative viral load determined, thus, 353 samples were included in viral load-specific analyses (Figure 2.1). RSV-B appeared to predominate on aggregate for these three respiratory illness seasons.

The median age of those with RSV detected was notably younger than those with influenza detected as well as those negative for both RSV and influenza (Table 2.1). Most participants in this study were female or white and not obese, across all infection comparison groups. Over two-thirds of adults with RSV detected had underlying multimorbidity. In contrast, 58% of adults with influenza detected or neither RSV nor influenza detected had underlying multimorbidity. Even with a significant presence of multimorbidity across all study participants, 92.6% (n=4,114) reported being in ‘Good Health’. The majority of participants with RSV or influenza detected had children under the age of twelve residing in their household.

2.5.2 Illness Outcomes

The overall response rate to the follow-up survey for all years combined was 63.2%. Few participants reported experiencing a length of illness lasting two days or less, and nearly 40% of participants with RSV detected reported a length of illness equal to seven days or longer (Table 2.3). Over three-quarters of patients with influenza detected reported experiencing a fever, compared to roughly half for participants with RSV or neither detected. A sore throat was more often reported by those with influenza or neither detected, and almost all participants reported congestion as a symptom. Nearly half of participants with influenza detected reported having all three symptoms, and a majority of those with RSV or neither detected reported the presence of two symptoms. Of those who responded to the follow-up survey, 11.6% of participants with RSV, 7.7% with influenza, and 8.8% with neither detected reported seeking subsequent care.

2.5.3 RSV Viral Characteristics

Among RSV-positive samples with successful subtyping (n=381), 62.2% (n=237) were RSV-B and 37.8% (n=144) were RSV-A (Figure 2.1). Among samples with viral load determined (n=353), quantitated viral load ranged between $2.09x10^1$-$1.10x10^9$ copies/mL and median viral load was $2.20x10^4$ copies/mL (log10-transformed = 4.3). Log10-transformed viral load was weakly but significantly correlated with viral subtype ($r = 0.23$, p-value < 0.0001) (Figure 2.2), and RSV-B samples had significantly higher viral loads (copies/mL) when compared to RSV-A samples (Mann-Whitney test, p-value <0.0001). We further evaluated the relationship between viral subtype and quantitated viral load using logistic regression, providing overall and age-stratified estimates (Table 2.4). After adjusting for age, gender, season, race, and time between illness onset and specimen collection, the odds were 1.88 times higher that RSV-B samples had a quantitated viral load $\geq 2.20x10^4$ copies/mL when compared to RSV-A samples

[OR_{adj} = 1.88 (95% CI: 1.14-3.11), p-value = 0.01]. When stratified by adults and children, the association became stronger in children (n=193) [OR_{adj} = 2.44 (95% CI: 1.25-4.77), p-value = 0.009], whereas it was attenuated and no longer significant in adults (n=160) [OR_{adj} = 1.22 (95% CI: 0.56-2.65), p-value = 0.61].

Next, we evaluated the relationship between viral load and illness outcomes (Table 2.5). After adjusting for age, sex, race, season, viral subtype, and time between illness onset and specimen collection, those with a viral load $\geq 2.20x10^4$ copies/mL had significantly higher odds of experiencing an extended length of illness when compared to those with a viral load < $2.20x10^4$ copies/mL (n=166) [OR_{adj} = 3.14 (95% CI: 1.25-7.93), p-value = 0.02]. After stratification by RSV subtype and adjusting for age, sex, race, season, and time between illness onset and specimen collection, this finding was no longer significant; however, participants with RSV-A detected (n=54) appeared to drive the overall association [OR_{adj} = 4.88 (95% CI: 0.82-29.1), p-value = 0.08] compared to participants with RSV-B detected (n=112) [OR_{adj} = 2.30 (95% CI: 0.75-7.06), p-value = 0.15]. We further tested for effect modification by including an interaction term for viral load and RSV subtype in all overall statistical models. The interaction term was not statistically significant for any illness outcome measured, thus, we concluded effect modification by viral subtype was not present.

2.5.4 Multimorbidity Analysis

There were 1,741 participants under the age of eighteen excluded from multimorbidity-specific analyses; however, children are included in overall demographic descriptions (Table 2.1), illness characteristic descriptions (Table 2.3), and RSV viral load, subtype, and illness outcome association measures (Table 2.4 and Table 2.5). For all three years, the top three prevalent MWI-ICD10 categories of conditions detected among adults were pulmonary,

endocrine, and cardiovascular. The top three prevalent conditions detected across all study years were elevated cholesterol hyperlipidemia, diabetes, and asthma.

Calculated non-zero MWI-ICD10 scores for RSV-positive adults ranged from 0.15-25.9, whereas scores ranged from 0.147-54.54 in influenza-positive adults and 0.147-36.57 in the negative control group. However, the median MWI-ICD10 score of RSV-positive adults in this sample was 1.62, which was significantly (p-value = 0.0001) higher when compared to the median MWI-ICD10 scores of influenza-positive adults and those negative for RSV and influenza (Table 2.6, Figure 2.3). Across all groups, median MWI-ICD10 scores were similar for those who sought subsequent care. Adults with RSV had significantly higher median MWI-ICD10 scores across various illness outcomes, including reporting a sore throat or congestion, reporting one or two symptoms total, experiencing a length of illness of zero to two days or more than seven, and not seeking subsequent care. After adjusting for age, sex, season, and race, no associations with respect to the impact of multimorbidity on illness outcomes among adults were statistically significant for any infection group (Table 2.7).

2.6 Discussion

We found that adults seeking medical care for symptomatic RSV-associated illness had significantly higher median multimorbidity scores when compared to adults seeking medical care for symptomatic ARI with influenza or neither RSV nor influenza detected. Those with RSV-B had significantly higher odds of having a viral load $\geq 2.20 \times 10^4$ copies/mL detected when compared to those with RSV-A. After adjusting for potential confounders, RSV-positive participants with a viral load $\geq 2.20 \times 10^4$ copies/mL had significantly higher odds of experiencing an extended illness when compared to participants with a viral load $< 2.20 \times 10^4$ copies/mL. Viral subtype does not appear to modify this association.

In a recent analysis comparing adults hospitalized with RSV or influenza, the authors reported that adults with RSV had significantly higher median Charlson Comorbidity Index scores (3 vs. 2, p-value < 0.001) when compared to those with influenza detected (Malosh et al., 2017a). Moreover, Malosh et al. found that clinical severity of RSV-associated hospitalization was similar to clinical severity experienced by those who tested positive for influenza (Malosh et al., 2017a). Our study extends this analysis to an ambulatory care setting where we have found that adults with RSV have significantly higher multimorbidity scores compared to adults with influenza or neither RSV nor influenza detected.

Prior studies describing the relationship between RSV subtype, viral load, and illness severity often focus on hospitalized infant populations and report conflicting results. Using molecular methods and standardized viral load measurements, our analysis demonstrated a significant relationship between quantitated RSV viral load and subtype. In contrast to our findings, a study of hospitalized infants by Rodriguez-Fernandez et al. found that RSV-A samples had significantly higher quantitative viral loads when compared to RSV-B samples (Rodriguez-Fernandez et al., 2018). A 2018 study by Walsh et al. assessed RSV illness severity in infants – measured via hospitalization, ICU admission, and the need for ventilation – and concluded viral load did not differ by illness severity (Walsh et al., 2018).

Our study does not evaluate severe illness requiring hospitalization and is not restricted to infants; however, we did find a significant relationship between viral load and length of illness in this ambulatory care population. Some earlier studies of hospitalized infants suggest that RSV-A is more likely to cause severe illness or necessitate intensive care when compared to RSV-B (Hall et al., 1990; McConnochie et al., 1990; Walsh et al., 1997). In contrast, and in line with multiple early studies, Monto and Ohmit found no differences in illness characteristics between

the two subtypes in a community setting (Hendry et al., 1986; McIntosh et al., 1993; Monto & Ohmit, 1990; Wang et al., 1995; Wilson et al., 1990).

The inclusion of influenza-positive and RSV-negative, influenza-negative participants was a major strength which allowed us to make meaningful comparative interpretations of RSV risk factors and severity. When stratifying analyses by age groups or RSV subtype, we attempted to compensate for small cell counts by using Firth-adjusted logistic regression models to adjust for potential bias (Firth, 1993). Additional strengths of this study include prospective screening and enrollment of participants who met a pre-established MAARI criteria as well as the use of highly sensitive and specific molecular testing for ARI detection and RSV subtype and viral load determination. Determination of viral characteristics, specifically viral load, are impacted by various factors including sample quality and storage conditions, PCR primer specificity, and timing of sample collection after the onset of symptoms. Nevertheless, our findings support the importance of differentiating RSV subtype in clinical and surveillance settings.

This is a novel investigative study using the MWI-ICD10 to assess the impact of multimorbidity on ARI in an outpatient clinic setting. Utilizing the MWI-ICD10, as opposed to CCI and EM, is a more meaningful metric for assessing the impact of multimorbidity on ARI in an ambulatory-care-seeking population. Future studies will provide insight about the clinical value and appropriateness of implementing the MWI-ICD10 in ambulatory care settings. Validation of the MWI-ICD10 for use in other populations could help researchers better understand the impact of multimorbidity on ARI. In conclusion, shifting our focus to regularly include older adults and those with multimorbidity in the identification and management of RSV-associated ARI may reduce disease burden as well as subsequent care utilization.

Consistent differentiation of RSV subtype will improve ARI surveillance efforts and may also improve a patient's course of clinical care.

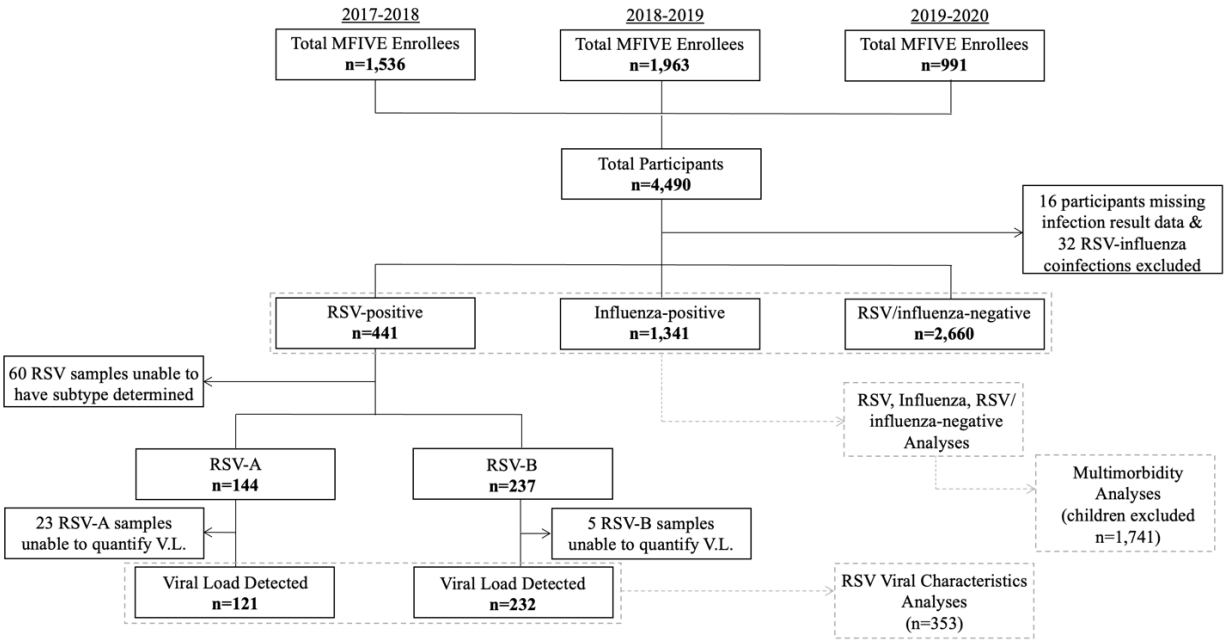

Figure 2.1 Aim 1 Analytic Flow Chart

Table 2.1 Characteristics of participants by infection detection status (2017-2020), n (col %)

	RSV-positive (n=441)	Influenza-positive (n=1,341)	RSV-negative, influenza-negative (n=2,660)
Age, median (IQR)	14 (2-54)	23 (8-54)	37 (10-57)
Age Group			
0-4 years	171 (38.8)	144 (10.7)	404 (15.2)
5-17 years	63 (14.3)	466 (34.8)	493 (18.5)
18-49 years	69 (15.7)	332 (24.8)	823 (30.9)
50-64 years	78 (17.7)	260 (19.4)	571 (21.5)
≥ 65 years	60 (13.6)	139 (10.4)	369 (13.9)
Sex			
Female	242 (54.9)	752 (56.1)	1,664 (62.6)
Race			
White	310 (70.3)	865 (64.5)	1,824 (68.6)
Black	59 (13.4)	246 (18.3)	471 (17.7)
Other	72 (16.3)	230 (17.2)	365 (13.7)
Education [a]			
< High School	14 (3.2)	42 (3.1)	85 (3.2)
Graduated High School/GED	62 (14.2)	145 (10.9)	358 (13.6)
Some college	127 (29.0)	382 (28.6)	776 (29.5)
Bachelor's degree	114 (26.0)	415 (31.1)	787 (29.9)
Advanced degree	121 (27.6)	352 (26.4)	627 (23.8)
BMI [b]			
Obese	95 (46.3)	341 (47.1)	854 (48.7)
Good health [c]			
Yes	401 (91.1)	1,262 (94.3)	2,451 (92.2)
Smoking [d]			
Every day	13 (6.4)	27 (3.7)	85 (4.9)
Some days	2 (0.98)	14 (1.9)	50 (2.9)
Never	189 (92.7)	682 (94.3)	1,615 (92.3)
Season			
2017/18	192 (43.5)	525 (39.2)	795 (29.9)
2018/19	169 (38.3)	478 (35.7)	1,306 (49.1)
2019/20	80 (18.1)	338 (25.2)	559 (21.0)
Multimorbidity [e]			
Yes	142 (68.6)	423 (58.0)	1,015 (58.2)

Children <12 in household			
Yes	256 (58.1)	718 (53.4)	1,234 (46.4)

[a] Missing education status (n=35); If participant < 18, parent's education was used
[b] Missing BMI data (n=30); Obesity not assessed in participants < 18
[c] Missing good health status data (n=4)
[d] Didn't know or refused (n=24); Smoking not assessed in participants < 18
[e] Adults missing data for calculating MWI-ICD10 (n=20); Excludes participants < 18

Table 2.2 Characteristics of adults in multimorbidity analyses by infection detection status (2017-2020), n (col %)

	RSV-positive (n=207)	Influenza-positive (n=730)	RSV-negative, influenza-negative (n=1,744)
Age, median (IQR)	56 (44-68)	52 (37-62)	51 (37-62)
Age Group			
18-49 years	69 (33.3)	331 (45.3)	811 (46.5)
50-64 years	78 (37.7)	260 (35.6)	567 (32.5)
≥ 65 years	60 (29.0)	139 (19.0)	366 (21.0)
Sex			
Female	142 (68.6)	458 (62.7)	1,204 (69.0)
Race			
White	146 (70.5)	487 (66.7)	1,206 (69.2)
Black	40 (19.3)	140 (19.2)	329 (18.9)
Other	21 (10.1)	103 (14.1)	209 (12.0)
Education [a]			
< High School	4 (1.9)	17 (2.3)	36 (2.1)
Graduated High School/GED	40 (19.4)	92 (12.7)	261 (15.1)
Some college	64 (31.1)	215 (29.6)	536 (31.0)
Bachelor's degree	49 (23.8)	211 (29.0)	488 (28.2)
Advanced degree	49 (23.8)	192 (26.4)	408 (23.6)
BMI [b]			
Obese	95 (46.3)	340 (47.0)	843 (48.6)
Good health [c]			
Yes	175 (84.5)	661 (90.8)	1,565 (89.8)
Smoking [d]			
Every day	13 (6.4)	27 (3.7)	84 (4.9)
Some days	2 (0.98)	13 (1.8)	50 (2.9)
Never	189 (92.7)	682 (94.5)	1,597 (92.3)
Season			
2017/18	81 (39.1)	312 (42.7)	466 (26.7)
2018/19	84 (40.6)	227 (31.1)	904 (51.8)
2019/20	42 (20.3)	191 (26.2)	374 (21.4)
Multimorbidity			
Yes	142 (68.6)	423 (58.0)	1,015 (58.2)
Children <12 in household			

Yes	33 (15.9)	194 (26.6)	427 (24.9)

[†] Adults missing multimorbidity data excluded from table (n=20)
[a] Missing education status (n=19)
[b] Missing BMI data (n=18)
[c] Missing good health status data (n=3)
[d] Smoking status, didn't know or refused (n=24)

Table 2.3 Illness characteristics by infection detection status (2017-2020), n (%)

	RSV-positive (n=441)	Influenza-positive (n=1,341)	RSV-negative, influenza-negative (n=2,660)
Length of Illness (days) *			
0-2	2 (0.45)	10 (0.75)	24 (0.90)
3-6	40 (9.1)	170 (12.7)	251 (9.4)
7+	176 (39.9)	479 (35.7)	941 (35.4)
Self-reported symptoms			
Fever	239 (54.2)	1,053 (78.5)	1,231 (46.3)
Sore throat	243 (55.1)	902 (67.3)	1,808 (68.0)
Congestion	417 (94.6)	1,194 (89.0)	2,267 (85.2)
Self-reported number of symptoms +			
1	87 (19.7)	169 (12.6)	596 (22.4)
2	226 (51.2)	503 (37.5)	1,236 (46.5)
3	120 (27.2)	658 (49.1)	746 (28.0)
Sought subsequent care **			
Yes	51 (11.6)	103 (7.7)	233 (8.8)
No	249 (56.5)	722 (53.8)	1,407 (52.9)

* n = 2,349 participants missing length of illness data
+ excludes those who reported 0 symptoms (n=101)
** n = 1,677 participants missing sought subsequent care data

