**Severe Illness Associated With Respiratory Viruses: Burden and Temporal Dynamics**

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Epidemiological Science)
in the University of Michigan
2020

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## Dedication

To my family and to Elizabeth. Without their unwavering support, none of this would have been possible

**Acknowledgements**

In writing my dissertation I have had time to dwell on the path that brought me here, and the many (many) people who have encouraged and supported me along the way. It is truly humbling to look back and recognize the substantial time and effort so many have directed towards my development. First and foremost, I would like to thank my advisor and committee chair, Dr. Aubree Gordon. Since I began working with you nearly five years ago, you have been an unwavering source of support; constantly encouraging me to be a better researcher. I would also like to thank the other members of my committee: Dr. Sara Adar, Dr. Adam Lauring, and Dr. Jon Zelner. Each of you gave generously with your time and provided crucial advice and feedback on numerous occasions. The quality of my work, and my understanding of the concepts that underpin it, have all been improved because of your insights.

I have also been fortunate to have a number of additional mentors outside of my committee. Dr. Mark Wilson was largely responsible for my coming to UM in the first place and has been a staunch supporter since day one. That this has continued unabated even after his retirement is something for which I remain truly grateful. I also count myself lucky to have had the opportunity to work closely with Dr. Lionel Gresh, Dr. Grace Noppert, and Dr. Eduardo Azziz-Baumgartner on several projects. Your thoughtful engagement throughout the research process has made me a far better researcher. I would also like to thank Dr. Lottie Adams, Dr. Pam Cox, and Dr. James Couch who helped me define the type of public health professional I wanted to become. Your encouragement over the years has meant the world to me.

Finally, I am so grateful to my friends and family for all of their patience and support throughout this process. Thank you to my amazing fellow PhD students and friends who have offered advice and suggestions on countless occasions, but more importantly made pursuing this degree so much more enjoyable. Thank you to my parents and sister for their support and encouragement. And a particular thank you to my partner Elizabeth who amazes me every day with strength and empathy that knows no bounds. You make me a better person.

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## List of Abbreviations

| | |
|---|---|
| ARI | Acute respiratory infections |
| ALRI | Acute lower respiratory tract infections |
| 95% CI | 95% confidence interval |
| CCA | Chimpanzee coryza agent |
| RSV | Respiratory syncytial virus |
| mAb | Monoclonal antibody |
| LMICs | Low- and middle-income countries |
| RT-PCR | Reverse transcriptase polymerase chain reaction |
| Ct | Cycle threshold |
| RNA | Ribonucleic acid |
| UR | Uncertainty range |
| PERCH | Pneumonia Etiology Research for Child Health |
| AIDS | Acquired immunodeficiency syndrome |
| PCV13 | Pneumococcal conjugate vaccine |
| CDC | U.S. Centers for Disease Control and Prevention |
| WHO | World Health Organization |
| FDA | U.S. Food and Drug Administration |
| CNDR | Centro Nacional de Diagnóstico y Referencia |
| HCSFV | Health Center Sócrates Flores Vivas |

| | |
|---|---|
| A/H1N1pdm09 | Influenza A H1N1 from 2009 swine flu pandemic |
| A/H1N1 | Influenza A H1N1 prior to 2009 swine flu pandemic |
| SARI | Severe acute respiratory infections |
| UCB | University of California, Berkeley |
| IRB | Institutional review board |
| SSI | Sustainable Sciences Institute |
| CDC-QCMD | U.S. Centers for Disease Control and Prevention Quality Control for Molecular Diagnostics |
| IMCI | Integrated Management of Childhood Illness |
| HIV | Human immunodeficiency virus |
| EPI | Expanded program on immunization |
| SD | Standard deviation |
| py | Person-years |
| OR | Odds ratio |
| mOR | Matched odds ratio |
| HR | Hazard ratio |
| GABRIEL | Global Approach to Biological Research, Infectious diseases and Epidemics in Low-income countries |
| EPIC | Etiology of Pneumonia in the Community |

**Abstract**

Acute lower respiratory tract infections (ALRI) are a substantial source of global morbidity and mortality, particularly among young children. Respiratory syncytial virus (RSV) and influenza are consistently observed to be significant drivers of this burden, but substantial gaps in our understanding remain. Greater understanding of the burden of these pathogens at the community-level, and their association with severe illness has the potential to substantially reduce the toll of ALRI, particularly among young children.

In chapter 2 of this dissertation we assess the community burden of RSV and RSV-associated severe illness among Nicaraguan children aged < 2 years. In chapters 3 and 4 we explore the individual-level association between influenza and subsequent pneumonia along with characterizing the risk period for pneumonia following symptomatic influenza infection. To explore these questions we use data from the Nicaragua Influenza Birth Cohort Study (2011-2016), the Nicaraguan Pediatric Influenza Cohort Study (2011-2018), and the Nicaragua Influenza Cohort Study (2007-2010).

In chapter 2 we observed the highest incidence of RSV occurred among children aged 6-11 months, while the highest incidence of RSV-associated severe ALRI was highest among those aged < 3 months and generally decreased as age increased. RSV was also associated with at least 25% of illness deaths that occurred in the cohort, highlighting its importance in reducing in infant mortality. In chapters 3 and 4 we established that influenza is associated with increased risk of subsequent pneumonia at the individual level across types and subtypes with the exception of seasonal H1N1. We also observed distinct periods of elevated pneumonia risk

following influenza among children aged <2 which is suggestive of multiple etiologic pathways existing between influenza and pneumonia. By better understanding the burden and temporal dynamics of RSV and influenza we can develop more effective interventions to prevent and mitigate their effects among children.