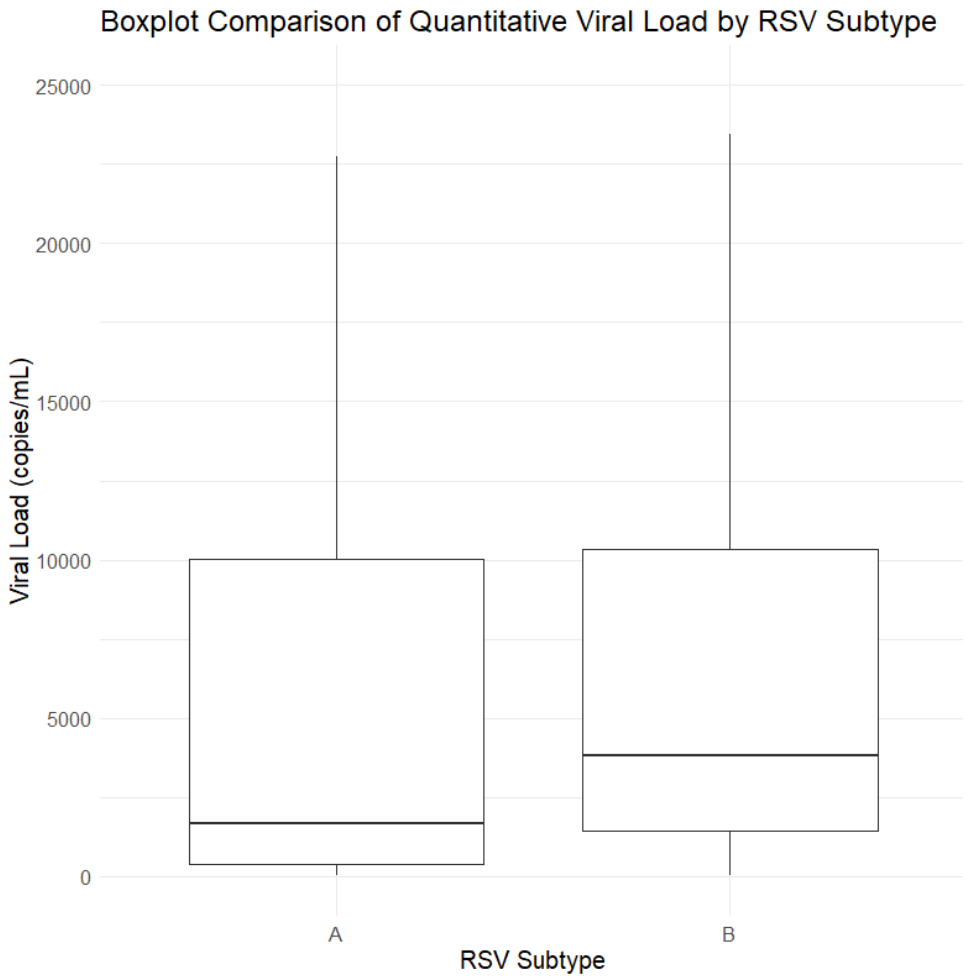

Figure 2.2 Comparison of Quantitative Viral Load (RSV-A versus RSV-B)

Table 2.4 Logistic regression analysis of viral load among those with RSV-B vs. RSV-A infection, stratified by adults and children

	Unadjusted OR (95% CI)	**p-value***	**Adjusted OR (95% CI)**	**p-value***
Overall (n=353) [b]				
Viral load (copies/mL)				
$\geq 2.20 \times 10^4$	1.71 (1.10-2.67)	0.02*	1.88 (1.14-3.11)	0.01*
18+ (n=160) [c]				
$\geq 2.20 \times 10^4$	1.26 (0.62-2.54)	0.53	1.22 (0.56-2.65)	0.61
<18 (n=193) [c]				
$\geq 2.20 \times 10^4$	2.48 (1.37-4.49)	0.003*	2.44 (1.25-4.77)	0.009*

[a] Reference group for all models $< 2.20 \times 10^4$ copies/mL

[b] Adjusted for age, sex, season, race, and time between illness onset and specimen collection

[c] Adjusted for sex, season, race, and time between illness onset and specimen collection

* Statistically significant at $\alpha = 0.05$

Table 2.5 Logistic regression analysis of outcomes of interest among those with high detected viral load compared to those with low detected viral load, stratified by viral subtype, (n) observations overall and included in model

	Unadjusted OR [a] (95% CI)	**p-value**	**Adjusted OR [a, §] (95% CI)**	**p-value**
Overall (n=353)				
Extended length of illness [b]	(n=166)		(n=166)	
Yes	1.96 (0.91-4.24)	0.09	3.14 (1.25-7.93)	0.02*
No. of reported symptoms	(n=353)		(n=353)	
High (≥ 2)	1.39 (0.82-2.36)	0.22	1.17 (0.67-2.03)	0.59
Sought subsequent care [c]	(n=236)		(n=236)	
Yes	0.84 (0.44-1.60)	0.59	1.03 (0.52-2.03)	0.94
RSV-A (n=121)				
Extended length of illness	(n=54)		(n=54)	
Yes	4.39 (0.95-20.2)	0.06	4.88 (0.82-29.1)	0.08
No. of reported symptoms	(n=121)		(n=121)	
High (≥ 2)	1.61 (0.64-4.03)	0.31	1.76 (0.66-4.67)	0.26
Sought subsequent care	(n=76)		(n=76)	
Yes	0.99 (0.31-3.12)	0.98	1.19 (0.31-4.53)	0.80
RSV-B (n=232)				
Extended length of illness	(n=112)		(n=112)	
Yes	1.22 (0.47-3.20)	0.68	2.30 (0.75-7.06)	0.15
No. of reported symptoms	(n=232)		(n=232)	
High (≥ 2)	1.26 (0.66-2.43)	0.48	0.97 (0.48-1.95)	0.93
Sought subsequent care	(n=160)		(n=160)	
Yes	0.77 (0.35-1.68)	0.51	1.09 (0.47-2.53)	0.84

[a] Reference group for extended illness and sought subsequent care outcomes was 'No'; reference group for number of reported symptoms was 'Low (< 2)'
[§] Adjusted for age, sex, race, season, and time between illness onset and specimen collection
[b] n = 187 individuals missing extended illness data
[c] n = 117 individuals missing sought subsequent care data

Table 2.6 Multimorbidity characteristics for RSV-positive, influenza-positive, and negative-for-both adults (2017-2020)

	RSV-positive (n=207)	Influenza-positive (n=730)	RSV-negative, influenza-negative (n=1,744)	p-value [a]
MWI-ICD10 score				
Max.	25.89	54.54	36.57	
Median (IQR)	1.62 (0-4.9)	0.40 (0-2.7)	0.64 (0-3.0)	0.0001*
Mean	3.29	2.16	2.37	
Median MWI-ICD10 score by reported symptom				
Fever	1.33	0.34	0.63	0.16
Sore throat	1.53	0.34	0.34	0.04*
Congestion	1.62	0.64	0.69	0.001*
Median MWI-ICD10 score by reported number of symptoms				
1	2.53	0.81	0.81	0.003*
2	1.62	0.34	0.40	0.05*
3	1.33	0.64	0.42	0.63
Median MWI-ICD10 score by length of illness (days) [b]				
0-2	1.62	0.64	0.81	0.02*
3-6	0.15	0.00	0.34	0.17
7+	1.62	0.34	0.34	0.01*
Median MWI-ICD10 score by sought subsequent care [c]				
Yes	1.62	1.32	1.30	0.30
No	1.62	0.34	0.34	0.002*

* p-value from Kruskal-Wallis test comparing median MWI scores

[b] length of illness data missing for n=127 RSV-positive, n=427 influenza-positive, and n=1,063 negative adults

[c] sought subsequent care data missing for n=79 RSV-positive, n=309 influenza-positive, and n=722 negative adults

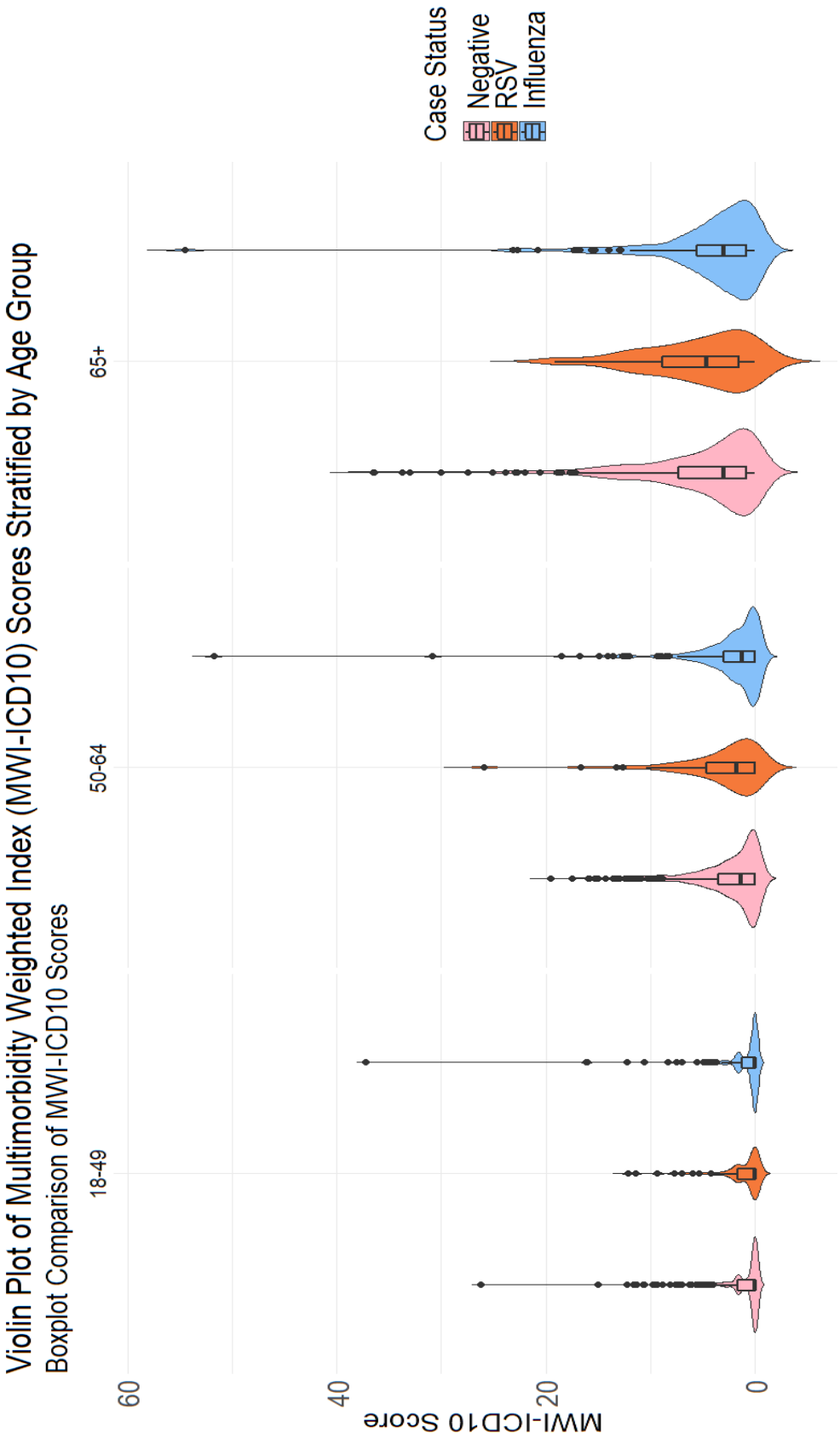

Figure 2.3 Multimorbidity Violin Plot

Table 2.7 Adjusted odds ratios of illness outcomes among adults with multimorbidity compared to adults without multimorbidity by infection status, OR_{adj} (95% CI) and Wald p-values, (n) observations overall and included in model [a, b]

	RSV-positive	p-value	Influenza-positive	p-value	RSV/FLU Negative	p-value
Overall [c]	(n=207)		(n=730)		(n=1,744)	
Extended illness [ǂ]	(n=80)		(n=304)		(n=695)	
Yes	1.63 (0.31-8.54)	0.56	1.03 (0.55-1.95)	0.93	0.92 (0.58-1.47)	0.73
No. of reported symptoms	(n=207)		(n=730)		(n=1744)	
High (≥ 2)	0.91 (0.43-1.93)	0.80	1.36 (0.89-2.08)	0.16	1.11 (0.87-1.41)	0.40
Subsequent care [§]	(n=128)		(n=422)		(n=1032)	
Yes	0.91 (0.29-2.86)	0.88	1.07 (0.59-1.94)	0.82	1.44 (0.98-2.10)	0.06

[a] Reference group for extended illness and sought subsequent care outcomes was 'No', reference group for number of reported symptoms was 'Low (< 2)

[b] Adjusted for age, sex, race, and season

[c] One influenza-positive adult and 19 adults negative for both did not have data to calculate an MWI-ICD10 score

[ǂ] length of illness data missing for n=1,049 negative, n=127 RSV-positive, and n=426 influenza-positive adults.

[§] sought subsequent care data missing for n=712 negative, n=79 RSV-positive, and n=308 influenza-positive adults.

CHAPTER 3

Comparisons of RSV and Influenza: Population Characteristics and Clinical Outcomes in Hospitalized Adults

3.1 Author Summary

In this chapter, I evaluate and compare epidemiologic characteristics and clinical outcomes of adults hospitalized with RSV, influenza, or neither using data from a multi-site adults hospitalized with ARI surveillance network. This network captures the most severe adult cases of RSV requiring hospitalization, and the inclusion of influenza cases provides a strong benchmark for comparison. We found that hospitalized adults with RSV were more likely to have underlying comorbidities and experience an extended length of stay as well as the need for mechanical ventilation. Higher levels of care among older patients with increased levels of pre-existing morbidity highlight the relevance of retaining clinical testing for RSV in this population.

3.2 Abstract

RSV is under-recognized in hospitalized adults and has not been as systematically described as influenza. Hospitalization and in-hospital outcomes, such as intensive care unit (ICU) admission and the need for mechanical ventilation, are widely accepted as markers of clinical severity with respect to acute respiratory illness (ARI). The objective of this study was to compare epidemiologic characteristics and clinical outcomes between adults (≥ 18 years) hospitalized with RSV, influenza, or neither RSV nor influenza detected from Sept 2016 to May 2019.

All hospitalized adults (≥ 18 years) who met a standardized case definition indicating ARI were prospectively enrolled across three respiratory seasons from nine hospitals participating across four sites of the U.S. Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN, 2016-2019). Multivariable logistic regression models were used to test associations between infection and characteristics as well as clinical outcomes, adjusting for age, sex, race, Charlson Comorbidity Index (CCI), body mass index (BMI), site, season, and days between symptom onset and admission.

10,311 adults who were admitted to a participating HAIVEN hospital with ARI were included, with 33.2% (n=3,423) aged 50-64 years and 44.5% (n=4,588) ≥ 65 years old. Six percent of adults tested positive for RSV (n=622), 18.8% positive for influenza (n=1,940), and 75.1% negative for both RSV and influenza (n=7,749). Obesity and age ≥ 65 years old were significantly associated with RSV detection when compared with participants negative for both RSV and influenza. Patients hospitalized with RSV had twice the odds of having a CCI ≥ 3 compared with patients hospitalized with influenza [OR=2.06 (95% CI: 1.49-2.90), p-value<0.0001]. The overall proportion of adults with CHF or COPD was significantly higher in those with RSV (37.3% CHF, 47.6% COPD, p-value <0.0001) compared with those with influenza (28.8% CHF, 35.8% COPD). Patients with RSV had a significantly higher odds of experiencing a length of stay ≥ 8 days [OR=1.38 (95% CI: 1.06-1.80), p-value=0.02] and need for mechanical ventilation [OR=1.45 (95% CI: 1.09-1.93), p-value=0.01] when compared with patients with influenza.

We found increased length of stay and an increased need for mechanical ventilation in individuals with RSV detected relative to influenza. Hospitalized adults with RSV infection were more likely to have underlying cardiopulmonary comorbidities and higher CCI scores. Our

findings suggest that RSV is associated with higher levels of care as older patients with increased levels of pre-existing morbidity are more likely to be hospitalized with RSV.

3.3 Introduction

Respiratory Syncytial Virus (RSV) is widely regarded as a disease of young children most severe in infants < two years (Hall et al., 2009; Zhou et al., 2012). However, clinically significant RSV infection occurs at all ages, and adults often present with unique complications compared to children infected with RSV (Falsey & Walsh, 2000; Walsh et al., 2007). Prior research has identified the following patient risk factors requiring hospitalization: the severely immunocompromised, those with underlying cardiopulmonary comorbidities including congestive heart failure (CHF) and chronic obstructive pulmonary disease (COPD), and elderly persons experiencing frailty (Englund et al., 1988; Falsey et al., 2005, 2006; Han et al., 1999; Walsh et al., 1999). Additional research is needed to support the ongoing evaluation of RSV hospitalization in adults. Moreover, results from such research have the potential to inform targeted vaccination strategies to protect high-risk adults from adverse health outcomes once a safe and effective vaccine is available.

Hospitalization and in-hospitalization outcomes, such as intensive care unit (ICU) admission and the need for mechanical ventilation, are widely accepted as markers of clinical severity with respect to acute respiratory illness (ARI). Moreover, hospitalization with influenza is well-documented and well-researched, making it a strong benchmark for comparing RSV hospitalization outcomes (Falsey et al., 1995; Ferdinands et al., 2019; Malosh et al., 2017; Van Kerkhove et al., 2011). A prior analysis of partial data from the HAIVEN study concluded patients hospitalized with RSV, compared to patients hospitalized with influenza, had more

comorbidities and experienced a longer time until hospital admission (days) (Malosh et al., 2017).

This study aims to characterize the frequency and clinical severity of RSV among hospitalized adults ≥ 18 years, for three respiratory illness seasons across all four sites participating in the nationally representative Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN) study. This study has two objectives: (1) identify population characteristics and key differences among adults (≥ 18 years) hospitalized with RSV, influenza, or neither within three respiratory illness seasons (2016-2019) from all sites participating in the HAIVEN study and (2) compare clinical outcomes between adults hospitalized with RSV, influenza, or neither.

3.4 Methods

3.4.1 Source Population

Data collected for this analysis comes from the HAIVEN study — a prospective study of adults hospitalized with ARI meeting a standardized case definition (Ferdinands et al., 2019; Malosh et al., 2017b; Petrie et al., 2016). The HAIVEN study was designed as a case-test negative study aimed at estimating vaccine effectiveness in the prevention of hospitalization associated with adult influenza cases (Ferdinands et al., 2019; Malosh et al., 2017b; Petrie et al., 2016). Adults ≥ 18 years old admitted to a participating HAIVEN site hospital were prospectively identified from September 2016 through May 2019 through either chief complaint(s) and/or admission diagnosis of an ARI. HAIVEN is comprised of eight (prior to 2018) or nine (2018 and after) hospitals located in Michigan, Pennsylvania, Texas, and Tennessee. HAIVEN study eligibility criteria has been described elsewhere (Ferdinands et al., 2019; Malosh et al., 2017b; Petrie et al., 2016). To participate in the study, written informed

consent is provided by patients or a proxy/surrogate. This study was approved by the Institutional Review Boards at all respective HAIVEN study sites.

3.4.2 Data Collection

Through structured enrollment interviews with HAIVEN research staff, consenting participants self-reported, or via proxy/surrogate where appropriate, demographic data, illness onset date, frailty score (0, 1, 2, 3, 4/5), and influenza vaccination status (yes/no). At the time of the enrollment interview, research staff collected throat and nasal swabs, which were combined in universal transport media (UTM). Specimens are transported to HAIVEN site laboratories and tested for RSV and influenza using real-time reverse transcription PCR (RT-PCR) with primers, probes, and protocols developed by the CDC Division of Viral Diseases and Influenza Division.

Electronic medical records (EMR) were reviewed to extract data for calculating Charlson Comorbidity Index (CCI) scores (0, 1-2, and ≥ 3), determining body mass index (BMI), as well as documented evidence of COPD, CHF, and asthma. Obesity was defined as having a BMI ≥ 30. The outcomes of interest were also extracted from participant EMR including length of stay, admission to the ICU, need for mechanical ventilation, and death prior to or 30-days after discharge. An extended length of stay was defined as ≥ eight days.

3.4.3 Statistical Analysis

Descriptive statistics were calculated for the following variables across each comparison group of interest: age group (18-49, 50-64, and 65+ years), sex, race/ethnicity (White non-Hispanic, Black non-Hispanic, Other non-Hispanic, and Hispanic), BMI (normal, overweight, and obese), CCI scores, asthma, CHF, COPD, frailty, site (Michigan, Texas, Pennsylvania, and Tennessee), season (2016/17, 2017/18, and 2018/19), and influenza vaccination status. Descriptive statistics were calculated for in-hospital outcomes of interest across each comparison

group of interest including extended length of stay, admission to the ICU, mechanical ventilation (any), and death (pre-discharge and 30 days post-discharge). CCI scores, time from illness onset to hospital admission, and time from onset to specimen collection were described with median and interquartile range (IQR) statistics. CCI scores of individuals who tested positive for RSV were compared to individuals who tested positive for influenza using Wilcoxon rank-sum tests, stratified by age group (18-49, 50-64, and 65+ years). Overall and age-stratified (18-49, 50-64, and 65+ years) proportions of CHF and COPD for RSV-positive participants were compared to proportions of CHF and COPD for influenza-positive participants using Chi-square statistics. Age-adjusted, Firth logistic regression models were used to test the association between CHF/COPD and RSV detection compared to influenza detection.

We evaluated characteristics and clinical outcomes by comparing RSV-positive versus influenza-positive and RSV-positive versus RSV-negative/influenza-negative cases separately. Multivariable logistic regression models were used to assess participant characteristics as risk factors – age, sex, CCI, BMI, site, season, time from illness onset to admission, and time from onset to specimen collection – associated with case detection status using the above comparison groups. Multivariable logistic regression models were used to test the association between clinical outcomes of interest – extended length of stay, ICU admission, need for mechanical ventilation, and death – and case detection status using the above comparison groups. Participant characteristic and clinical outcome multivariable models were adjusted for age, race/ethnicity, sex, BMI, CCI, site, season, and time from illness onset to admission. To account for small cell counts resulting from stratification, all logistic models used Firth's adjustment (Firth, 1993). Profile-likelihood confidence intervals and Wald p-values were used to determine statistical

significance. A p-value less than 0.05 was considered statistically significant for all analyses, and all analyses were performed using SAS software version 9.4 (SAS Institute, Cary, NC).

3.5 Results

3.5.1 Population Characteristics & RSV-Influenza Epidemiology

11,369 adults were enrolled in HAIVEN across the three respiratory seasons included in this study. Individuals with subsequent enrollments within a given respiratory illness season (n=602) and/or with missing influenza or RSV laboratory results (n=499), and those presenting with an RSV-influenza co-infection (n=21) were excluded from analysis. The final study population for this analysis was comprised of 10,311 patients, 26.0% (n=2,679) from 2016-2017, 37.7% (n=3,885) from 2017-2018, and 36.3% (n=3,747) from 2018-2019 (Table 1).

Overall, RSV was detected in 6.0% (n=622) and influenza in 18.8% (n=1,940) of eligible participants. The remaining 75.1% (n=7,749) of included participants had neither RSV nor influenza detected. RSV peaked in January for the 2016-2017 and 2017-2018 seasons, whereas RSV peaked in December in 2018-2019 (Figure 3.1). On the other hand, the peak for influenza tended to vary with peaks occurring in February, January, and March, respective to each season included in this analysis.

Just over half (52.4%) of all RSV cases detected in this study were among adults ≥ 65 years of age (Table 3.1). The age-specific proportion of RSV among adults hospitalized with ARI increased with each age group with 4.7% among those 18-49, 5.5% among those 50-64, and 7.1% among those ≥ 65 years of age. Whereas the age-specific prevalence of influenza was relatively consistent among those aged 18-49 (17%) and 50-64 (17.3%) with slightly higher (20.9%) detection among those ≥ 65 years of age. The median time from illness onset to hospital admission among RSV-positive patients was 3 days (IQR: 1-5), and the median time from illness

onset to hospital admission among influenza-positive patients was 3 days (IQR: 1-4). Median time from illness onset to specimen collection for patients with influenza or RSV detected was 3 days (IQR: 2-5) (Table 3.1).

Overall, 90% (n=9,278) of included participants had a CCI ≥ 1, indicating the presence of at least one major underlying comorbid condition (Table 3.1). The overall proportion of adults with CHF or COPD was significantly (p-value<0.0001, X^2 test) higher in those with RSV detected (37.3% CHF, 47.6% COPD) compared with those with influenza detected (28.8% CHF, 35.8% COPD) (Table 3.1). When stratified by age group, the proportions of CHF and COPD between RSV-positive (31.0% CHF, 47.1% COPD) and influenza-positive (25.0% CHF, 43.4% COPD) adults aged 50-64 years were comparable. RSV-positive adults aged 18-49 years had higher proportions of CHF and COPD (26.6% CHF, 29.4% COPD) when compared to influenza-positive adults aged 18-49 years (14.3% CHF, 13.6% COPD). In adults aged 65 years and older, those with RSV detected had higher proportions of CHF and COPD (44.5% CHF, 54.0% COPD) when compared to those with influenza detected (37.0% CHF, 40.1% COPD). Adjusting for age, when compared to those without CHF, those with CHF have significantly higher odds of having RSV detected compared to influenza detected [OR_{adj}=1.40 (95% CI: 1.15-1.70), p-value=0.0007]. Adjusting for age, when compared to those without COPD, those with COPD have significantly higher odds of having RSV detected compared to influenza detected [OR_{adj}=1.57 (95% CI: 1.31-1.89), p-value<0.0001]. In age groups 18-49 and 65+, those with RSV detected had significantly higher median CCI scores when compared to those with influenza detected (Table 3.2, Figure 3.2).

3.5.2 Clinical Outcomes

Among RSV-positive patients, 16.6% experienced a length of hospital stay ≥ 8 days, 12.4% were admitted to the ICU, and 15% required some type of mechanical ventilation — either invasive or noninvasive (Table 3.3). In contrast, among patients with influenza detected, 11.3% experienced a length of stay ≥ 8 days, 9.9% were admitted to the ICU, and 11.1% required some type of mechanical ventilation (Table 3.3). With respect to deaths prior to discharge, nine were recorded in patients with RSV detected, 25 in patients with influenza, and 105 in the negative group (Table 3.3).

After adjusting for potential confounders, patients with RSV detected had a significantly higher odds of experiencing a length of stay ≥ 8 days [OR_{adj}=1.40 (95% CI: 1.08-1.82), p-value=0.01] and need for mechanical ventilation [OR_{adj}=1.46 (95% CI: 1.09-1.94), p-value=0.01] when compared to patients with influenza detected (Table 3.3). Patients with RSV detected, compared to patients with influenza detected, had a higher odds of ICU admission although this finding was not statistically significant [OR_{adj}=1.27 (95% CI: 0.95-1.69), p-value=0.11]. There were no significant clinical outcome associations when comparing patients with RSV detected to the RSV-negative, influenza-negative group or when assessing death as an outcome comparing patients with RSV detected to either group.

3.5.3 Participant Characteristics as Risk Factors

Patients with RSV detected had twice the odds of having a CCI ≥ 3 compared with patients with influenza detected, and this finding was statistically significant [OR_{adj}=2.10 (95% CI: 1.50-2.93), p-value<0.0001] (Table 3.4). Obesity [OR_{adj}=1.29 (95% CI: 1.02-1.63), p-value=0.03] and age ≥ 65 years old [OR_{adj}=1.65 (95% CI: 1.30-2.10), p-value<0.0001] were significantly associated with RSV detection when compared to participants negative for both RSV and influenza. Female sex was significantly associated with RSV detection when compared

to patients with influenza detected [OR_{adj}=1.44 (95% CI: 1.19-1.75), p-value=0.0002] as well as RSV-negative, influenza-negative [OR_{adj}=1.38 (95% CI: 1.16-1.63), p-value=0.0003].

3.6 Discussion

The aim of this study was to compare characteristics and clinical outcomes of adults hospitalized with RSV versus influenza and RSV versus neither RSV nor influenza detected between 2016 and 2019 from all sites participating in the HAIVEN study. Hospitalized adults with RSV detected had a greater overall proportion of underlying cardiopulmonary comorbidities and higher CCI scores when compared to those with influenza detected. With respect to clinical outcomes, hospitalized adults with RSV detected had higher odds of experiencing an extended length of hospital stay as well as the need for mechanical ventilation when compared to those with influenza detected. Female sex and a slight increase in time from illness onset to hospital admission were associated with RSV detection when compared to those with influenza detected. Female sex, age 65 and older, and obesity were also associated with RSV detection when compared to those with neither influenza nor RSV detected.

Adults aged 18-49 and 65+ with RSV detected had significantly higher median CCI scores when compared to those with influenza detected, and the proportion of adults with CHF or COPD was significantly higher in those with RSV detected compared with those with influenza detected. Findings related to CCI in our study are consistent with the findings of Malosh et al (Malosh et al., 2017a). Reproducibility in a larger, geographically diverse population is essential for strengthening criteria to identify adults who should be considered at high-risk of experiencing severe illness when infected with RSV. Further, our findings corroborate prior research in identifying adults with a history of CHF and/or COPD as a priority high-risk group for infection with RSV (Falsey et al., 2006; Walsh et al., 1999).

Results from our clinical outcomes analysis indicate that RSV-associated ARI may be more severe than influenza-associated ARI in some instances. Fifty percent of adults with influenza detected had received an influenza vaccine, which may offer protection against severe influenza-associated outcomes. However, there were no differences in clinical outcomes when comparing the RSV-positive group to the RSV-negative/influenza-negative group, which may be due to these two groups being more similar, especially with regards to proportions of CHF and COPD detected. We found some differences in the demographics of the hospitalized RSV population as well. When compared to the negative control group, those with RSV detected were more likely to be obese and aged 65 and older. Our obesity result is consistent with the findings from Malosh et al.'s comparison of RSV and RSV-negative, influenza-negative patients (Malosh et al., 2017a). Interestingly, the association measured among those aged ≥ 65 is unique, albeit expected, and this association may have been detected due to the expanded sample size of our study.

When compared to both those with influenza detected as well as RSV-negative, influenza-negative, female sex was significantly associated with RSV detection. A study of 2,225 adults aged ≥ 50 with medically attended ARI reported a null association between RSV detection and sex from adjusted logistic regression models (Sundaram et al., 2014). Our finding could be in part due to a larger sample size as well as the inclusion of adults 18 and older. While we do not have data on whether or not our participants work with children or have children residing in their household, it is possible that women are at higher risk of contracting RSV, considering they disproportionally assume roles with greater time spent among children, both personally and professionally (Gould E., 2015; Swinkels et al., 2019). Alternatively, prior research suggests that biologic sex – through various mechanisms including hormone levels,

environmental factors such as smoking, nutrition, and vaccine hesitancy, as well as the presence of underlying chronic conditions – plays a key role in differential incidence, immune response, and severity of ARI between males and females (Falagas et al., 2007; Klein, 2012; Klein et al., 2012; Klein & Flanagan, 2016).

The novelty of this research stems from the expansion to include all sites as well as more recent seasons of the HAIVEN study. In the future, this study could be improved by including influenza and RSV viral subtype data to explore the impact of subtype on in-hospital outcomes. The greatest strength of our analysis was the considerable size and geographic coverage of the sample population due to use of multisite data. Compared to a six-year retrospective study of adults hospitalized with RSV conducted in one U.S. city, we captured 133 more cases of RSV from the three seasons included in our analysis (Schmidt et al., 2019). Another strength of using the HAIVEN study is the implementation of prospective, active participant enrollment that does not depend on clinical sample testing for case status determination (Ferdinands et al., 2019). Missing data was not a restrictive analytic issue due to data collection from participant EMR; however, 'death 30 days post-discharge' was missing a considerable number of observations, which is expected given the potential for loss-to-follow-up in large, hospital-based studies, limiting our analysis of that outcome. Generalizability of this study is limited, and precaution should be taken when interpreting these results for populations that are not predominantly white.

Multiple RSV vaccine candidates in various stages of clinical trials have demonstrated promising results of safety and efficacy. Determining which populations to focus on during initial vaccine rollout is a high-priority discussion that should happen prior to availability of a vaccine. Yamin et al. posits that targeting children < 5 years as a vaccine strategy would be the most efficient method of reducing RSV cases in children as well as adults and the elderly

through indirect protection (Yamin et al., 2016). In a review by Stephens and Varga, the authors emphasize the need for a vaccine tailored to elderly populations that will elicit a high immune response to overcome immune dysfunction often associated with aging (Stephens & Varga, 2021). Our analysis supports previous research through the identification of adults with underlying cardiopulmonary comorbidities being at high risk of experiencing severe RSV illness. To reduce medical costs and resource burden associated with RSV hospitalization, consideration should be given to the immunocompromised, elderly, and young children in any targeted vaccination campaign.

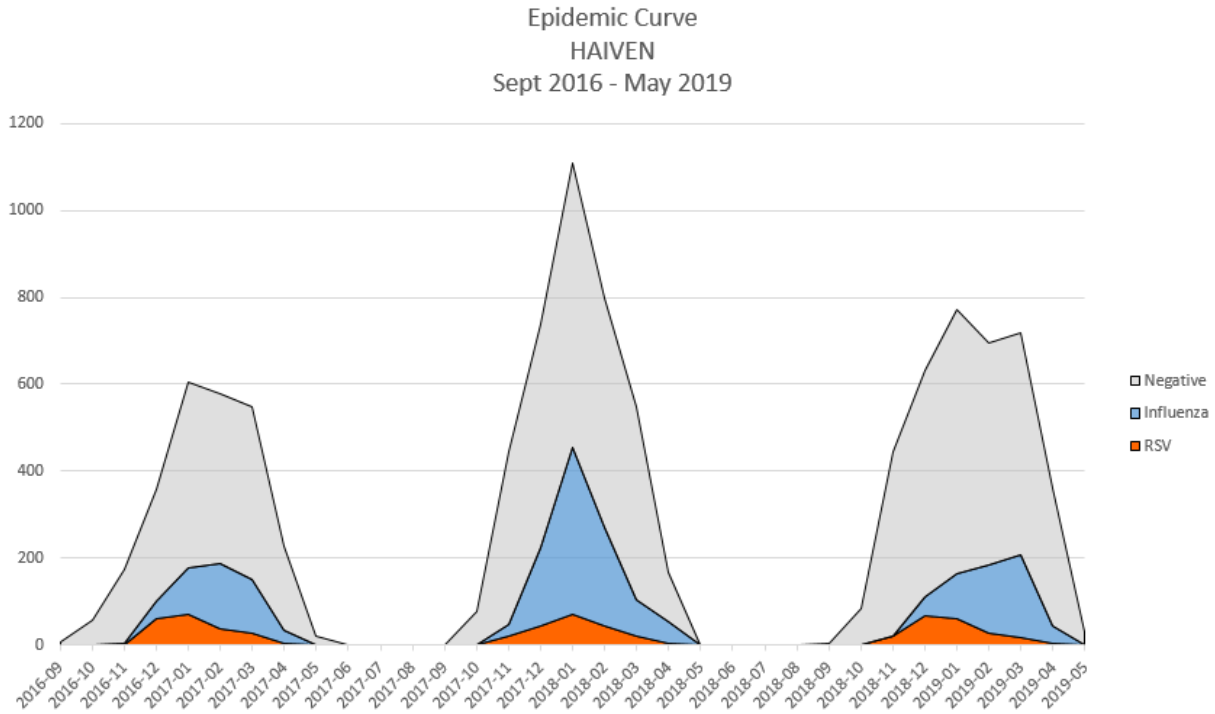

Figure 3.1 Modified HAIVEN RSV-Influenza Epidemiologic Curve

Table 3.1 Frequencies of Epidemiologic Characteristics Among Participants

	Totals (n=10,311)	RSV+ (n=622)	Flu+ (n=1,940)	RSV-/Flu- (n=7,749)
Age group, n (%)				
18-49	2,300 (22.3)	109 (17.5)	391 (20.2)	1,800 (23.2)
50-64	3,423 (33.2)	187 (30.1)	592 (30.5)	2,644 (34.1)
≥ 65	4,588 (44.5)	326 (52.4)	957 (49.3)	3,305 (42.7)
Sex				
Female	5,760 (55.9)	395 (63.5)	1,086 (56.0)	4279 (55.2)
Race Ethnicity, n (%)				
White, Non-Hispanic	6,675 (64.7)	444 (71.4)	1,225 (63.1)	5,006 (64.6)
Black, Non-Hispanic	2,852 (27.7)	137 (22.0)	569 (29.3)	2,146 (27.7)
Other, Non-Hispanic	310 (3.0)	15 (2.4)	57 (2.9)	238 (3.1)
Hispanic	474 (4.6)	26 (4.2)	89 (4.6)	359 (4.6)
BMI, n (%) [a]				
Normal (18.5-24.99)	2,090 (20.3)	122 (19.6)	403 (20.8)	1,565 (20.2)
Overweight (25-29.99)	1,771 (17.2)	100 (16.1)	380 (19.6)	1,291 (16.7)
Obese (≥ 30)	3,271 (31.7)	223 (35.9)	699 (36.0)	2,349 (30.3)
Charlson score, n (%) [b]				
0	1,033 (10.0)	50 (8.0)	281 (14.5)	702 (9.1)
1-2	3,361 (32.6)	202 (32.5)	658 (33.9)	2,501 (32.3)
≥ 3	5,917 (57.4)	370 (59.5)	1001 (51.6)	4,546 (58.7)
Asthma, n (%)	2,820 (27.3)	177 (28.5)	508 (26.2)	2,135 (27.6)
CHF, n (%)	3,805 (36.9)	232 (37.3)	558 (28.8)	3,015 (38.9)
COPD, n (%)	4,506 (43.7)	296 (47.6)	694 (35.8)	3,516 (45.4)
Frailty score, n (%) [c]				
0	1,918 (18.6)	110 (17.7)	465 (24.0)	1,343 (17.3)
1	2,222 (21.6)	161 (25.9)	420 (21.7)	1,641 (21.2)
2	2,166 (21.0)	117 (18.8)	369 (19.0)	1,680 (21.7)
3	1,785 (17.3)	102 (16.4)	324 (16.7)	1,359 (17.5)
4/5	2,090 (20.3)	123 (19.8)	332 (17.1)	1,635 (21.1)
Site, n (%)				
MI	2,596 (25.2)	137 (22.0)	566 (29.2)	1,893 (24.4)
TX	2,963 (28.7)	175 (28.1)	447 (23.0)	2,341 (30.2)
PA	2,437 (23.6)	180 (29.0)	527 (27.2)	1,730 (22.3)
TN	2,315 (22.5)	130 (20.9)	400 (20.6)	1,785 (23.1)
Season, n (%)				
2016-2017	2,679 (26.0)	207 (33.3)	457 (23.5)	2,015 (26.0)
2017-2018	3,885 (37.7)	211 (33.9)	948 (48.9)	2,726 (35.2)
2018-2019	3,747 (36.3)	204 (32.8)	535 (27.6)	3,008 (38.8)