# Chapter 1 Introduction

## 1.1 Burden of acute respiratory infections

Acute lower respiratory infections (ALRI), like pneumonia and bronchiolitis, remain a significant source of morbidity and mortality worldwide, especially among young children.[1-3] In 2010 there were an estimated 11.9 million episodes of severe ALRI, and roughly 265,000 in hospital deaths among children under 5.[1] It is also estimated that in-hospital deaths represent only 19% of overall mortality bringing the total estimated mortality from ALRI among young children to roughly 1.4 million deaths each year.[4] In 2017, pneumonia alone was responsible for the deaths of over 800,000 children under five—15% of all deaths in this age group worldwide.[5] Additionally, the global burden is not evenly distributed, with an estimated 99% of ALRI-mortality occurring in developing countries.[1] Nicaragua is a prime example of this disparity as acute respiratory infections are the leading communicable cause of death for children under five.[6] While there are many respiratory viruses that cause illness in humans, respiratory syncytial virus (RSV) and influenza are substantial contributors to the overall burden of morbidity and mortality from ALRI, and thus are the focus of this dissertation.[7, 8]

## 1.2 RSV

### 1.2.1 History and burden

First discovered in chimpanzees in 1956, RSV was initially named chimpanzee coryza agent (CCA) owing to its frequent presentation of rhinorrhea and nasal inflammation.[9] However, its isolation from infants with respiratory illness in 1957 led to a new name,

respiratory syncytial virus (RSV), a reference to the multinucleated syncytial giant cells caused by viral replication in bronchial epithelial cells.[9, 10] There are two known serotypes, A and B, with evidence showing RSVA viral loads are frequently higher than RSVB.[10, 11] RSV is also one of the most commonly occurring viral infections, with an estimated 97% of children having at least 1 infection by age 2.[12]

RSV has been established as a significant source of morbidity and mortality among young children [2, 13, 14], the elderly[15, 16], and the immunocompromised.[17, 18] In 2015 there were an estimated 33.1 million cases of RSV-ALRI among children under five worldwide, 3.2 million of which were severe enough to require hospitalization.[2] That same year there were nearly 60,000 in-hospital deaths from RSV-ALRI, though the authors suggested that the overall mortality from RSV (including deaths occurring in the community) could be up to twice as high.[2] Despite this substantial burden, few options exist for the prevention or treatment of RSV illness.

### 1.2.2 Treatment and prevention

RSV has long been a target for vaccination, however, its ability to elicit such a strong host immune response has complicated efforts. In the 1960s clinical trials of a formalin-inactivated RSV vaccine were conducted in which the vaccine was found to enhance RSV illness, contributing to the death of two study participants.[19-22] Vaccine efforts have continued since then, trying to strike a balance between inducing sufficient immunity while not causing enhanced disease.[19, 21] While some vaccine candidates have shown promise in early phases of clinical trials, none have yet met their phase 3 primary outcome and thus there are still not any licensed RSV vaccines.

Development of interventions to prevent RSV has not been limited to vaccines. Temporary protection during especially high-risk periods has been explored through the use of monocolonal antibodies (mAb) like Palivizuvab (Synagis®)—a monoclonal antibody to the RSV F-protein.[23, 24] Currently Palivizumab exists as the only market approved preventive measure for RSV[25], and the American Academy of Pediatrics recommends it be considered only for young children most susceptible to severe RSV illness (e.g. premature infants, immunocompromised).[26] Studies surrounding its cost-effectiveness are somewhat controversial as they rely heavily on cost-savings accrued over the life course from asthma/recurrent wheezing averted by preventing severe RSV infection.[26] Regardless, the substantial cost of the intervention, both monetarily and through repeated healthcare visits, places it beyond the reach of many low- and middle-income countries (LMICs), leaving essentially no viable preventative measures for RSV available in those countries. While preliminary results from several interventions in development appear promising, addressing gaps that persist in our understanding of risk factors for RSV will be crucial in mitigating the substantial burden associated with RSV until such a time when an effective treatment or preventative are available. These gaps in the literature are especially prevalent in LMICs where even basic information regarding seasonality, burden, and transmission remain unknown.

### 1.2.3 Research gaps

Given the sheer ubiquity of RSV, research on risk factors tends to focus on severe RSV illness (e.g. hospitalization, pneumonia)[27], and as such are frequently conducted in hospitals or other in-patient settings.[28-34] Such hospital-based studies on their own, fail to capture the overall RSV burden in a given population. While the number of community-based studies continues to grow, it still lags far behind that of hospital-based studies.[13, 14, 35]

Still, published studies have consistently shown an association between factors like prematurity, low birth weight, and comorbidities and severe RSV.[27] However, the paucity of community-based studies provides less opportunity to examine social and environmental factors that may contribute to children developing severe illness. In their 2015 review, Shi et al. call for an increase in "large-scale, high-quality multivariable studies" to improve understanding of the risk factors for severe RSV.[27] However, without addressing the imbalance between hospital and community-based studies, the role of social and environmental risk factors for severe RSV illness will remain poorly understood.

## 1.3 Influenza

### 1.3.1 History and Burden

Influenza refers to a group of single-stranded RNA viruses in the *Orthomyxoviridae* family of which influenza types A and B commonly infect humans.[36, 37] Until the viruses were first isolated in the 1930s, little was known about the pathogen responsible for influenza illness, with some mistakenly attributing it to a bacterium (particularly *Haemophilus influenzae* as evident in its name).[36, 38, 39] Influenza A was isolated first by researchers in the United Kingdom [38], while influenza B was isolated soon after by Thomas Francis at the University of Michigan.[39]

Influenza A viruses are classified into subtypes based on two surface proteins on the virus: hemagglutinin and neuraminidase.[37] These are abbreviated (H for hemagglutinin and N for neuraminidase), numbered (H:1-18 and N:1-11), and combined giving rise to familiar subtypes like H1N1 or H3N2.[37] While H1N1 and H3N2 are the subtypes currently causing seasonal epidemics, other subtypes, particularly avian subtypes like H7N9 and H5N1, have also been known to infect humans.[40, 41] Influenza B, conversely, is classified into distinct lineages,

specifically B/Yamagata and B/Victoria.[37] Both influenza A and B are then further categorized into clades and sub-clades.[37]

Influenza B has exhibited far less genetic variation than influenza A, remaining relatively homogenous until the 1970s when antigenically distinct lineages began to diverge.[42, 43] A number of influenza A subtypes, however, have circulated since the virus was first isolated, with new subtypes frequently leading to severe epidemics, and even pandemics. Since the beginning of the 20$^{th}$ century the world has experienced four influenza pandemics (1918, 1957, 1968, and 2009).[44] The 1918 and 2009 pandemics were caused by A/H1N1 strains while those in 1957 and 1968 marked the emergence of H2N2 (no longer believed to be circulating), and H3N2 respectively.[44] Influenza-associated pneumonia has been implicated as a substantial driver of mortality in these pandemics, but our understanding of the inter-pandemic burden and the importance of influenza's interactions with bacteria remain limited.

Influenza has been associated with both primary viral pneumonia (caused directly by the virus itself) and secondary bacterial pneumonia (caused indirectly by facilitating bacterial colonization and/or infection.[45] Laboratory studies have identified likely mechanisms through which influenza may facilitate bacterial pneumonias, but the exact process remains uncertain.[45, 46] Regardless, we would expect primary viral pneumonias to occur approximately concurrent with influenza infection, and secondary bacterial pneumonias to occur following some lag.[46-48] Davis et al. explained the logic behind this assumption stating:

> "…we would expect to see a relatively long lag time between influenza infection and the presence of CAP [community acquired pneumonia] because of the time needed for the influenza to reduce the ability of the lungs to clear a bacterial infection...The bacterial

agent would then have to proliferate within the host before the host began to show symptoms."[46]

This trend has been observed at a population level with studies showing cases of pneumonia tend to peak 1-3 weeks after influenza cases.[47, 49] However, such studies are inherently limited in the causal inferences that can be drawn from them. Particularly, we cannot be certain that these population-level correlations hold true at the individual-level (ecologic bias). A small number of studies have tried to assess this association at an individual-level, but have been limited by small sample size and seasonal confounding.[50, 51]

While the temporal dynamics between influenza and severe illness remain uncertain, it is clear that influenza remains a substantial source of severe illness and death worldwide, particularly among young children.[8] A 2020 systematic review and modeling study reported that in 2018 there were an estimated 109.5 million (uncertainty range [UR]: 63.1-190.6 million) influenza episodes that occurred globally among children aged < 5 years. Of these, approximately 10% were classified as ALRI and 870,000 (UR: 543,000-1,415,000) were severe enough to require hospitalization.[52] The same study also estimated there were up to 34,800 deaths (UR: 13,200-97,200) resulting from influenza-associated ALRI.[52] As with RSV, the majority (82%) of in-hospital deaths associated with influenza occurred in LMICs.[52] It's notable that such a substantial burden persists despite having greater means of prevention and treatment than RSV.

### 1.3.2 Treatment and Prevention

Multiple antiviral medications against influenza have been developed since the virus was first isolated. The FDA approved amantadine as a prophylactic against H2N2 in 1966, and for use against other influenza A subtypes ten years later.[53] This was followed by Rimantadine, a

derivative of amantadine was approved for use in the US in 1993, and the first neuraminidase inhibitor, Oseltamivir (Tamiflu®), in 1999.[53] While the spread of influenza strains resistant to Oseltamivir has not yet become a substantial problem, it remains a concern.[53, 54] Past experience with resistance to amantadine and rimantadine underscores the importance of continuing to develop new therapeutics, particularly those which operate through different mechanisms. Fortunately, antiviral drugs are not the only means by which to combat influenza, as vaccination continues to be an integral component prevention and mitigation strategies.

Vaccination has been the central tool in the effort to reduce seasonal influenza burden for decades. In fact, the first influenza vaccines were developed and licensed for use by the 1940s, the decade after influenza was first isolated.[55, 56] However, influenza vaccine effectiveness has varied widely over time. A recent meta-analysis showed that vaccine effectiveness varies by influenza type/subtype as well. Vaccine effectiveness was highest for influenza A H1N1pdm09 at 61% (95% CI: 57, 65), followed by influenza B with 54% (95% CI: 46, 61), and A/H3N2 with 33% (95% CI: 26, 39).[57] This is not to say that our current influenza vaccines are without value. Multiple studies have shown that influenza vaccination results in millions of fewer influenza illnesses and tens of thousands fewer hospitalizations.[58-60] Despite this, influenza is still estimated to be responsible for approximately 10% of respiratory hospitalizations globally among children aged <18 years.[8] This persistent burden highlights the importance of exploring the mechanisms involved with severe influenza illness.

Antibiotics also play an important role in treating influenza-associated ALRIs like pneumonia as influenza may have facilitated the development of a bacterial infection that is actually causing the illness.[45, 46] However, with the growing challenge of antibiotic resistance, it is crucial that antibiotics only be used in those illnesses that are bacterial in nature.

Unfortunately, this is challenging even with advanced diagnostics[61], leading acute respiratory infections to become one of the largest sources of antibiotic misuse. A 2016 analysis of U.S. prescribing behavior in 2010-2011 reported that ARIs were responsible for 221 antibiotic prescriptions per 1000 population annually (95% CI: 198-245), but only 111 prescriptions per 1000 were deemed appropriate.[62] Better understanding of the relationship between influenza and subsequent viral and bacterial pneumonias may lead to intervention strategies that decrease inappropriate use of antibiotics.