Median time to admission in days (IQR)	-	3 (1-5)	3 (1-4)	2 (1-5)
Median time to specimen collection in days (IQR)	-	3 (2-5)	3 (2-5)	3 (1-5)
Influenza vaccination, n (%) [d]	5,381 (52.2)	361 (58.0)	981 (50.6)	4,039 (52.1)

[a] 30.8% (n=3,179) of individuals were missing BMI data

[b] 1.3% (n=134) missing Charlson score data

[c] 1.3% (n=130) of individuals were missing frailty data

[d] 1.9% (n=195) missing influenza vaccination status data

[§] One-way ANOVA comparison of means

Table 3.2 Comparison of median (IQR) CCI among RSV-positive and influenza-positive hospitalizations, stratified by age-group

	RSV+	*Flu+*	p-value [a]
18-49 years	2 (1-4)	1 (1-3)	0.02*
50-64 years	3 (1-6)	3 (1-5)	0.17
65+ years	4 (2-6)	3 (1-6)	0.02*

[a] p-values from Wilcoxon rank-sum test

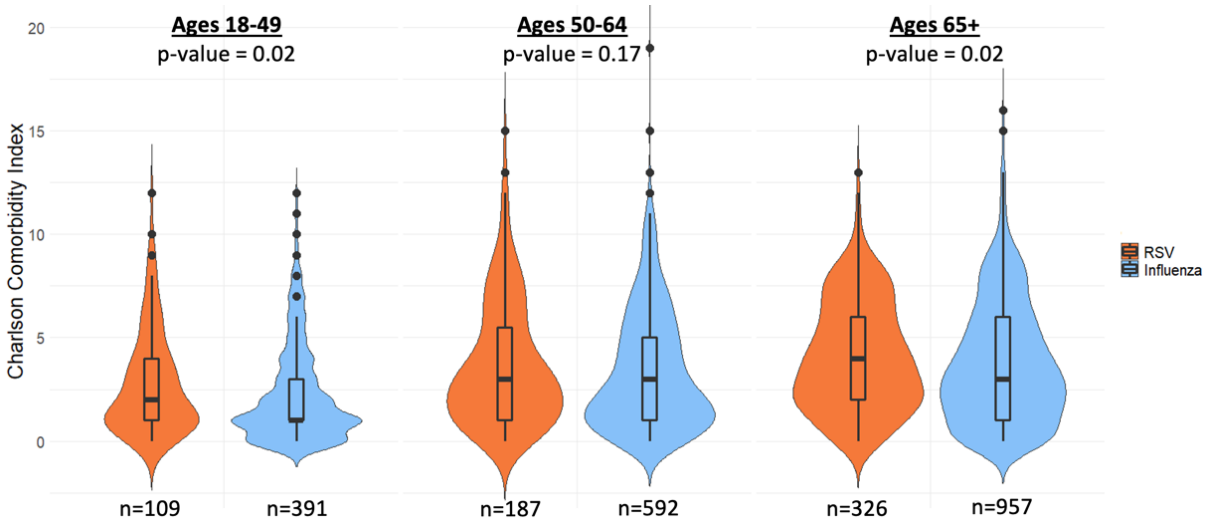

Figure 3.2 Violin Plot Comparing RSV and Influenza Participant CCI Stratified by Age

Table 3.3 Frequencies and Odds Ratios of Clinical Outcomes Comparing Hospitalized ARI Adults, (n) observations overall and included in model

	n (%)			
Frequencies of Outcomes	Total (n=10,311)	RSV (n=622)	Influenza (n=1,940)	RSV-/Flu- (n=7,749)
Extended LOS (≥ 8 days)	1,616 (15.7)	103 (16.6)	220 (11.3)	1,293 (16.7)
ICU Admission [x]	1,340 (13.0)	77 (12.4)	192 (9.9)	1,071 (13.8)
Mechanical ventilation, any [x]	1,447 (14.0)	93 (15.0)	215 (11.1)	1,139 (14.7)
Death				
Pre-discharge [§]	139 (1.4)	9 (1.5)	25 (1.3)	105 (1.4)
30 days post-discharge [ǂ]	222 (2.2)	11 (1.8)	23 (1.2)	188 (2.4)
	Adjusted logistic regression models OR (95% CI) [a]			
	RSV vs. RSV-/Flu-	p-value [b]	RSV vs. Influenza	p-value [b]
Extended LOS (≥ 8 days)	(n=8371)		(n=2562)	
	1.03 (0.82-1.28)	0.83	1.40 (1.08-1.82)	0.01*
ICU Admission [x]	(n=8359)		(n=2558)	
	0.95 (0.74-1.22)	0.70	1.27 (0.95-1.69)	0.11
Mechanical ventilation, any [x]	(n=8359)		(n=2558)	
	1.17 (0.91-1.49)	0.21	1.46 (1.09-1.94)	0.01*
Death				
Pre-discharge	(n=8325)		(n=2537)	
	1.19 (0.61-2.31)	0.61	0.94 (0.46-1.94)	0.88
30 days post-discharge	(n=6516)		(n=1863)	
	0.72 (0.38-1.37)	0.32	1.36 (0.65-2.84)	0.42

[x] 0.14% (n=14) individuals missing ICU and mechanical ventilation data

[§] 0.6% (n=63) individuals missing death prior to discharge data

[ǂ] 23.0% (n=2376) missing death after discharge data

[a] Adjusted for age, sex, race, Charlson score, BMI, site, season, and days to admission

[b] Wald p-values

Table 3.4 Factors associated with case detection using adjusted logistic regression models, OR (95% CI), (n) observations included in model

	RSV+ vs. Flu+ [a, b]	p-value [c]	RSV+ vs. RSV-/Flu- [a, b]	p-value [c]
Charlson score	(n=2562)		(n=8371)	
0	Ref		Ref	
1-2	1.69 (1.20-2.40)	0.003*	1.01 (0.73-1.40)	0.94
≥ 3	2.10 (1.50-2.93)	<0.0001*	0.93 (0.68-1.28)	0.67
Age group	(n=2562)		(n=8371)	
18-49	Ref		Ref	
50-64	1.04 (0.79-1.37)	0.80	1.18 (0.92-1.51)	0.19
≥ 65	1.02 (0.78-1.32)	0.89	1.65 (1.30-2.10)	<0.0001*
Sex	(n=2562)		(n=8371)	
Male	Ref		Ref	
Female	1.44 (1.19-1.75)	0.0002*	1.38 (1.16-1.63)	0.0003*
BMI [d]	(n=1927)		(n=5650)	
Normal (18.5-24.99)	Ref		Ref	
Overweight (25-29.99)	0.90 (0.66-1.22)	0.48	1.00 (0.76-1.32)	0.99
Obese (≥ 30)	1.13 (0.87-1.47)	0.35	1.29 (1.02-1.63)	0.03*
Site	(n=2562)		(n=8371)	
MI	Ref		Ref	
TX	1.43 (1.07-1.92)	0.02*	0.90 (0.70-1.15)	0.40
PA	1.14 (0.86-1.53)	0.36	1.35 (1.06-1.72)	0.02*
TN	1.11 (0.82-1.49)	0.51	0.90 (0.70-1.16)	0.43
Season	(n=2562)		(n=8371)	
2016-2017	Ref		Ref	
2017-2018	0.47 (0.38-0.59)	<0.0001*	0.75 (0.61-0.92)	0.006*
2018-2019	0.80 (0.57-1.11)	0.18	0.62 (0.50-0.76)	<0.0001*
Time from symptom onset to hospital admission, days	(n=2562) 1.05 (1.01-1.09)	0.01*	(n=8371) 1.02 (0.99-1.05)	0.17
Time from symptom onset to specimen collection, days	(n=2562) 1.07 (0.99-1.15)	0.08	(n=8371) 1.00 (0.94-1.06)	0.94

[a] Firth penalized logistic regression with profile-likelihood confidence intervals

[b] Adjusted for age, race/ethnicity, sex, BMI, Charlson score, study site, season, and time from illness onset to admission

[c] Wald p-values

[d] 2721 individuals with missing BMI data were excluded

* Statistically significant p-value at $\alpha = 0.05$

CHAPTER 4

RSV Epidemiology in a Longitudinal Southeast Michigan Cohort with Genomic Community Comparisons

4.1 Author Summary

In this chapter of the dissertation, I evaluate RSV illness epidemiology over the past decade from cases observed in an ARI surveillance study of households with young children (HIVE). The repetitive sampling frame and longitudinal follow-up of HIVE participants allowed for a characterization of repeat infections by subtype, age, and interval between infections. Additionally, I incorporate sequencing data from HIVE RSV samples – and include specimens from a regional ARI surveillance study as community sample reference strains – to depict a picture of annual RSV circulation at the genotype level in Southeast Michigan from the past ten years.

4.2 Abstract

A significant number of studies on RSV-associated illness are based on data from hospitals where infants and children often present with severe illness; however, less is known about mild to moderate RSV infections in community settings, particularly within households in the United States. Historical and present-day household ARI surveillance studies of RSV characterization are typically limited to reporting the frequency and fundamental demographics of RSV-associated ARI, and historical studies were further limited by less sensitive serologic and viral culture methods of ARI detection. Moreover, immunity to RSV is short-lived and repeat

infections are common throughout life; yet there is limited research on reinfections in longitudinal, household settings. The objective of this study was to characterize RSV illnesses, repeat infections, and strain circulation using epidemiologic and genomic data from a household ARI surveillance study between 2010 and 2020.

Data for this aim comes from the Household Influenza Vaccine Effectiveness (HIVE) study — an ongoing, prospective household study with active, year-round ARI surveillance. Real-time polymerase chain reaction (RT-PCR) including specific primers and probes was used to detect non-influenza ARI pathogens and determine RSV subtype using extracted viral RNA. Whole genome amplification sequencing on the Illumina platform was implemented to construct genomic RSV data. RSV-positive specimens from a regional ARI ambulatory clinic cohort study between 2017 and 2020 were included as community reference strains in the sequencing-based strain circulation analysis. For statistical comparisons, non-parametric tests and adjusted binomial-distributed, logit-linked Generalized Estimating Equation (GEE) models were used as appropriate.

In HIVE, 9,822 acute respiratory illnesses detected between 2010 and 2020 were eligible for study inclusion. Five percent of illnesses (n=494) were RSV-positive, of which 36% (n=180) were RSV-A, 34% (n=167) RSV-B, and 30% (n=147) had no subtype determined. The median age of those with RSV-B (5 years, IQR: 3-11) was significantly (p-value = 0.03) lower than the median age of RSV-A (7 years, IQR: 4-15) illnesses. Adjusting for gender, children aged 0-4 years and children aged 5-17 years had significantly higher odds of testing positive for RSV compared to adults [ages 0-4 versus 18+ OR_{adj}: 3.32, p-value<0.0001; ages 5-17 versus 18+ OR_{adj}: 1.72, p-value<0.0001]. The mean interval between first-detected and repeat RSV infection was one and a half years (median: one year and one month). Sixty-five percent of repeat

infections with complete subtype data were heterologous pairs, meaning they were infected with a subtype opposite of their previously detected infection. Within the past decade, RSV-A genotype ON-1 and RSV-B genotype BA-11 were the predominant circulating strains in this community.

Our research provides an in-depth characterization of RSV illnesses and strain circulation across ten recent years of the HIVE study, highlighting household surveillance studies as a valuable tool for conducting a wide-range of ARI research. Reinfection with RSV, particularly with differing subtypes, within one year is common; however, our RSV reinfection analysis helps build upon sparse existing literature on repeat infection intervals by subtype distribution, expanding our understanding of infection-acquired immunity against RSV. In conclusion, upcoming RSV vaccines must provide long-lasting immunity and equal effectiveness across subtypes. Moreover, the importance of household exposure relative to ARI transmission dynamics indicate that households with young children should be considered in the early phases of RSV vaccine distribution.

4.3 Introduction

Respiratory Syncytial Virus (RSV) is a significant cause of acute respiratory infection (ARI) worldwide, chiefly recognized as an etiologic agent of severe disease in children under the age of five (Hall et al., 2009). Many epidemiologic studies of RSV-associated ARI are based on data from hospital settings where infants and children often present with severe illness (Gilca et al., 2006; González-Ortiz et al., 2019; Hall et al., 2013; Laham et al., 2017; Papadopoulos et al., 2004). However, less is understood about mild to moderate RSV infections in the community, particularly within households in the United States.

A pioneering household ARI surveillance study, The Tecumseh Study of Respiratory Illnesses, conducted an RSV analysis between 1965 and 1981 (Monto et al., 1971; Monto & Ohmit, 1990). This study implemented culture and serologic methods and found no difference in age distribution or illness characteristics between RSV subtypes (Monto & Ohmit, 1990). However, similar U.S. studies in the contemporary era of molecular virus detection have been scarce with a few notable exceptions (Byington et al., 2015; Emanuels et al., 2020; Ohmit et al., 2013; Stockwell et al., 2014). Household surveillance studies are difficult to establish and maintain in the United States in part due to their labor and expense, and contemporary studies focus on broad characterizations of ARI frequency typically with an emphasis on influenza (Byington et al., 2015; Emanuels et al., 2020; Monto et al., 2014; Ohmit et al., 2013; Petrie et al., 2017; Stockwell et al., 2014). With updated enrollment, sampling, and laboratory methods, the Household Influenza Vaccine Effectiveness (HIVE) study provides longitudinal specimens and data to support the molecular characterization of RSV illnesses in a single community over ten years. A comprehensive analysis of RSV epidemiology in household settings could better inform non-therapeutic approaches to control RSV transmission and identify priorities for future vaccines and therapeutics.

In the absence of a vaccine, understanding the duration of infection-acquired immunity to RSV infection plays a key role in evaluating the epidemiology of the virus as well as risk factors and correlates of protection. Immunity to RSV is not long-lasting and reinfection is common throughout life; yet, there is limited research on repeat infections in longitudinal, community-based settings (Bont et al., 2002; Glezen et al., 1986; Rossey et al., 2014; Wong et al., 2021). Assessing the interval between repeat infections is one approach to characterizing the role of

waning natural immunity against RSV infections. Understanding the impact of age and other factors on reinfection may provide important information for vaccine development.

We aim to characterize RSV illness epidemiology along with repeat infections using a decade's worth of data from the HIVE study. We depict population characteristics and make statistical comparisons between RSV-positive illnesses and non-influenza RSV-negative illnesses captured in the HIVE study between 2010 and 2020. Further, we complement RSV epidemiology data using whole genome sequencing of detected RSV viruses. We sought to characterize annual strain circulation in our cohort between 2010-2020 at the subtype and genotype level, and sequenced HIVE samples were compared to samples from a regional ARI surveillance network drawing from the same community.

4.4 Methods

4.4.1 Source Population

Data and specimens from this project were available from the Household Influenza Vaccine Effectiveness (HIVE) study from participants who provided informed consent across all years between 2010/11 and 2019/20. HIVE is an ongoing, prospective household study with active, year-round ARI surveillance (Monto et al., 2019). Prior to 2014-2015, surveillance was conducted within the influenza illness season between October and May, and surveillance was conducted year-round beginning in the summer of 2015. HIVE enrolled between 213 - 385 households in Southeastern, MI and 890 - 1,526 participants in Southeastern, MI during this time period.

From 2010-2014, the HIVE study defined a household as 'at least four individuals who received primary care from the University of Michigan Health System and had at least two children < 18 years old' (Monto et al., 2019). Since the 2014-2015 illness season, the HIVE

eligibility criteria were updated to include three-person households (Monto et al., 2019). Participants were asked to come into the HIVE clinic to complete an illness visit within seven days of onset of two or more of the following ARI symptoms: cough, fever, nasal congestion, chills, headache, body aches, or sore throat. The median time between symptom onset and specimen collection in the HIVE study was two days (IQR 1-4 days) (Malosh et al., 2021). Nasal and throat swab specimens were collected and processed at the on-site laboratory at the Michigan Center for Respiratory Virus Research and Response. Participating households completed annual surveys to provide data on household factors as well as demographic data for each participating member of the household.

To compare viral genomes from our household setting to viral sequences in the broader community, RSV samples were obtained from the Michigan Henry Ford Influenza Vaccine Effectiveness (MFIVE) study, located in southeast Michigan, for the study years 2017/18, 2018/19, and 2019/20 seasons. Patients presenting to ambulatory care outpatient clinics for medically attended acute respiratory illness (MAARI) lasting ≤ seven days with a cough and who have not taken antiviral treatment for their current illness were eligible for participation. Research staff collect throat and nasal swab specimens and deliver them to the Michigan Center for Respiratory Virus Research and Response laboratory for processing.

4.4.2 Laboratory — Virus Detection

Initial detection of RSV cases, among other viral pathogens, was accomplished using viral RNA from participant samples, extracted with QIAamp Viral RNA Kits (Qiagen), and RT-PCR respiratory assays on an ABI 7500 PCR system platform (Life Technologies). Prior to the 2016/17 seasons, singleplex assays were used to detect RSV with primers and probes developed by the CDC Division of Viral Diseases (Ohmit et al., 2013). Beginning in the 2016/17 season

and onward, the FTD Respiratory Pathogen 33 multiplex PCR kit (Fast Track Diagnostics) panel was used for detection of RSV (Sakthivel et al., 2012).

For specimens positive for RSV, viral subtype was determined using a multiplex RT-PCR assay (Kuypers et al., 2004; Martin et al., 2008). Forward and reverse primers specific to RSV as well as fluorescent probes specific to RSV-A and RSV-B were included in the RT-PCR reaction master mix. A negative control, nuclease-free water, and two positive controls–conserved RSV plasmids for both RSV-A and RSV-B–were included on each assay plate for quality control. All samples that tested negative for RSV–even if positive for a different pathogen detected by the FTD panel–were classified as an RSV-negative case for comparison.