### 1.3.3 Research Gaps

While there are many important gaps in our understanding of influenza, the manner in which influenza and pneumonia are associated remains a particularly important challenge. Though pneumonia is frequently used as a measure of severe influenza, the research examining pneumonia etiology has substantial limitations. In 2011, the Pneumonia Etiology Research for Child Health (PERCH) study was started in an effort to update understanding of pneumonia etiology.[63] Knowledge to that point had come largely from studies conducted between the 1970s and the 1990s, using data before the AIDS epidemic or the introduction of important vaccines—both of which altered populations' susceptibility to pneumonia.[63, 64] A multi-site case-control study conducted at 9 sites in 7 countries, PERCH used more sophisticated molecular diagnostics to explore pneumonia etiology to a depth not previously possible. By the time of its completion in 2014, 4232 cases and 5325 controls had participated.[7] The scale of this undertaking is a striking indicator of the importance of understanding pneumonia etiology. Still, the challenges associated with such an effort are substantial.[65] Though the initial results were published in June 2019, the case-control design utilized in the study limits causal inference and any detailed assessment of the temporal dynamics between respiratory pathogens and subsequent

pneumonia.[7] These questions can still only be effectively answered through large-scale, prospective cohort studies.

## 1.4 Dissertation Aims

### 1.4.1 Aim 1 - Assess and describe the burden of symptomatic RSV illness among Nicaraguan children aged < 2 years.

In chapter two, I will discuss Aim 1 of my dissertation, focused on describing the burden of symptomatic RSV illness within a prospective, community-based study of Nicaraguan children aged < 2 years. Acute respiratory infections (ARI) are the most common communicable cause of death among Nicaraguan children, responsible for 15% of deaths in children under five.[6] However, the etiology of these infections remains poorly explored. With RSV estimated to be responsible for more than 1/5 of ALRI cases worldwide, it is important that the nature of RSV's burden in Nicaragua be examined.[66] This analysis, provides the first in-depth look into the burden of RSV in Nicaragua, its seasonality, and the frequency of severe outcomes associated with it.

### 1.4.2 Aim 2 - Explore whether influenza infection is temporally associated with pneumonia at a participant level among Nicaraguan children aged 0-14 years.

In chapter three, I will discuss Aim 2 of my dissertation, exploring whether acute viral respiratory infection (specifically influenza) is associated with pneumonia at a participant level among Nicaraguan children aged 0-14 years. Influenza is considered a substantial contributor to the global burden of pneumonia—directly through viral pneumonia [61, 67], and indirectly through secondary bacterial pneumonia [46, 49], though as previously described, pneumonia etiology remains poorly understood. Multiple studies have suggested that secondary bacterial

pneumonia was a primary driver of mortality in influenza pandemics including that in 1918 [68, 69], and more recently, 2009.[49, 70-72] However, it is unclear whether this association extends to inter-pandemic periods. By studying influenza and subsequent pneumonia among Nicaraguan children between 2011 and 2018, we aim to better understand the importance of influenza in driving pneumonia burden and identify temporal variation in pneumonia risk following influenza.

### 1.4.3 Aim 3 – Characterize the temporal dynamics between influenza and clinical pneumonia before and after the introduction of the pneumococcal conjugate vaccine

Bacterial pneumonia caused by pathogens like *Streptococcus pneumoniae* has been a considerable source of global morbidity and mortality.[73] Fortunately the introduction of pneumococcal conjugate vaccines (PCV) has led to major decreases in this burden.[74, 75] Since S. pneumoniae has been implicated as a key source of secondary bacterial pneumonias following influenza [50, 68], we might expect that the introduction of PCV would also lead to a reduction in the occurrence of influenza-associated pneumonias. However, this has been little studied in the literature. In chapter 4, I will discuss Aim 3 of my dissertation, where we characterize the risk and temporal dynamics of pneumonia following influenza in two cohorts of Nicaraguan children—one prior to the introduction of PCV to Nicaragua, and the other after its introduction and near universal adoption. Comparing the relationship between influenza and pneumonia in these two groups will provide important insights into the interaction of influenza with bacterial pathogens which contribute to severe illness.

Respiratory Syncytial Virus (RSV) and influenza are two of the most substantial contributors to the burden of ALRI, responsible for an estimated 22% and 13% respectively of all cases in children, and more than 200,000 deaths in a given year.[1, 76, 77] My dissertation, as

aims explore several important questions that remain regarding the burden of RSV, and the temporal dynamics between influenza and pneumonia in Nicaragua.

# Chapter 2 Assessing the Incidence of Symptomatic Respiratory Syncytial Virus (RSV) Illness Within a Prospective Birth Cohort in Managua, Nicaragua

## 2.1 Author Summary

We found a substantial burden of RSV among Nicaraguan children under 2 years. Around 1/3 of deaths from medical causes were RSV-associated, suggesting it's an important driver of infant mortality in highly vaccinated populations with little HIV or malaria.

## 2.2 Abstract

Respiratory syncytial virus (RSV) causes substantial morbidity and mortality among children worldwide, commonly through acute lower respiratory tract infections (ALRI). To assess the incidence of symptomatic RSV illness among young children, we conducted a prospective birth cohort study following children from 0-2 years of age in Managua, Nicaragua.

Children meeting the testing criteria (fever, history of fever, or severe respiratory symptoms [apnea, stridor, nasal flaring, wheezing, chest indrawing, and/or central cyanosis]), were tested for RSV infection using real-time reverse transcriptase-polymerase chain reaction. Acute lower respiratory infection was defined as diagnosis of pneumonia, bronchiolitis, bronchitis, or bronchial hyper-reactivity. Incidence was calculated, and 95% confidence intervals estimated using a Poisson distribution.

A total of 833 children participated in the cohort, 289 (34.7%) had at least one episode of laboratory-confirmed RSV, and 156 (18.7%) of RSV-associated ALRI (RSV-ALRI). The

incidence of symptomatic RSV was 248.1 cases per 1000 person-years (95% confidence interval [CI]: 223.2, 275.7). While infants aged 6-11 months had the highest incidence of symptomatic RSV (361.3/1000 person-years, 95% CI: 304.4, 428.8), infants <3 months had the highest incidence of severe RSV (RSV-associated hospitalizations and/or severe ALRI). RSV was also associated with 25.0-37.5% of deaths from medical causes (n=8).

A substantial burden of RSV exists among children aged <2 years in Nicaraguan communities. RSV was also a leading cause of infant mortality among study participants. Development and implementation of effective RSV prevention and treatment measures represent an opportunity to substantially reduce severe illness and death among children worldwide.

## 2.3 Introduction

Respiratory syncytial virus (RSV) is an important cause of acute lower respiratory tract infections (ALRI) like pneumonia and bronchiolitis, particularly among children.[66] In 2015, there were an estimated 33.1 million cases of RSV-associated ALRI (RSV-ALRI) worldwide, of which 3.2 million required hospitalization.[2] This burden is especially pronounced among young children, with an estimated 1.4 million RSV-ALRI hospitalizations and 27,300 in-hospital deaths among infants aged < 6 months.[2]

Significant disparities exist in the distribution of RSV-associated mortality, with an estimated 99% of in-hospital deaths occurring in low- and middle-income countries (LMICs).[3] In Nicaragua, severe acute respiratory infections (SARIs) remain the leading communicable cause of death among children aged <5 years.[6] While increasing attention has been given in recent years to improving our understanding of the global burden of RSV, substantial knowledge gaps remain in LMICs. Many studies have used hospital-based populations to study RSV burden [28-34], but community-based studies are less common.[13, 14, 67, 78, 79]

Clinically, RSV infection often presents with respiratory symptoms like cough, rhinorrhea, and difficulty breathing. As many as 97% of children are infected with RSV by age 2.[12] RSV has also been associated with the development of severe illness and is considered the most common viral cause of pneumonia among children aged <5 years.[80] In Nicaragua, the respiratory illness season can last from June through February. While the seasonality of influenza in Nicaragua has been documented [81], the seasonality of RSV and other respiratory viruses is not well-defined.

RSV has long been a target for vaccine development because of its ubiquity and potential for causing severe illness. An overview of RSV vaccines and monoclonal antibodies in development reported 21 candidates in clinical trials.[25] Addressing knowledge gaps about the burden of RSV is crucial to the investment case for these interventions, and their successful future implementation. This study aims to assess the incidence of RSV among young children in Nicaragua, a lower-middle income tropical country in Central America.[82] We used the Nicaraguan Influenza Birth Cohort Study [83], originally designed to examine the incidence of influenza, it provides a unique opportunity to investigate other respiratory pathogens such as RSV.

## 2.4 Methods

### 2.4.1 Ethics statement

This study was conducted as a collaboration between the Sustainable Sciences Institute, the Nicaraguan Ministry of Health, the University of California, Berkeley (UCB), the University of Michigan, and the US Centers for Disease Control and Prevention (CDC). The study was approved by the Institutional Review Boards (IRBs) of the Nicaraguan Ministry of Health,

University of Michigan, and UCB. The CDC's IRB relied on the UCB IRB for approval. Written informed consent was obtained from a parent/guardian of all participants.

### 2.5 Study population

A detailed description of this study has been previously published.[83] The Nicaraguan Influenza Birth Cohort Study was a prospective cohort study conducted year-round from 2011-2016 in the catchment area of the Health Center Sócrates Flores Vivas (HCSFV) in Managua, Nicaragua. Continuous enrollment of newborns was conducted between September 8, 2011, and September 5, 2014 (Figure 2.1). Eligible subjects were identified when brought to the HCSFV for their first well-baby visit, or by home visits. Those who met the enrollment criteria, and for whom informed consent was received, were enrolled into the study. To be included, (1) infants had to be ≤ 4 weeks of age at enrollment, (2) live in the HCSFV catchment area, (3) infants' guardians had to plan to live in the area during the following 2 years, and (4) guardians had to be willing to attend HCSFV for all the infant's medical visits. Infants who required continued hospitalization directly after birth for ≥4 weeks were not eligible. Enrolled participants remained in the study until their 2nd birthday, they were withdrawn, or were lost to follow-up.

#### 2.5.1 Data

Baseline information about demographics, risk factors, and socioeconomic status were collected through surveys conducted by study staff at enrollment and yearly in March/April. Daily symptom diaries were completed by parents and were collected by study staff during weekly home visits. Respiratory samples were collected from infants who met the testing definition by presenting with (1) influenza-like illness (ILI) — fever (temperature ≥37.8°C) or history of fever and rhinorrhea and/or cough [84]; (2) fever or history of fever without defined focus; (3) severe respiratory symptoms (i.e., apnea, stridor, nasal flaring, wheezing, chest

indrawing, and/or central cyanosis) as judged by a study physician, regardless of the presence of fever/history of fever; or (4) those hospitalized with respiratory symptoms (previously listed) or sepsis.[83]

### 2.5.2 Sample collection and RSV testing

Oropharyngeal specimens collected with un-flocculated polyester tipped plastic swabs (Fisher Scientific, catalog number: 23-400-111) were obtained from infants aged <6 months who met the testing definition, while combined nasal and oropharyngeal swabs were collected from infants aged ≥6 months. Laboratory testing for RSV was conducted by the National Virology Laboratory at the National Center for Diagnosis and Reference (CNDR) of the Nicaraguan Ministry of Health. The CNDR has demonstrated proficiency in RSV testing through CDC-QCMD (Quality Control for Molecular Diagnostics) External Quality Assessment.[85] RNA was extracted (QIAamp Viral RNA Mini Kit, Qiagen) and tested by real-time RT-PCR for RSV using CDC protocols.[86]