4.4.3 Statistical Analysis of RSV positivity

RSV-positive, RSV-negative, and RSV subtype-specific illnesses were characterized by median age at infection, age at infection category (0-4, 5-17, 18-49, 50-64, and 65+ years of age), sex, race, and number of children present in the household (1-2, 3-5, 6+). Those with missing illness laboratory data were excluded from analyses. Statistical comparisons were made using Chi-square, N-1 Chi-square, and Median Two-Sample Test, where appropriate. To control for repeat sampling of participants in statistical analyses, binomial-distributed, logit-linked Generalized Estimating Equations (GEE) were used to study the effects of gender (males vs. females), age (0-4 vs. 5-17, 0-4 vs. 18+, and 5-17 vs. 18+), and children in household (1-3 vs. 4+) on RSV-positivity. In GEE models comparing gender and age categories, adult illnesses aged 18 and older were collapsed into one age category for comparisons, and child illnesses were separated by ages 0-4 years and 5-17 years. Models assessing the effect of number of children residing in a household (categorical: 1-3 and 4+) were restricted to adults aged 18 and older and adjusted for gender and continuous age. A p-value=0.05 for all statistical comparisons was

considered significant. The seasonality of RSV-positive illnesses was examined for each year, and an epidemiologic curve was generated. All statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC) and figures were created using R (version 4.0.2).

Repeat infections were defined as a new case of RSV detected 14 days or more between confirmed infections within the same individual. Per study protocol, individuals were not resampled during continued illnesses unless they reported new or worsening symptoms. Repeat RSV illnesses were characterized by viral subtype, interval between detected infection, and age at first-detected and repeat infection. Individual infection pairs were considered heterologous if a different subtype was detected at the time of repeat sampling compared to their first-detected infection.

4.4.4 Laboratory — RSV Sequencing Protocol

After viral subtype determination, samples with a subtype-specific cycle threshold (Ct) cutoff of ≤ 30 were sequenced using a previously published six-segment amplification method with subtype-specific forward and reverse primers (Agoti et al., 2015). This method, intended to improve genome coverage by increasing segment overlap, was implemented for all eligible samples (Figure 4.1 (Agoti et al., 2015)). To increase yield, viral RNA was reextracted from all eligible samples just prior to amplification using the MVP_2Wash_200_Flex_med protocol with a KingFisher Sample Purification System (Thermo Fisher Scientific).

The amplification process is subtype-specific, and specific forward and reverse primers for both RSV-A and RSV-B were used for respectively subtyped samples (Table 4.1). Primer names and corresponding nucleotide sequences are described elsewhere (Agoti et al., 2015). Complementary DNA (cDNA) was generated for each segment using RT-PCR. First, 2µL of freshly harvested RNA was combined with 8µL segment-specific forward primer working stock

(1µL dNTP mix + 1µL segment specific primer mix at 2µM per forward primer + 6µL DEPC-treated H_2O per reaction). Plates were placed on thermocyclers for 10 min at 65°C followed by ice for 1 min. Next, 10µL of SuperScript™ First-Strand Synthesis System (Thermo Fisher Scientific) master mix (2µL RT buffer + 4µL $MgCl_2$ + 2µL DTT + 1µL RNase OUT + 1µL SSIII enzyme per reaction) were added to each reaction well and plates were placed back on thermocyclers for 42°C for 50 min followed by 85°C for 5 min.

After cDNA was generated, all segments were amplified using subtype-specific, segment-specific forward and reverse primers, Phusion® High-Fidelity DNA Polymerase (New England Biolabs, Thermo Scientific®), and cDNA. The Phusion master mix added to each reaction well contained 5µL 5x Phusion GC buffer + 0.5µL of 10µM dNTPs + 1.25µL of 10µM forward primer mix + 1.25µL of 10µM reverse primer mix + 0.25µL Phusion + 11.75µL DEPC-treated H2O. For each segment, 5µL of cDNA were added to 20µL of Phusion master mix and mixed via pipetting. Plates were amplified using the following protocol: 98°C for 30 sec, 40 cycles of [98°C for 10 sec, 68°C for 30 sec, 72°C for 3 min], 72°C for 10 min, 4°C hold. Once amplification was complete, six segments for each sample were pooled and post-PCR bead cleaning was performed. Samples were run in individual wells on a 1% agarose gel to visually inspect for the presence of PCR product. In preparation for sequencing on the Illumina platform, samples were barcoded using Nextera® DNA Library Preparation Kits (Illumina). After library prep was completed, an additional post-PCR bead clean was performed and indexed samples were pooled for sequencing on a MiSeq platform with read length (2x250) chemistry (v2) by the Michigan Microbiome Sequencing Core (Ann Arbor, MI).

For the sequencing data analysis pipeline, subtype-specific zero-indexed bed files were created for mapping sample sequences to subtype-specific reference genomes. Subtype-specific

Snakefiles were generated for use as the primary analytic pipeline and run for all samples on the University of Michigan (Ann Arbor, MI) Advanced Research Computing (ARC) cluster using the Snakemake 6.12.3 module. Sample sequences were aligned to reference genomes using BWA (RSV-A reference genome FJ948820.1, RSV-B reference genome JQ582843.1), and primers were trimmed using iVar. Genome coverage was determined using samtools, and iVar was used to get consensus sequences. A minimum quality score of zero was used for base calling, with a minimum depth of 10, and the minimum whole genome length for inclusion was 13,500 base pairs. For filtering G-gene sequences, we aligned consensus sequences using MAFFT (online Version 7) to G-gene reference genomes and required no missing data. For assessing quality of sequencing runs, subtype-specific stepwise-line cutoff and genome coverage plots were generated in R Studio (v1.1.453). Subtype-specific phylogenetic trees with branch support indicated by bootstrap values were created using IQ-TREE (stable Version 1.6.12) and visualized with FigTree (v1.4.4) for whole genome and G-gene specific sequences. Tabulations of subtype-specific genotypes were calculated by study and year.

4.5 Results

4.5.1 RSV Epidemiology in Households

Between 2010-2020, 9,989 illnesses were captured in the HIVE study, and 5.0% (n=494) were cases of RSV (Table 4.2). Among RSV-positive illnesses, 36% (n=180) were RSV-A, 34% (n=167) were RSV-B, and 30% (n=147) had no subtype determined. RSV-negative illnesses (n=9,328) included those who tested positive for any respiratory virus, or combination of respiratory viruses, excluding RSV using the FTD panel as well as those who tested pan-negative. There were 167 illnesses missing respiratory viral laboratory data and were excluded from analyses, making the total analytic sample size equal to 9,822. The presence of children in a

household was a requirement for HIVE enrollment, thus, the median age of HIVE illnesses was young (Median: 12 years old), and only 3% (n=302) were 50 years of age or older (Table 4.2).

Relative to children aged 0-4 years, the proportion of RSV illnesses was lower among children aged 5-17 years and adults aged 18–49-years (Table 4.2). Among 1,983 illnesses in children 0-4 years old, 9.1% were due to RSV, and 43 RSV illnesses were identified in infants ≤ one year of age. By comparison, 5.0% of 4,078 illnesses in children aged 5-17 years and 2.9% of 3,459 illnesses in adults aged 18-49 years were due to RSV. When stratified by RSV subtype, the median age of those with RSV-B (5 years, IQR: 3-11) was significantly (p-value = 0.03) lower than the median age of RSV-A (7 years, IQR: 4-15) cases. Among households with higher numbers of children present, the proportion of RSV illnesses detected increased as well (Table 4.2). Among infant RSV illnesses, 58% (n=25) were female and 42% (n=18) were male, and this difference was not statistically significant (N-1 Chi-square test, p-value=0.14).

Controlling for categorical age (0-4, 5-17, and 18+), there was no difference in the odds of testing positive for RSV between males and females [OR_{adj}=1.09, (CI: 0.91, 1.31), p-value=0.36]. Adjusting for gender, there were significant differences in the odds of testing positive for RSV when comparing age groups. Children aged zero to four had significantly higher odds of testing positive for RSV when compared to children between the ages of 5-17 [OR_{adj}=1.93, (CI: 1.57, 2.37), p-value<0.0001] and adults aged 18 and older [OR_{adj} =3.32, (CI: 2.61, 4.22), p-value<0.0001]. When compared to adults, children aged 5-17 also had significantly higher odds of testing positive for RSV [OR_{adj}=1.72, (CI: 1.35, 2.19), p-value<0.0001]. Controlling for sex and continuous age, there was no significant difference in the odds of testing positive for RSV among adults in households with 1-3 children compared to

adults in households with four or more children [n=3,761, OR_{adj}=0.73, (CI: 0.40, 1.33), p-value=0.31].

The seasonal percentage of RSV illnesses detected in HIVE was relatively consistent, apart from the lowest estimate (3.7%) in 2014-2015 and highest (7.6%) in the 2012-2013 season (Table 4.3, Figure 4.2). The latest seasonal peak of RSV cases in HIVE occurred in March in the 2010-2011 season (n=28 cases), and the highest peak occurred in January of the 2012-2013 (n=41 cases). In subsequent seasons, peaks occurred earlier in the season typically between the months of December and February. When HIVE surveillance was expanded to year-round beginning in June 2015, no cases of RSV were captured between June and September of any year with the exception of one repeat RSV infection detected in June 2019.

4.5.2 Repeat RSV Infections

A repeat infection was defined as a new case of RSV detected with ≥ 14 days between confirmed infections, and individuals were not resampled unless they reported new or worsening symptoms. Among 446 first-detected RSV infections, 48 repeat infections were detected in HIVE, with seven of those infections in individuals with two prior infections. The average interval between first-detected and repeat infection was one and a half years (median: one year and one month). Among those who experienced a repeat infection, the average age at first-detected infection was seven years and nine months (Median: four years of age, Range: 2.5 months to 44.5 years of age), and the average age at repeat infection was eight and a half years (Median: five years and three months, Range: 9.5 months to 45.5 years).

The minimum interval between detected infections in our sample was 14 days with a maximum of four years and ten months (Median: one year and one month) (Figure 4.3). Among individuals with repeat infections who were able to have all infections subtyped (n=20), 65%

(n=13) were infected with a different subtype compared to their earlier detected infection. One individual that experienced multiple repeat infections of the same subtype was infected by RSV-A at all points of sampling (2012, 2013, and 2017). There were nine household clusters represented by individuals who experienced repeat infections, and four household pairs experienced their first detected and second detected infections at the same time.

4.5.3 RSV Sequencing Analysis

Of all available subtyped RSV samples (n=748), 50.4% (n=377) had adequate quantity for sequencing. Eighty-three RSV-A and 88 RSV-B complete (90% completeness) sequences were generated and included in respective phylogenetic trees (HIVE versus Community Reference Samples "MFIVE") (Figure 4.4, Figure 4.5). Genotype-specific phylogenetic trees (Figure 4.6, Figure 4.7) comprised of samples with complete (100% completeness) G-gene sequence data (n=127 RSV-A, n=65 RSV-B) and reference strains were also included.

Among all samples sequenced (n=377), HIVE accounted for 27.6% (n=104) RSV-A and 17.5% (n=66) RSV-B samples. Fifty-two RSV-A and 27 RSV-B complete genomes (Figure 4.4, Figure 4.5) and 61 RSV-A and 20 RSV-B G-gene sequences (Figure 4.6, Figure 4.7) representing HIVE were generated. Between 2010-2011, two HIVE RSV-A samples were most closely related to genotype NA1. Between 2011-2013, seven HIVE RSV-A samples were equally closely related to genotypes NA-1 and ON-1, and in 2012, two HIVE samples were most closely related to genotype GA-5. Between 2012-2020, 50 HIVE RSV-A samples were most closely related to the ON-1 genotype. For RSV-B, no samples from 2010 or 2012 had complete G-gene sequences available for inclusion. In 2011, one HIVE RSV-B sample was most closely related to genotype BA-12, and three samples between 2011-2014 were most closely related to BA-8. Between 2015-2020, 16 HIVE RSV-B samples were most closely related to genotype BA-

11. Four individuals with detected repeat infections had sequencing data available for both infections, and three of those individuals had heterologous infections detected. One individual, who experienced multiple repeat infections of RSV-A, only had sequencing data available from their first-detected and second repeat infection. Both their first-detected (Sample ID: MH2437) and second repeat infection (Sample ID: MH14845) were most closely related to genotype ON-1.

Using previously collected samples available from MFIVE (community reference samples), 6,491 were tested for RSV, and 7.3% (n=473) were positive for RSV among this MAARI population between 2017/18 and 2019/20 (Chung et al., 2020; Dawood, 2020; Flannery et al., 2018). In MFIVE, 32.6% (n=154) of RSV-positive samples were RSV-A, 51.8% (n=245) were RSV-B, and 15.6% (n=74) had no subtype determined. Among all samples sequenced (n=377), MFIVE accounted for 25.7% (n=97) RSV-A and 29.2% (n=110) RSV-B samples. Thirty-one RSV-A and 61 RSV-B complete genomes (Figure 4.4, Figure 4.5) and 66 RSV-A and 45 RSV-B G-gene sequences (Figure 4.6, Figure 4.7) representing MFIVE were generated. Between 2017-2020, all (n=66) MFIVE RSV-A G-gene sequences were most closely related to ON-1, and all (n=45) RSV-B G-gene sequences were most closely related to BA-11.

4.6 Discussion

The objective of this study was to characterize RSV illness epidemiology and repeat infections in-depth using data from the HIVE longitudinal household cohort. We further supplemented this analysis with sequencing data to describe RSV strain circulation at the genotype level. Approximately 5% of illnesses in HIVE between 2010 and 2020 were RSV-positive, and RSV percent positivity demonstrated a U-shaped curve across age groups. We found significant differences in age across subtypes as well as in the odds of testing positive for RSV. Our reinfection analysis provided a comprehensive profile of interval between infections

detected and subtype distribution among 41 individuals. Finally, our phylogenetic analysis afforded insight into genotype strain circulation within the community.

A challenge of household studies in general is the high cost and extensive labor input associated with conducting them as well as loss to follow-up of study participants, thus RSV data from U.S. households using molecular methods is limited; however, multiple household ARI surveillance studies have been established (Byington et al., 2015; Cohen et al., 2021; Emanuels et al., 2020; Nokes et al., 2008; Ohmit et al., 2013; Scott et al., 2019; Stockwell et al., 2014). U.S.-based household studies typically focus on describing the frequency of RSV detection along with other ARIs, and the Seattle Flu Study reported the incidence of primary and repeat RSV infections in their analysis (Byington et al., 2015; Emanuels et al., 2020; Monto et al., 2014; Stockwell et al., 2014). A birth cohort household study in Kilifi, Kenya has reported a high burden of RSV among infants and young children, and through whole genome sequencing they identified school-aged children as the source of household RSV introduction and infection among infants (Agoti et al., 2012; Munywoki et al., 2014; Nokes et al., 2004; Ohuma et al., 2012; Okiro et al., 2008). Conversely, from a sequencing-based household transmission analysis in Nepal, Scott et al. reported that preschool-aged children were the source of household RSV introduction (Scott et al., 2019). The PHIRST cohort study in South Africa has indicated future plans to assess RSV burden and transmission (Cohen et al., 2021).

We found significant (p-value<0.0001) differences in the odds of testing positive for RSV when comparing young children (ages 0-4) to older children (ages 5-17) and adults as well as older children to adults, indicating that young age is an important risk-factor for RSV-associated ARI which is in-line with previous research (Hall et al., 2009; Nokes et al., 2008). Our data also showed that the median age of those infected with RSV-B was significantly

younger compared to the median age of those infected with RSV-A (p-value=0.03), and this finding differs from previous studies. In a recent meta-analysis assessing RSV surveillance data from 15 countries, the authors reported no global difference in age between subtypes (Staadegaard et al., 2021). Interestingly, community care data from the Netherlands found that the median age of RSV-A cases was significantly lower than RSV-B cases (Staadegaard et al., 2021). We found that both RSV-A and RSV-B are significant contributors to illness in children and adults, and vaccine development must focus on ensuring that effectiveness holds across subtypes. Studies that include a community-representative range of ages would also provide better insight into differences in age across RSV subtypes.

In the assessment of repeat infections, our data indicated that the average interval between first-detected (median age: four years) and repeat (median age: five years) infection was one and a half years (range: two weeks to four years and ten months). Further, among those with a complete subtype profile, 65% were infected with a different subtype compared to their earlier detected infection. A hallmark immunology study on repeat RSV infections among hospitalized infants in the Netherlands found that T-cell response at primary infection did not completely protect against subsequent infection in the following illness season (Bont et al., 2002). An analysis from the Kenya birth cohort study found that the mean interval between primary and repeat infections was one year which was within the interval range in our data, and we found a higher proportion of heterologous reinfection pairs (Agoti et al., 2012). Our findings underscore the importance of developing a vaccine that offers long-term immunity with cross-subtype protection.

Whole genome sequencing is rapidly becoming a widely available tool for surveillance of ARI; however, less than 500 complete RSV sequences from the last decade in North America are

currently available as many efforts target specific gene regions rather than the entire genome (Choudhary et al., 2013; Eshaghi et al., 2012; Trento et al., 2006). The predominance of RSV subtype changes with each respiratory season, but several intra-group viral genotypes can co-circulate in the same season (Agoti et al., 2015; Do et al., 2015; Rebuffo-Scheer et al., 2011; Schobel et al., 2016; Tan et al., 2013). Our work contributed 171 additional complete sequences to the U.S. RSV genome profile, and our G-gene phylogenetic analysis identified ON-1 as the predominate RSV-A genotype and BA-11 as the predominate RSV-B genotype circulating in HIVE during this period. Since its emergence in 2010, an increasing prevalence of the ON-1 genotype has been reported in North America and is reflected in our data (Duvvuri et al., 2015; Eshaghi et al., 2012; Jackson et al., 2021; Schobel et al., 2016).

Data for this project comes from a well-established ARI household surveillance study lending to multiple strengths of our analysis, yet, our project faced several limitations as well. Representation in HIVE is limited to households with children and those residing in zip codes that HIVE targets for enrollment. To assess how RSV sequence data in HIVE varied from sequences from RSV infections detected in adjacent regional settings, we included eligible representative samples from MFIVE to establish a community reference population of individuals enrolled without restrictions on household membership. Between 2017 and 2020, genotype strain relatedness for both RSV-A and RSV-B were virtually identical among HIVE and MFIVE samples, indicating that the HIVE study is accurately representative of community RSV circulation in southeast Michigan. Our use of ambulatory clinic-collected samples made for a more representative comparison with respect to mild to moderate illness in the community compared to a hospital-based study, which would likely capture more severe cases of RSV. Due to lapses in re-enrollment among households, it is plausible we missed some RSV illnesses, yet

we were able to identify a substantial number of RSV illnesses, including reinfections, during this period. Notably, we identified reinfections, in some cases multiple and within the same household, among children and adults, implicating RSV as significant cause of ARI across the life course. The implementation of highly sensitive and specific PCR assays for ARI identification reduced the likelihood of misclassification bias, and the supplementation of sequencing data allowed us to more precisely characterize strain circulation at the genomic level.