### 2.5.3 Clinical definitions

Clinical care was provided to all study participants at the HCSFV by study personnel, and data were collected for each encounter, regardless of the reason for the visit. Laboratory-confirmed cases of RSV were classified as symptomatic RSV illness. Samples positive for RSV occurring ≥14 days from symptom onset for a previous RSV illness were considered new illness episodes. Symptomatic RSV illness was further classified as ALRI (RSV-ALRI) if study physicians diagnosed acute illness affecting the lower respiratory tract (i.e., pneumonia, bronchiolitis, bronchitis, or bronchial hyper-reactivity). Pneumonia diagnosis was made by study physicians according to the Integrated Management of Childhood Illness (IMCI) guidelines.[87] Severe ALRI was used instead of severe pneumonia as done by Shi et al.[2] Cases of ALRI,

severe ALRI, and hospitalization occurring within 14 days of symptom onset of a laboratory-confirmed RSV illness episode were considered associated with RSV.

### 2.5.4 Statistical analysis

Person-time was calculated as the number of weeks between participant enrollment and their exit from the study (at their 2nd birthday, or when withdrawn or lost to follow-up). Infants were not considered to be at risk for the 14 days following symptom onset for an RSV illness episode and were thus excluded from contributing person-time—except for measures intended to assess severe RSV (RSV-ALRI, RSV-severe ALRI, and RSV-hospitalization). A Poisson distribution was used to calculate 95% confidence intervals for incidence rates. Statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc.). Figures were created using R version 3.4.4 (R Foundation for Statistical Computing).

## 2.6 Results

Between the start of enrollment in September 2011 and study conclusion in September 2016, 833 infants were enrolled into the cohort and included in this analysis. The mean follow-up time for participants was 1.7 years (19.9 months) (Table 2.1). A total of 9 (1.1%) infants died during the study, with 8 (88.9%) deaths associated with medical illnesses and 1 (11.1%) resulting from an unknown cause. Over 75% of infants completed the study (n=629), while 23.4% (n=195) were withdrawn or were lost to follow-up before study completion. The most common reason infants were withdrawn from the study or were lost to follow-up (60.3%, n=123) was because the child moved away from the study area. We did not observe any significant differences between the demographics of those who completed the study and those who did not (Table 2.2).

There were a total of 17,209 visits to the study clinic; of these, 15,508 (90.1%) were for acute illness. The median number of clinic visits per participant was 18 (IQR: 20), and 814 (97.7%) of participants had at least 1 visit.

### 2.6.1 Incidence of symptomatic RSV illness

Participants contributed a total of 1,417.3 person-years and experienced 344 laboratory-confirmed episodes of symptomatic RSV illness, 11 (3.2%) of which were co-infected with influenza A. We did not observe differential illness severity among those co-infected. Of the 833 infants, 289 (34.7%) had at least 1 documented episode of symptomatic RSV illness. Of these, 50 (17.3%) infants had recurrent (≥2) episodes of symptomatic RSV illness, and 5 (1.7%) experienced 3 episodes of symptomatic RSV illness. The crude incidence of symptomatic RSV illness was 248.1 cases per 1000 person-years (95% CI: 223.2, 275.7) (Table 2.3). Incidence of symptomatic RSV illness increased steadily with age, peaking among infants aged 6-11 months at 361.3 cases per 1000 person-years (95% CI: 304.4, 428.8), before falling to 249.2 per 1000 person-years (95% CI: 214.0, 290.1) among those aged 12-23 months (Table 2.3 and Figure 2.2). One-hundred and seventy-six (51.2%) symptomatic RSV illnesses did not present with nurse/physician measured fever (≥38°C); including measured fever in the symptomatic RSV illness case definition decreased rates by 37-66% (Table 2.4). RSV epidemics started as early as May, and as late as September, lasting an average of 6.9 months (range: 4-7 months) (Figure 2.3).

### 2.6.2 Incidence of RSV-associated ALRI and severe ALRI

Of the 344 laboratory-confirmed cases of symptomatic RSV illness identified in the study, 170 (49.4%) were classified as ALRI (Table 2.5), resulting in an overall incidence rate for RSV-ALRI of 119.9 cases per 1000 person-years (95% CI: 103.2, 139.4). Incidence of RSV-

ALRI followed a similar trend across age groups as that of symptomatic RSV illness with incidence increasing with age until peaking among participants aged 6-11, with 181.8 cases per 1000 person-years (95% CI: 143.1, 231.0). While children aged <3 months had the lowest overall RSV illness rates, they had the highest rate of RSV-severe ALRI (Figure 2.4, Table 2.5)—though the differences between age groups were not statistically significant.

Among the 170 cases of RSV-ALRI, 21 (12.4%) had severe illnesses (Table 2.5), with an incidence of RSV-severe ALRI of 14.8 cases per 1000 person-years (95% CI: 9.7, 22.7). Participants aged <3 months had the highest incidence of RSV-severe ALRI, with 31.4 cases per 1000 person-years (95% CI: 13.1, 75.5). Except for a sharp decline among those 3-5 months of age, the incidence of RSV-severe ALRI decreased as age increased (Figure 2.4). While episodes of symptomatic RSV illness were less frequent among the youngest participants—aged <3 months— (Table 2.3 and Table 2.5), those that did occur were more likely to be severe. Eighty percent of children aged <3 months with symptomatic RSV illness had RSV-ALRI (vs. 56.8% among 3-5 months, 51.2% among 6-11 months, and 44.6% among 12-23 months, chi-square p=0.1); additionally, 50% of children aged <3 months with symptomatic RSV illness had RSV-severe ALRI (vs. 2.7% among 3-5 months, 6.1% among 6-11 months, and 4.2% among 12-23 months, chi-square p<0.0001).

### 2.6.3 Incidence of RSV-associated hospitalizations

The incidence of RSV-associated hospitalizations was 22.6 cases per 1000 person-years (95% CI: 16.0, 31.9). Aside from a precipitous drop among those aged 3-5 months, incidence of RSV-associated hospitalization steadily decreased as age increased, with infants aged <3 months having the highest incidence (37.7 cases per 1000 person-years, 95% CI: 16.9–83.9) (Table 2.6).

### 2.6.4 RSV-associated deaths

Of the 8 infants who died from medical causes during the study, 3 (37.5%) died of severe pneumonia (all in-hospital) and were RT-PCR-positive for RSV in the weeks preceding their death. Two (25.0%) of these deaths occurred within 2 weeks (1 and 14 days) of symptom onset; the infant who died 1 day after testing positive for RSV was 4 months old, while the infant who died 14 days after symptom onset was aged 10 months. One additional infant (aged 11 months) died of severe pneumonia 46 days after symptom onset. The RSV-associated mortality rate among infants ranged from 2.8 deaths per 1000 person-years (95% CI: 0.7, 11.1) using a 14 day risk period to 4.2 per 1000 person-years (95% CI: 1.3, 12.9) when considering the deaths that occurred up to 46 days after laboratory-confirmation with RSV.

## 2.7 Discussion

Using data from a community-based prospective birth cohort study, we found a high incidence of symptomatic RSV illness in Nicaragua in children aged <2 years. Infants aged <3 months had the highest rates of severe RSV infection outcomes, including severe ALRI and hospitalization. In our birth cohort, laboratory-confirmed RSV illness was associated with one third of deaths. In this population many common contributors to infant mortality in LMICs are missing as >98% children received WHO recommended immunizations [88], the prevalence of HIV is low [89], and malaria is absent, suggesting RSV is a significant contributor to infant mortality. This finding has important implications for a number of countries that have full coverage under the Expanded Program on Immunization (EPI), but still struggle to lower infant mortality.

Our findings are consistent with published estimates from other parts of the world.[13, 14, 90] In a review of the 2015 global burden of RSV-ALRI, Shi et al. reported incidence rates

ranging from 26.6–343.8 per 1000 person-years among children aged 0–5 months, 18.0–338.1 among those aged 6-11 months, and 21.8–304.3 among those aged 12-23 months.[2] One study, conducted in the Peruvian highlands, was responsible for the highest estimates in all age groups—reporting rates approximately double those of the next highest estimates (Table 2.7).[2, 91] Our RSV-associated ALRI estimates were similar to the majority of studies referenced by Shi et al. (i.e., 67, 160, and 93 per 1000 person-years among children aged 0-5, 6-11, and 12-23 months, respectively). We did observe rates of symptomatic RSV and ALRI that peaked later (among infants 6-11 months) than other studies. It is possible that our age-specific estimates of symptomatic RSV are biased from the inclusion of reported/measured fever as RSV illness presenting with fever increases with age. However, a study in Guatemala [35] showed a similar pattern suggesting that regional variations might impact the age distribution of RSV incidence.

There are limited published data about the incidence of RSV in community settings in low- and middle-income countries (LMICs), especially in Central America.[2] Shi et al. compiled data from 329 studies, of which only 14 (4%) were community-based with active case-ascertainment.[2] Of these 14, only one was from Central America (Guatemala).[35] The Guatemalan study reported an incidence of RSV pneumonia among children aged ≤18 months of 143.6 per 1000 person years (116.2, 177.3).[35] While the Guatemala estimate was higher than the estimate in our study (70.6 cases per 1000 person-years (95% CI: 58.0, 85.8)—Table 2.7), this is likely because in the Guatemalan study RSV-ALRI cases were identified only from children with physician diagnosed pneumonia, not the overall study population.

Identifying and quantifying RSV-associated mortality is challenging, and the most appropriate time period to use in classifying deaths associated with RSV remains a subject of debate.[92] RSV-associated mortality might peak weeks after the original RSV infection and

perhaps be associated with secondary bacterial infection.[93-95] A recent examination of RSV mortality in Minnesota included deaths that occurred within an 8-week period of laboratory confirmation.[96] Moreover, quantification of RSV-associated mortality in community-based studies is limited by the fact that only a relatively small number of deaths are expected. However, in our study, out of 8 deaths from medical causes, 2 deaths seemed clearly associated with RSV because they occurred within two weeks of the onset of laboratory-confirmed RSV illness, and one could argue that a third death (approximately 6 weeks following RSV laboratory-confirmation) was also associated with RSV illness. Thus, 25% or 37.5% of deaths from medical causes were associated with RSV.

This study has a number of strengths. This community-based study provides insight about the largely undocumented burden of RSV in communities in LMICs where a substantial proportion of the population might not seek hospital care for severe illness. The study enrolled children from birth and actively monitored them each week throughout the year for respiratory illnesses. As a prospective, longitudinal cohort study, we were able to calculate incidence and examine four seasonal RSV epidemics. Finally, by including neonates, we document RSV rates in a younger age group than much of the existing literature.