Multiple RSV vaccine candidates are currently in clinical trials, and there are open questions surrounding who should be targeted in an effective vaccination campaign. Our data suggests that households with young children should be considered a priority group for vaccination once available. In a resource-limited scenario, one proposal suggests targeting children under the age of five would be the most efficient method of reducing RSV cases in children as well as adults, especially the elderly, through indirect protection (Yamin et al., 2016). Our findings on reinfection stress the importance of developing a vaccine that provides lasting immunity and is of equal effectiveness across both subtypes. Further, with regards to young children, particularly infants, and older adults, any successful vaccine would have the capacity to illicit an immune response strong enough to overcome immunocompromised states (Stephens & Varga, 2021).

In conclusion, we present a robust description of RSV illnesses across ten years of the HIVE study. Households serve as a valuable setting to conduct a wide-range of ARI research, and our analysis highlights the relevance of ongoing RSV epidemiologic characterization within established household studies. Our repeat infection analysis helps build the sparse existing literature on reinfection intervals by subtype distribution and expands our understanding of RSV infection-acquired immunity dynamics. The inclusion of sequencing data was a considerable

contribution to the field as genomic data is essential for examining transmission and annual circulatory patterns of respiratory viruses; our results highlight the value of incorporating sequencing-based analyses in household ARI surveillance studies. Once a safe and effective vaccine is available, households with young children should be targeted early in vaccine distribution.

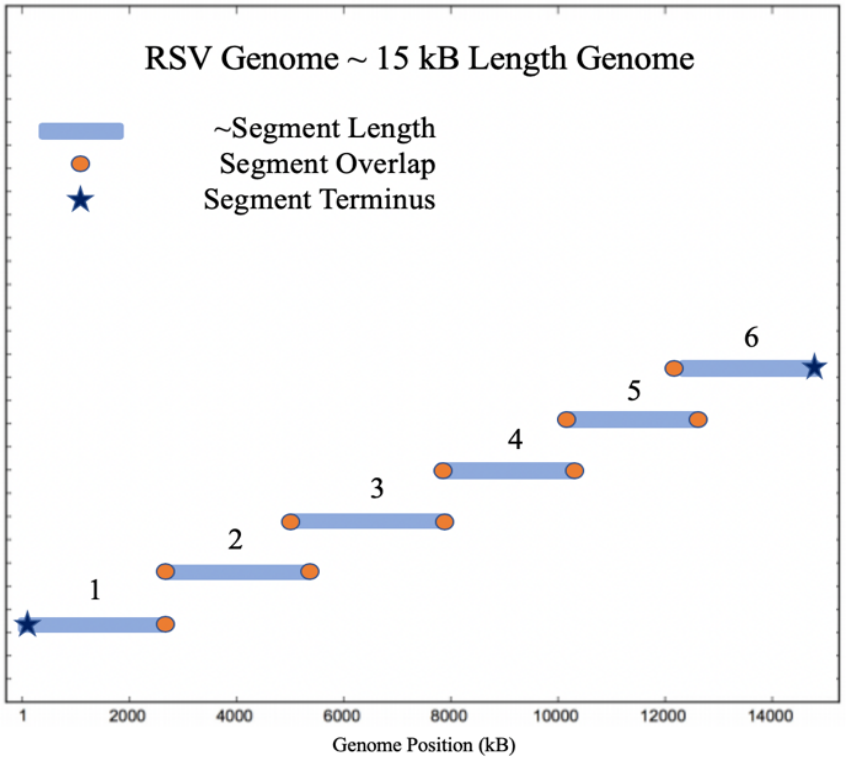

Figure 4.1 Six-segment amplification genome coverage (Agoti et al., 2015)

Table 4.1 Summary of RSV Primers (Agoti et al., 2015)

Target	Primer	Sequence (5′ to 3′)	Position /Strand
RSVA	rsvas	ACGCGAAAAAATGCGTACAAC	1/+
RSVA	rsva52	TGTGCATGTTATTACAAGTAGTGATATTTG	266/+
RSVA	rsva50	GCATGTTATTACAAGTAGTGATATTTGCC	269/+
RSVA	rsva117	ATAAGAGATGCCATGGTTGGTTTAAGA	2849/+
RSVA	rsva86	AAGAGATGCCATGGTTGGTTTAAGA	2851/+
RSVA	rsva175	TTCTCTTAAACCAACCATGGCATCT	2878/-
RSVA	rsva39	CTTCTCTTAAACCAACCATGGCATC	2879/-
RSVA	rsva1820	GCAGCATATGCAGCAACAATC	5207/+
RSVA	rsva1914	CAGCATATGCAGCAACAATCCAA	5208/+
RSVA	rsva1644	CAACTCCATTGTTATTTGCCCC	5674/-
RSVA	rsva1688	CAACTCCATTGTTATTTGCCCCA	5674/-
RSVA	rsva704	ATGTGTTGCCATGAGCAAACTC	7893/+
RSVA	rsva731	GCCATGAGCAAACTCCTCACT	7900/+
RSVA	rsva341	TTGTCAGGTAGTATCATTATTTTTGGCATG	8196/-
RSVA	rsva312	AGGATATTTGTCAGGTAGTATCATTATTTTTGG	8203/-
RSVA	rsva374	AAGAGAACTCAGTGTAGGTAGAATGTTT	10360/+
RSVA	rsva350	AGAACTCAGTGTAGGTAGAATGTTTG	10363/+
RSVA	rsva497	GCTTGATTGAATTTGCTGAGATCTGT	10620/-
RSVA	rsva539	ATGCTTGATTGAATTTGCTGAGATCTG	10622/-
RSVA	rsva1220	GATTGGGTGTATGCATCTATAGATAACAAG	12386/+
RSVA	rsva1232	ATTGGGTGTATGCATCTATAGATAACAAG	12387/+
RSVA	rsva364	TTATATATCCCTCTCCCCAATCTTTTTCAAA	13070/-
RSVA	rsva385	ATCAGTTATATATCCCTCTCCCCAATCTT	13075/-
RSVA	rsva4066	GTTGTATAACAAACTACCTGTGATTTTAATCAG	14983/-
RSVA	rsva5632	TAACTATAATTGAATACAGTGTTAGTGTGTAGC	15063/-
RSVA	rsvae	ACGAGAAAAAAAGTGTCAAAAACTAATA	15223/-
RSVB	rsvbs	ACGCGAAAAAATGCGTACTACA	1/+
RSVB	rsvb3	TGGGGCAAATAAGAATTTGATAAGTGC	44/+
RSVB	rsvb1021	GGGGCAAATAAGAATTTGATAAGTGCTATT	45/+
RSVB	rsvb33	ATATTAGGAATGCTCCATACATTAGTAGTTG	2777/+
RSVB	rsvb71	TAAGAGATGCTATGGTTGGTCTAAGAGA	2841/+
RSVB	rsvb50	AGTCTTGCCATAGCCTCTAACCT	2937/-
RSVB	rsvb95	CCATTTTTTCGCTTTCCTCATTCCTA	2963/-
RSVB	rsvb7884	AGTATATGTGGCAACAATCAACTCTG	5202/+
RSVB	rsvb7996	TATGTGGCAACAATCAACTCTGC	5206/+
RSVB	rsvb7442	GATGTGGAGGGCTCGGATG	5548/-
RSVB	rsvb7423	CCATGGTTATTTGCCCCAGATTTAAT	5662/-
RSVB	rsvb3762	AGAGGTCATTGCTTGAATGGTAGAA	7642/+
RSVB	rsvb3712	AAGAGCATAGACACTTTGTCTGAAATAAG	7762/+
RSVB	rsvb3652	GCTTATGGTTATGCTTTTGTGGATATCTAAT	8130/-
RSVB	rsvb3660	GCAATCATGCTTTCACTTGAGATCAA	8247/-

RSVB	rsvb32	AAGAAGAGTACTAGAGTATTACTTGAGAGATAA	10236/+
RSVB	rsvb52	AAATCCAAATCTTAGCAGAGAAAATGATAG	10412/+
RSVB	rsvb47	CCATGCAGTTCATCTAATACATCACTG	10673/-
RSVB	rsvb168	TGCATGTCTATATGTACATATTATTGTGACAAG	10746/-
RSVB	rsvb651	ATCGACATTGTGTTTCAAAATTGCATAAG	12640/+
RSVB	rsvb165	TTCAAAATTGCATAAGTTTTGGTCTTAGC	12653/+
RSVB	rsvb27	TTAATGAACATATGATCAGTTATATACCCCTCT	13088/-
RSVB	rsvb60	AACTTAAAACTGTGACAGCCTTTTATTCT	13325/-
RSVB	rsvb1199	ATAGTACACTACCTGTTATTTTAATCAGCTTCT	14977/-
RSVB	rsvb989	TATAGTACACTACCTGTTATTTTAATCAGCTTC	14978/-
RSVB	rsvbe	ACGAGAAAAAAAGTGTCAAAAACTAATGT	15216/-

Table 4.2 RSV and RSV-Negative Illness Characteristics in HIVE (2010-2020), n (row %)

	Overall (n=9,822)	**RSV-Positive (n=494)**	**RSV-negative (n=9,328)**	**RSV-A (n=180)**	**RSV-B (n=167)**
Age at Infection Median (IQR)	12 (5-37)	6 (3-16)	12 (6-37)	7 (4-15)	5 (3-11)
Age Group (years)					
0-4	1983	180 (9.1)	1803 (91.0)	73 (3.7)	83 (4.2)
5-17	4078	202 (5.0)	3876 (95.0)	67 (1.6)	56 (1.4)
18-49	3459	100 (2.9)	3359 (97.1)	36 (1.0)	25 (0.7)
50-64	274	10 (3.7)	264 (96.3)	3 (1.1)	3 (1.1)
≥ 65	28	2 (7.1)	26 (92.9)	1 (3.6)	0 (0.0)
Sex					
Female	5425	266 (4.9)	5159 (95.1)	90 (1.7)	84 (1.5)
Male	4397	228 (5.2)	4169 (94.8)	90 (2.0)	83 (1.9)
Race [a]					
White	7565	382 (5.0)	7183 (95.0)	139 (1.8)	126 (1.7)
Black	390	23 (5.9)	367 (94.1)	5 (1.3)	8 (2.1)
Other	1284	59 (4.6)	1225 (95.4)	29 (2.3)	19 (1.5)
Children in household [b]					
1-2	5186	252 (4.9)	4934 (95.1)	91 (1.8)	82 (1.6)
3-5	4425	227 (5.1)	4198 (94.9)	80 (1.8)	81 (1.8)
6+	133	11 (8.3)	122 (91.7)	6 (4.5)	4 (3.0)

[a] Missing or unknown reported for race (n=583)
[b] Missing children in household count (n=78)

Table 4.3 RSV in Households (HIVE 2010-2020) by Year

	10/ 11	**11/ 12**	**12/ 13**	**13/ 14**	**14/ 15**	**15/ 16**	**16/ 17**	**17/ 18**	**18 /19**	**19/ 20**
Households (n) [a]	328	213	321	232	343	226	297	291	351	385
Participants (n)	1441	943	1426	1049	1435	992	890	1187	1488	1526
RSV+ Participants (n) [b]	58	21	86	41	51	36	38	54	55	54
RSV-A (n)	16	12	39	3	22	24	13	19	16	16
RSV-B (n)	26	6	16	25	26	3	13	19	15	18
Total Illnesses (n)	979	398	1133	676	1392	926	803	1152	1311	1052
RSV Illness (%)	5.9%	5.3%	7.6%	6.1%	3.7%	3.9%	4.7%	4.7%	4.2%	5.1%

[a] Households may be enrolled in more than one year
[b] n=147 had no subtype determined

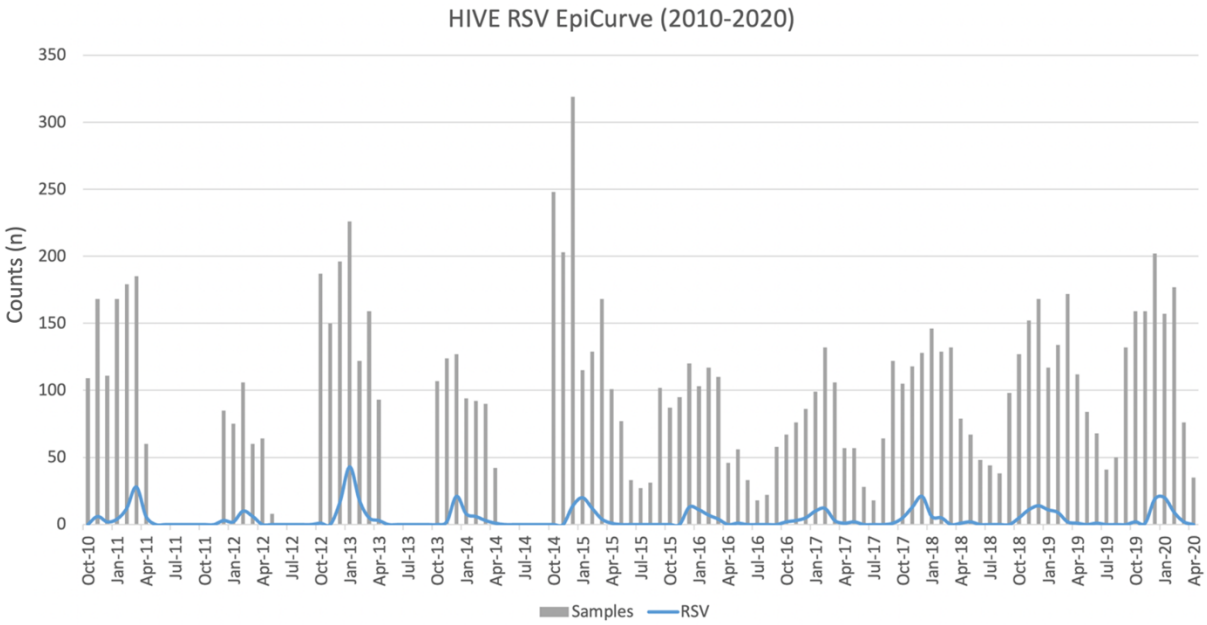

Figure 4.2 *HIVE RSV Epidemiologic Curve*

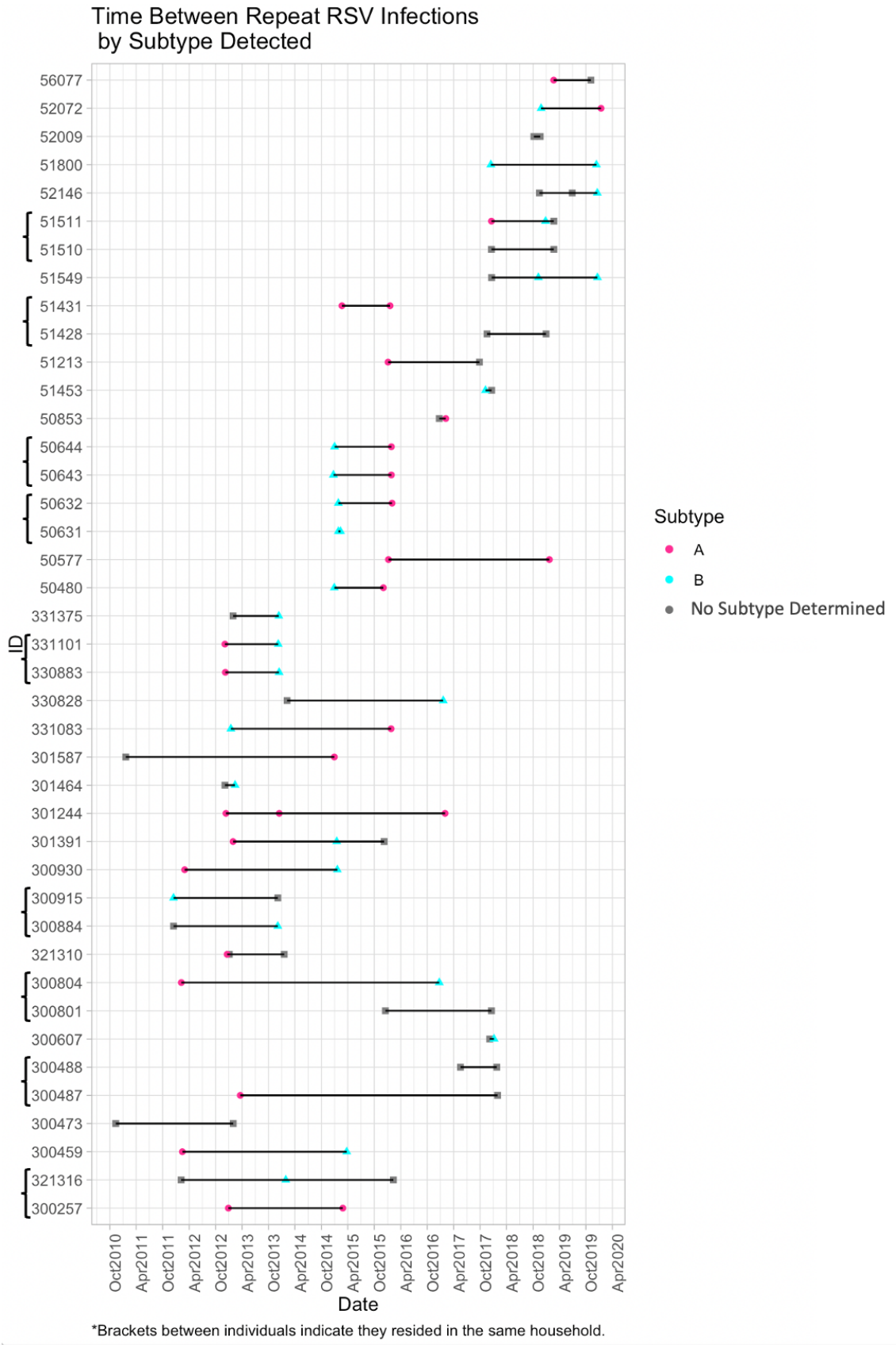

Figure 4.3 Repeat RSV Infection Interval by Subtype

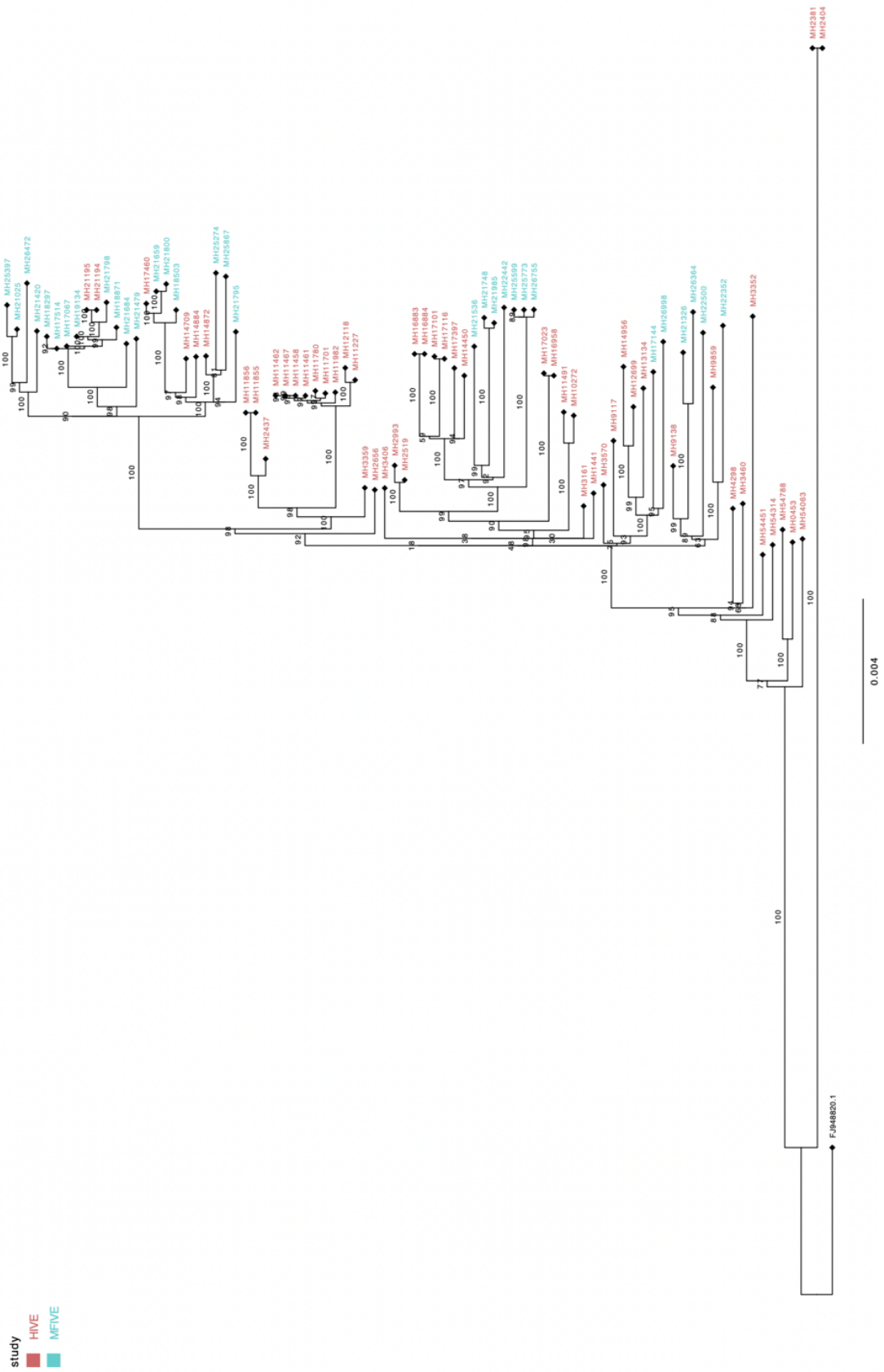

Figure 4.4 RSV-A Phylogenetic Tree

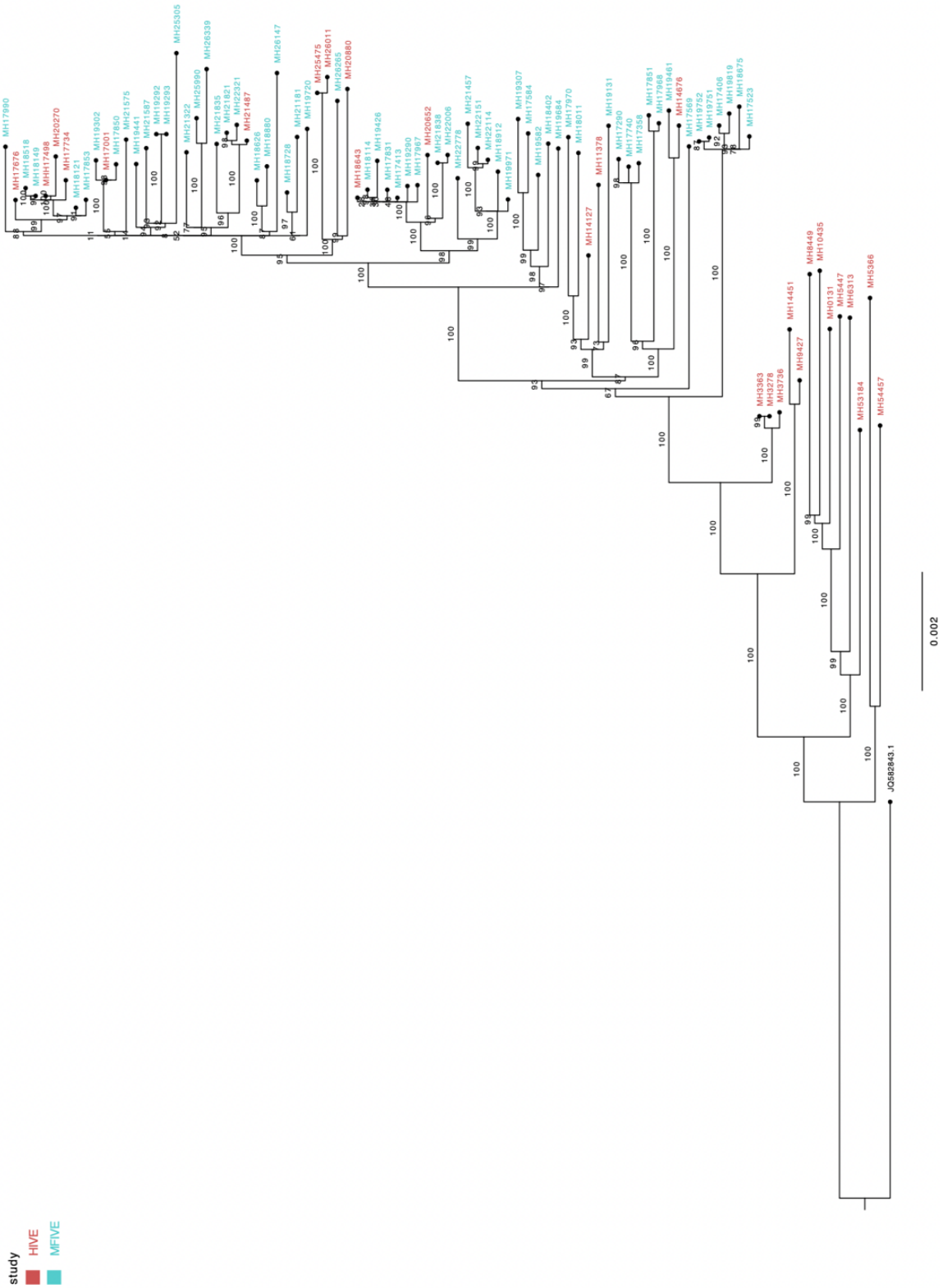

Figure 4.5 RSV-B Phylogenetic Tree

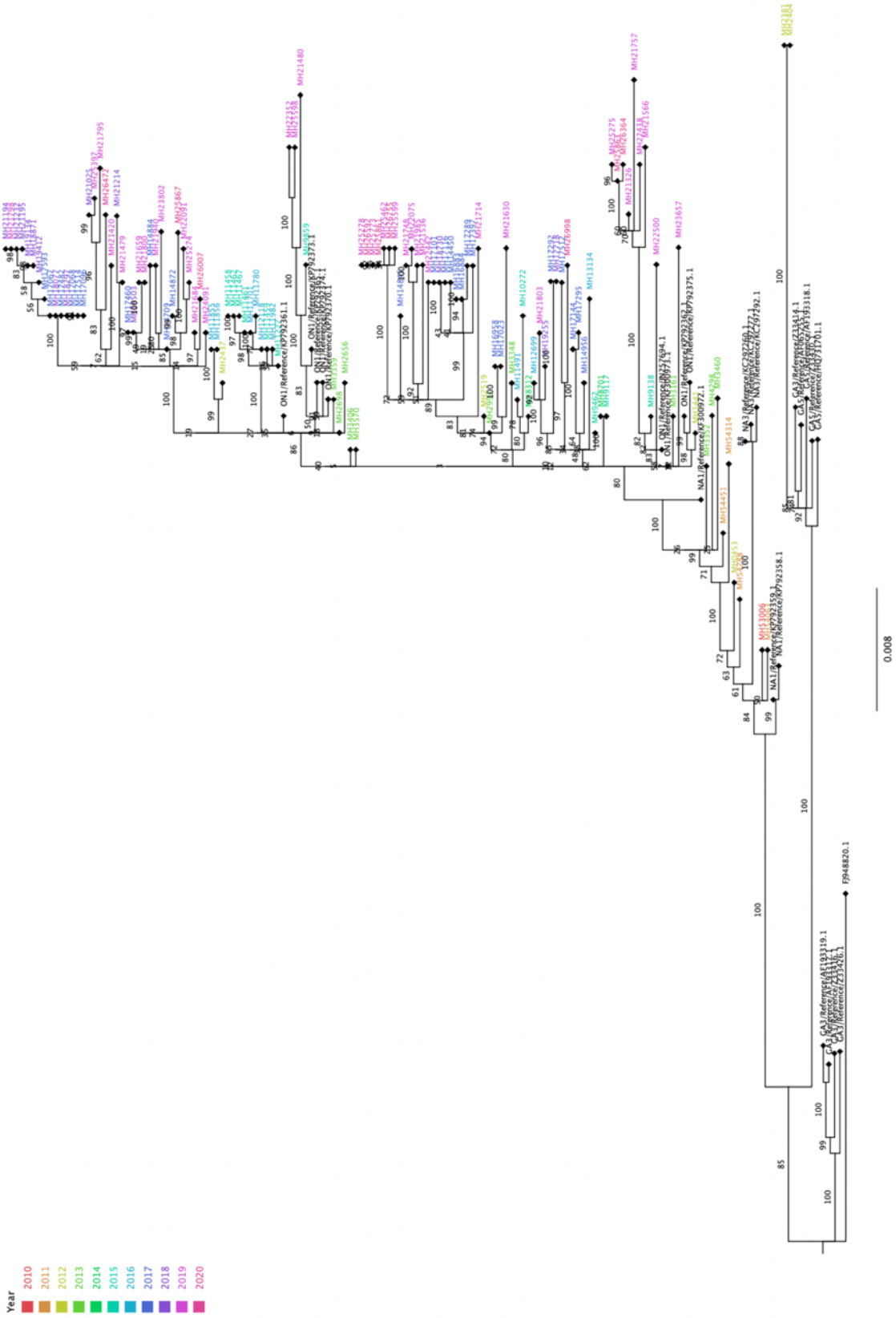

Figure 4.6 RSV-A G-Gene Genotype Reference Phylogenetic Tree by Year

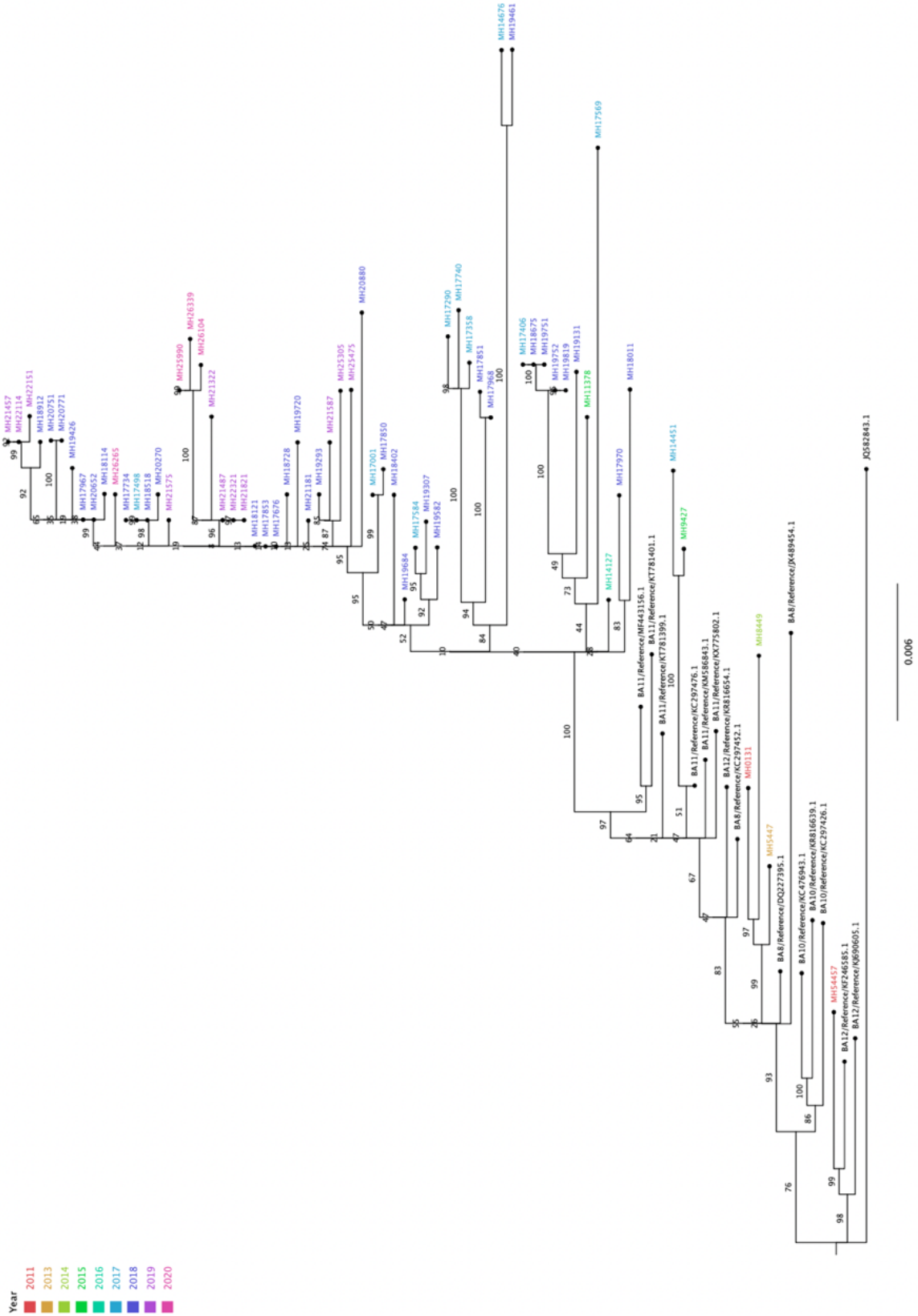

Figure 4.7 RSV-B G-Gene Genotype Reference Phylogenetic Tree by Year

CHAPTER 5

Summary and Conclusions

5.1 Summary of Findings

RSV is a significant cause of respiratory infections among the very young, elderly, and immunocompromised, and there is no vaccine currently available. Overall, this dissertation focused on three topics: the impact of multimorbidity and viral characteristics on RSV illness in ambulatory settings, the burden of RSV hospitalization assessed through in-hospital outcomes, and the epidemiology of RSV in a longitudinal study supplemented with genomic data. In each aim, we used alternate ARI control groups to provide descriptive comparisons. In chapter two, we evaluated length of illness, self-reported symptoms, and subsequent care seeking behavior as outcomes of interest. RSV subtype and viral load were measured using highly sensitive and specific PCR assays, minimizing potential bias in our viral characteristic analysis. In chapter three, outcomes of interest shifted to in-hospital outcomes such as extended length of stay, ICU admission, and the need for mechanical ventilation. With a robust analytic sample, we were able to describe and compare factors associated with ARI among comparison groups. In the final chapter, we provided the first epidemiologic analysis of RSV infections and repeat infections spanning an entire decade of the HIVE study. The inclusion of community sample reference strains for the last three years of the study helped develop a picture of circulating community strain characterization. The results from these analyses are summarized below, highlighting their strengths and limitations as well as contribution to the field.

5.1.1 Aim 1

Links between RSV-associated ARI and specific chronic conditions have been described in prior research; however, less is known about RSV outcomes across the spectrum of multimorbidity (Falsey et al., 2005, 2006; Walsh et al., 2004). To better understand the impact of RSV detection in the outpatient setting, research assessing the effect of underlying conditions on RSV illness outcomes among adults is needed. Additionally, results from previous studies regarding associations between RSV viral load, subtype, and illness outcomes vary and many were limited to hospitalized infants (Hall et al., 1990; McConnochie et al., 1990; Monto & Ohmit, 1990; Rodriguez-Fernandez et al., 2018; Walsh et al., 1997, 2018; Wang et al., 1995; Wilson et al., 1990) Using data from an ambulatory care study (MFIVE), we aimed to evaluate the relationship between multimorbidity and ARI outcomes among adults. To measure multimorbidity, we applied a patient-centric, condition-weighted index (MWI-ICD10) validated for use in outpatient settings. Further, we assessed the relationship between viral load and illness outcomes, as well as whether subtype acted as an effect modifier, among all RSV-positive participants in this population.

Our findings indicate that RSV-B samples had significantly higher viral loads (copies/mL) when compared to RSV-A samples (Mann-Whitney U test, p-value <0.0001). In contrast, from a recent analysis of infants hospitalized with bronchiolitis, Rodriguez-Fernandez et al. found that RSV-A samples had significantly higher quantitative viral loads when compared to RSV-B samples (Rodriguez-Fernandez et al., 2018). The inverse relationship between age and viral load may explain these contradictory findings, and we did not restrict our study to infants or young children (Kuypers et al., 2004; Martin et al., 2008).

Using median sample viral load as a threshold and after adjusting for potential confounders, RSV-positive participants with a viral load $\geq 2.20x10^4$ copies/mL had significantly higher odds of experiencing an extended length of illness when compared to participants with a viral load $< 2.20x10^4$ copies/mL. Viral subtype did not appear to modify this association after stratification by subtype or when included as an interaction term in statistical models. The use of standard curves in RT-PCR is a more precise method for determining viral load compared to estimations from the inverse of sample Ct values; however, our threshold cutoff was a subjective decision and other factors, such as time of sampling and specimen storage conditions, affect viral load measurements as well.

We found that adults with RSV had significantly higher median multimorbidity scores – indicating a higher presence of underlying morbidity – when compared to adults with influenza or neither RSV nor influenza detected. However, no findings from our impact of multimorbidity on illness outcome regression analysis were significant, and this analysis was limited by two factors. The first limitation was the exclusion of those under the age of 18, since the MWI-ICD10 has not been validated for use in that group; yet, children do not typically experience multimorbidity nor do they need significant underlying morbidity to be at risk of RSV infection. Next, approximately 37% of participants did not complete the follow-up survey, which is where we capture most illness outcomes of interest, such as length of illness and subsequent seeking of care. This loss to follow-up, in addition to missing data from those who did respond, limited our illness outcome statistical analyses by producing wide confidence intervals.

Two key strengths of this study were the inclusion of the influenza-positive and RSV-negative/influenza-negative control groups and the prospective enrollment of individuals who met a pre-established MAARI criteria. This was the first study of its kind to apply the MWI-

ICD10 to assess the impact of multimorbidity on ARI in an outpatient setting. Interestingly, we found higher median multimorbidity scores among those with RSV when compared to those with influenza or neither. Further, our findings describe associations between viral load and subtype as well as viral load and length of illness; however, additional research assessing the relationships between viral load, subtype, and illness outcomes across all ages is needed. In conclusion, a shift to regularly include older adults and those with multimorbidity in the identification and management of RSV-associated ARI may reduce overall disease burden, and consistent differentiation of RSV subtype will improve ARI surveillance efforts and has the potential to improve a patient's course of clinical care.

5.1.2 Aim 2

Hospitalization of adults with RSV has not been as thoroughly documented compared to adults with influenza who experience hospitalization. The immunocompromised – particularly those with underlying CHF or COPD and the elderly – are at greater risk of experiencing severe illness associated with RSV infection. Extended length of hospital stay, admission to the ICU, and need for mechanical ventilation are well-recognized markers of ARI severity. Using data from all four sites (MI, TX, PA, and TN) of the large, nationally representative HAIVEN study, we sought to compare population characteristics and clinical outcomes between hospitalized adults with either RSV, influenza, or neither detected.

With respect to underlying comorbidity, we found that adults aged 18-49 and 65+ with RSV detected had significantly higher median CCI scores compared to those with influenza detected. These findings are consistent with a previous comparison of RSV and influenza hospitalization burden using limited data from the Michigan site of the HAIVEN study (Malosh et al., 2017a). Further, the proportion of adults with CHF or COPD was significantly higher in

those with RSV detected compared to those with influenza detected. Our findings identify CHF and COPD as substantially prevalent high-risk conditions among those experiencing severe RSV infections that led to hospitalization in this population, and this is consistent with prior research (Falsey et al., 2006; Walsh et al., 1999).

For the clinical outcomes analysis, our data demonstrated that hospitalized adults with RSV detected had significantly higher odds of experiencing an extended length of hospital stay (≥ 8 days) as well as the need for mechanical ventilation. The odds of ICU admission were also higher in the RSV versus influenza comparison group; however, this finding was not statistically significant. These findings suggest that RSV illness requiring hospitalization in adults may be as severe as influenza illness requiring hospitalization.

Some of our results with respect to associations between participant demographics and infection status were statistically significant as well. When compared to the negative control group, those with RSV were more likely to be obese and aged 65 and older. The relationship for obesity is consistent with the findings from Malosh et al., but our association measured for those aged 65 and older differs and may have been detected due to the expanded sample size of our study. When compared to both those with influenza detected as well as RSV-negative, influenza-negative, female sex was significantly associated with RSV detection. This may be explained by biologic mechanisms or through due to extended interactions with children, either personally or professionally.

A strength of this work was finding similar results in an expanded analysis of Malosh et al. (Malosh et al., 2017a). Reproducibility in a larger, geographically diverse population is essential for establishing criteria to identify adults considered high-risk for experiencing severe illness when infected with RSV. Aside from the benefits of a large analytic sample, HAIVEN

was an ideal study to utilize due to its prospective, active enrollment strategy. Additionally, HAIVEN laboratory testing is performed by site-specific research laboratories, as opposed to relying on clinical testing, which ensures we were collecting accurate RSV and influenza data for all participants enrolled. Loss to follow-up – as with any large, hospital-based study – was a potential limitation in this analysis, particularly with the outcome 'death 30 days post-discharge'. However, data collection from participant EMR ensured we were missing few variables of interest.

With multiple vaccine candidates showing promising results in clinical trials, determining which at risk groups receive vaccination priority must be determined well in advance. A tailored vaccine that elicits a stronger immune response may be necessary for older or immunocompromised adults with weakened immune systems (Stephens & Varga, 2021). Our findings highlight the importance of retaining testing for RSV in older adults hospitalized with ARI. In conclusion, this research stresses the importance of recognizing these individuals at risk of experiencing severe RSV-associated ARI and in need of an effective vaccine.

5.1.3 Aim 3

Typically, epidemiologic studies of RSV are conducted in hospitals where infants and young children often present with severe illness; however, less is known about RSV in community settings, particularly within households in the United States. Historical household studies in the U.S. primarily calculated frequencies of ARI using less sensitive laboratory assays compared to what is available today. Even with molecular methods of viral detection widely available today, household-based studies remain an uncommon approach for conducting ARI surveillance – in part due to their high cost and labor required for operation and maintenance –

with few exceptions (Byington et al., 2015; Cohen et al., 2021; Emanuels et al., 2020; Monto et al., 2014; Nokes et al., 2004; Scott et al., 2019; Stockwell et al., 2014).

Moreover, immunity to RSV is short-lived and reinfection throughout life is common, yet, limited research is available regarding repeat infections in longitudinal, community-based settings (Bont et al., 2002; Glezen et al., 1986; Wong et al., 2021). Evaluating the interval between detected infections is one approach to understanding the role of waning immunity against RSV infection. Assessing the impact of age and other factors on reinfection may provide valuable information for vaccine development. The central aim of this study was to characterize RSV illness epidemiology and repeat infections using ten years of data from the HIVE study. We describe characteristics of, and make statistical comparisons between, RSV-positive illnesses and non-influenza RSV-negative illnesses. Additionally, we set out to characterize annual strain circulation at the subtype and genotype level, and sequenced HIVE samples were compared to sequenced samples from a regional ARI surveillance study that we designated as community reference strains.

We found that the median age of those infected with RSV-B was significantly younger compared to the median age of those infected with RSV-A (p-value=0.03). After adjusting for gender, young children (aged 0-4) compared to older children (aged 5-17) and adults (aged 18+) had significantly (p-value<0.0001) higher odds of testing positive for RSV. Similarly, there were significant (p-value<0.0001) differences in the odds of testing positive for RSV when comparing older children (aged 5-17) to adults (aged 18+). However, after adjusting for age, there was no difference in the odds of testing positive for RSV when comparing males and females. Adjusting for age and sex, there was no difference in the odds of testing positive for RSV when comparing

adults residing in households with one to three children compared to adults in households with four or more children present.

We identified 41 individuals (age range at first infection detected: 2.5 months to 44.5 years of age) who experienced repeat infections with RSV, and seven individuals had more than one reinfection detected. Nine pairs of individuals with repeat infections resided in the same household, and four household pairs experienced their first detected and repeat infection at the same time. Our data indicated that the average interval between first-detected (median age: four years) and repeat (median age: five years) infection was one and a half years. Among those with a complete subtype profile, 65% were subsequently infected with a different subtype compared to their earlier detected infection. Our phylogenetic analysis showed that ON-1 was the predominant RSV-A genotype and BA-11 was the predominant RSV-B genotype circulating in HIVE during this period. Since its emergence in 2010, the ON-1 clade has increased in prevalence in North America, and a high prevalence of this genotype is reflected in our data from 2012 onward.

We sourced data from a well-established ARI household surveillance study which provided multiple strengths to this analysis. Across ten years of data, we were able to capture a considerable number of RSV illnesses and repeat infections within households. The use of GEE models to account for repeat sampling of individuals allowed us to conduct robust statistical analyses of longitudinal data. The inclusion of community reference samples in our sequencing analysis provided a more comprehensive picture of community strain circulation and demonstrated that the HIVE study is an accurate representation of the community at large. However, this study was not without limitations. Like any longitudinal cohort study, loss to follow-up of participants is a common problem, and due to lapses in household re-enrollment it

is possible we missed cases of RSV, both first detected and reinfections. Lastly, not all RSV-positive illnesses were eligible to be sequenced which restricted our final sequencing analysis sample.

There remain open questions surrounding who should be targeted in an effective RSV vaccination campaign, and our data suggests that households with young children are an important population for consideration. Moreover, our findings on reinfection stress the importance of developing a vaccine that provides lasting immunity and is of equal effectiveness across subtypes. When considering infants and older adults, any successful vaccine would have to have the capacity to illicit a response strong enough to compensate for less robust immune systems (Stephens & Varga, 2021). From this analysis, we provided a robust characterization of RSV illnesses across ten years of the HIVE study. Households serve as a valuable setting to conduct a wide-range of ARI research, and our repeat infection analysis builds upon the sparse existing literature on reinfection intervals and subtype distribution. Additionally, our work highlights the value of incorporating sequencing-based analyses in household ARI surveillance studies. Once a vaccine is available, households with young children are an important population to be included in the early phases of any vaccination campaign.

5.2 Significance of Findings

5.2.1 Strengths of Dissertation

The use of highly specific and highly sensitive molecular assays across all aims was a significant strength of this work. The use of PCR respiratory panel assays for initial detection of pathogens reduced the potential for misclassification bias and ensured we could appropriately exclude detected RSV-influenza coinfections in the second and third chapters. When comparing illness and in-hospital outcomes across similarly presenting ARIs, excluding coinfections gives

us confidence that a single pathogen of interest is in part responsible for the outcomes measured. Many studies and clinicians rely on extrapolating viral load from the inverse of Ct value output from standard PCR (e.g., lower Ct values correspond to higher viral loads); however, the units of Ct value are arbitrary thus a threshold cannot be reliably established. In chapter two, we measured RSV quantitative viral load using standard curves which provided a more meaningful assessment of viral load that could be assigned units of measurement (viral RNA copies/mL). This quantitative method is not only a more accurate reflection of viral load but allowed us to analyze the results in a wide range of statistical analyses.

Accurate determination of RSV subtype is important for epidemiologic research as severity may differ by subtype and subtype prevalence varies by season which may indicate the need for a subtype-specific vaccine. In chapter two, we contributed to the pool of knowledge regarding relationships between viral load, subtype, and illness outcomes. While our findings alone are not definitive of these associations, they help expand our understanding of a dynamic virus that differentially impacts certain populations experiencing varying risk factors. In chapter four, viral subtyping was required prior to determining sequencing eligibility as implementation of the segment amplification protocols was entirely dependent on defining sample subtype. Chapter three was the only aim that did not evaluate subtype data because not all HAIVEN sites performed subtyping assays. We believe results from this dissertation further emphasize the value of RSV subtype differentiation in ARI research.

Whole genome sequencing is the gold standard molecular assay, and its application in research extends beyond the data presented in chapter four. Moreover, sequencing techniques are flexible with respect to genome regions targeted and amplification protocols and sequencing platforms available allowing researchers to choose a method that best suits their goals and

budget. We increased publicly available, complete North American RSV sequences in the last decade by nearly 40% from work contributing to this dissertation. We characterized local genotype circulation by conducting a focused analysis on the hypervariable G gene region of the genome. While we believe consistent subtype determination across RSV research to be a priority, our chapter four findings also support a shift to expanding sequencing-based analyses in community settings.

While no findings from our impact of multimorbidity on illness outcomes analysis were significant, we are confident that multimorbidity was adequately defined through the application of the MWI-ICD10 in this outpatient setting. Implementing a multimorbidity measure that weights the impact of conditions on physical functioning – as opposed to measures that predict mortality, such as CCI – was appropriate for individuals seeking care in an ambulatory setting. In contrast, we used CCI scores to measure underlying comorbidity in our hospitalized adult study, and, even though not validated for in-patient use, the MWI-ICD10 would have been a biased measure of morbidity in a hospitalized patient study.

Finally, the inclusion of influenza-positive and RSV-negative/influenza-negative participants in chapters two and three and RSV-negative illnesses in chapter four increased respective analytic samples. The overall prevalence of RSV is low which has the potential to limit analyses, even more so when stratified by subtype, age group, or other categories of interest. More importantly, using these groups for comparison provided meaningful benchmarks for comparison when interpreting results.

5.2.2 Public Health Relevance

Diagnostic stewardship is a somewhat novel concept that encourages appropriate clinical laboratory testing to guide patient management and treatment through minimizing unnecessary

test orders (Patel & Fang, 2018). This model serves as an attempt to reduce result turnaround times and excess labor on already strained healthcare systems while keeping patient-oriented decision making at the core. Multiple studies have found that rapid molecular tests for RSV have high sensitivity and specificity similar to their non-rapid counterparts (Chartrand et al., 2015; Hassan et al., 2018). In cases of similarly presenting ARIs, it is crucial to quickly differentiate causal pathogens for appropriate patient care and improving outcomes. In chapter three, research laboratory testing – as opposed to clinical diagnostic testing – helped us identify pathogens of interest. We believe our findings support the retention of testing for RSV in all populations, but hospital settings are one area where we cannot afford to make exceptions.

Over the decades, RSV vaccine development has faced many challenges, but researchers are closer now than ever to making a safe and effective product that affords adequate protection. Early failed vaccine candidates often targeted the postfusion (postF) protein that would appear after the virus fused with host cell membranes (Powell, 2021). The current frontrunning RSV vaccine candidates (GlaxoSmithKlein, Pfizer, Moderna, and Janssen) include a stabilized version of the prefusion (preF) protein which produces strong neutralizing antibodies that prevent the virus from entering host cells (McLellan et al., 2013; Powell, 2021). A mAb treatment that targets stabilized preF, nirsevimab, was tested by AstraZeneca and Sanofi and proved effective at reducing RSV infections in infants (MedImmune LLC, 2021a, 2021b). Promising results are also emerging from clinical trials in important at-risk groups including the very young, elderly, and pregnant women (Powell, 2021).

Interestingly, mRNA platform vaccines for RSV were in development prior to the COVID-19 pandemic, and existing RSV mRNA vaccine research helped scientists rapidly develop vaccine technology against SARS-CoV-2 (Powell, 2021). The SARS-CoV-2 spike

protein and RSV preF protein are similar in their respective function of mediating virus-host cell attachment (McLellan et al., 2013; Wrapp et al., 2020). The rapid and successful progression in the fight against COVID-19 is in exchange helping RSV vaccine development become more efficient (Powell, 2021). Due to the varying immune response capacity of at-risk populations, the most optimistic outlook is to have multiple candidates approved for use. Infants and children are understandably a significant group for RSV vaccine consideration, but our research emphasizes the need to target elderly adults and those with underlying morbidity as a priority as well.

Results from the third aim demonstrate Agoti et al.'s six-segment amplification sequencing approach is well-suited for community public health RSV research (Agoti et al., 2015). The widespread application of sequencing RSV isolates will expand our understanding of RSV epidemiology and immunology as manufacturers develop and improve vaccine products and treatments as they become available. The benefits of using a whole genome sequencing approach are two-fold. First, the construction of complete sequences would allow researchers to assess transmission and circulation more accurately, which would benefit epidemiologic-based transmission studies. Second, researchers can extract specific gene regions from complete sequences to evaluate potential therapeutic targets. In the event that subtype, or genotype-specific vaccines are necessary, having a broad database of sequences available would support those efforts tremendously.

5.3 Future Work

Future directions for the second chapter would involve analyzing illness outcomes, RSV subtype distribution, and viral load stratified by individual category of underlying conditions. This type of analysis would be most interesting and well-powered for more common conditions, such as diabetes, asthma, and cardiovascular-related conditions. There would be value in

conducting a sensitivity analysis in this population comparing various thresholds for the categorization of MWI-ICD10 scores to look at different levels of multimorbidity. Such an analysis would allow us to evaluate potential selection bias introduced by the dichotomization of MWI-ICD10 scores and lend to the validation of this index in a MAARI population. Assessing influenza viral load using the same quantitative methods to determine RSV viral load would also provide interesting data for comparing the impact of RSV and influenza viral characteristics on illness outcomes.

Regarding data from the third chapter, it would pose beneficial to compile future seasons into this analysis to provide an ongoing assessment of hospitalized RSV and influenza patient characteristics and clinical outcomes in this network, however, the HAIVEN study recently concluded. Additional large-scale, nationally representative ARI hospital surveillance networks have since been developed, such as the Influenza and Other Viruses in the Acutely Ill (IVY) network, and we encourage similar research be conducted in these studies (Grijalva et al., 2021; Tenforde, Patel, et al., 2021; Tenforde, Self, et al., 2021). Monitoring potential demographic and underlying comorbidity shifts within these populations as well as identifying trends of RSV cases leading to hospitalization would advance our understanding of RSV epidemiology in hospitalized adults. Detecting and monitoring novel trends in populations experiencing severe illness would provide valuable information for RSV vaccine and mAb recommendations.

Results from the fourth chapter are indicative of the value of evaluating RSV primary and repeat infections over time and expanding RSV sequencing to future seasons of the HIVE study as well as other longitudinal household surveillance networks. A noteworthy extension of this data might include geo-coding households linked to their sequence data to assess community transmission. HIVE does not assess points of contact between study participants, so this could be

used as a method to visualize clusters of RSV infections in the community. In the absence of sequencing feasibility, it would be valuable for ARI studies and surveillance networks to regularly incorporate RSV subtyping into their laboratory testing. It is common practice to subtype influenza infections, and consistently assessing RSV infections by subtype would provide additional information on circulation variation by season and illness outcomes with respect to RSV-A or RSV-B.

5.4 Final Remarks

It is clear why RSV is predominantly considered a disease of childhood, yet our data supports clinical intervention and surveillance efforts to be more proactive in the monitoring and prevention of adult RSV cases, particularly among the elderly and those with underlying morbidity. Including individuals infected with influenza as a benchmark comparison group in the second and third chapter allowed us to draw meaningful conclusions on the severity of RSV-associated ARI relative to influenza. A reduction in adult RSV misdiagnoses could help alleviate adult RSV illness burden as well as added stress on healthcare systems — such as hospitalization and ventilator-use. Once a safe and effective vaccine against RSV is available, it may prove beneficial to prioritize the elderly and immunocompromised adults for vaccine intervention as these individuals are at risk of experiencing severe RSV-associated illness due to their underlying conditions and overall immune function. In the event that initial vaccine availability is limited, those with specific morbid conditions, such as CHF and COPD, should be considered high priority. Recommending these populations – in addition to children and those who reside in households with them – preferentially receive the vaccine could reduce the overall burden of RSV for the individual and healthcare systems. Finally, results from the final aim help shape

future RSV community epidemiology research by framing sequencing as a valuable public health-oriented approach.

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