Multiple hospital-based studies have demonstrated that the inclusion of fever (measured--≥38°C--or reported) in case definitions results in an underestimate of RSV cases—particularly among children aged < 1 year.[97-99] Studies using data from cohorts initially designed to study influenza (like this one) are susceptible to such underestimates as case definitions like influenza-like illness and severe acute respiratory illness reflect influenza's more frequent presentation with fever. While we were unable to make the direct comparisons across a variety of case definitions like Saha [98], Nyawanda [97], and Rha et al. [99], we did conduct sensitivity

analyses examining the effect of including nurse/physician measured fever (≥38°C) on RSV rates. Had measured fever been included as a required criteria for sampling and/or testing, our estimated incidence rates would have been 30-70% lower depending on participants' age. Such findings suggest the value of developing RSV-specific case definitions—like those pursued through the WHO's Global RSV Surveillance Pilot.[100] While our testing definition likely missed some cases of symptomatic RSV infection—particularly among those aged < 1 year—the majority of any missed cases were most likely among those with less severe illness. The inclusion of any severe respiratory symptoms (regardless of fever/history of fever) in this study's testing criteria suggests that our assessments of more severe manifestations of RSV are good approximations of the true severe RSV burden in our study community. Future studies in this population are underway to examine the specific risk and prognostic factors contributing to this burden.

This study demonstrates that a substantial burden of RSV exists among children aged <2 years in Nicaragua. This coupled with the high proportion of infant deaths associated with RSV illness underscores the importance of RSV in such communities. Such findings demonstrate the merit of exploring the cost-benefit of current interventions, and continued support for those being developed for pregnant women and young children, to prevent RSV illness among this high-risk group. Development and implementation of effective RSV prevention represents a prime opportunity to substantially reduce morbidity and mortality of young children in Nicaragua and other LMICs.

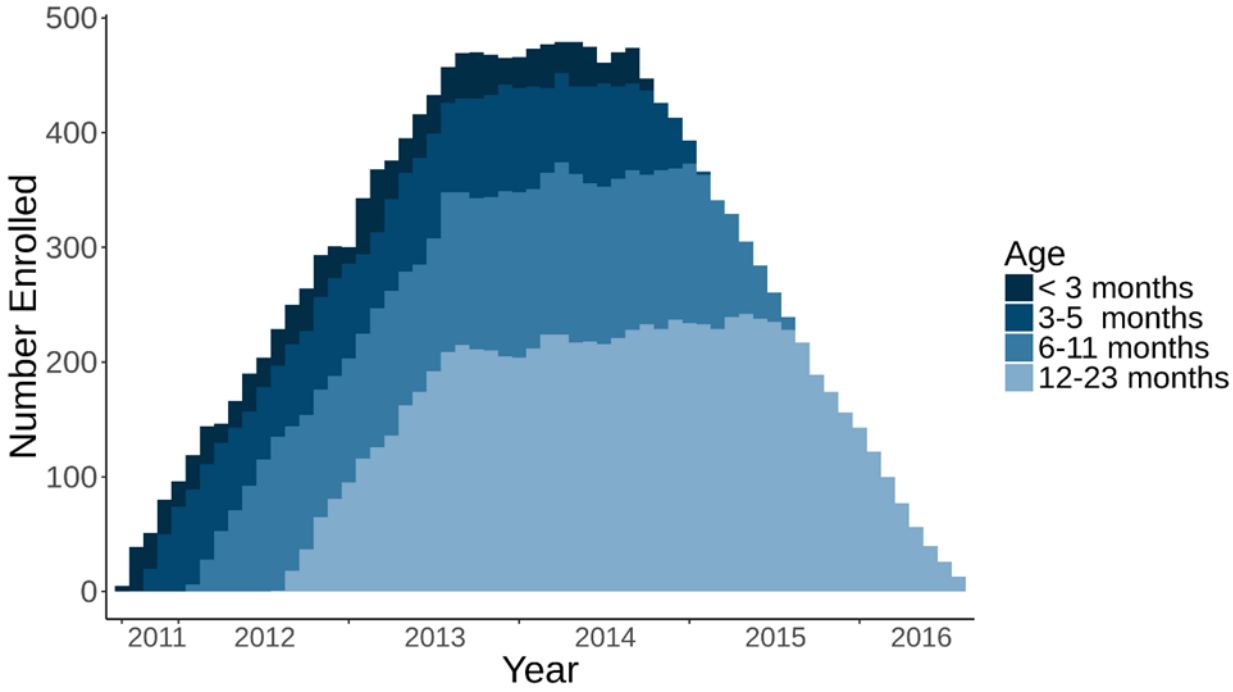


*Figure 2.1: Plot of Categorical Age Distribution Over the Course of the Study*

Count of enrolled participants by age category across study duration.

*Table 2.1: Characteristics of Study Participants*

| Characteristic | | Total No. = 833[a] |
|---|---|---|
| Age at enrollment | 0-2 weeks | 581 (69.8) |
| | 3-4 weeks | 249 (29.9) |
| | 5-6 weeks | 3 (0.4) |
| Male | | 415 (49.8) |
| Mean follow-up time, person-years | | 1.7 (0.6[b]) |
| Smoking in household | | 249 (29.9) |
| Mean number of persons in household | | 8.7 (4.4[b]) |
| Mothers with secondary or tertiary education (No. = 830) | | 677 (81.3) |
| Fathers with secondary or tertiary education (No. = 810) | | 644 (77.3) |
| Water tap location | Outside | 291 (35.0) |
| | Inside | 541 (65.0) |
| Dirt floor | Yes | 94 (11.3) |

[a]Data are presented as No. (%) unless otherwise indicated.
[b]Standard deviation

*Table 2.2: Characteristics of Study Participants by Completion Status*

| Characteristics | | Total | Completed study | Withdrawn/Lost to follow-up | p-value |
|---|---|---|---|---|---|
| | No. | 833 | 629 | 204 | |
| Age at enrollment | 0-2 weeks | 581 (69.8) | 442 (70.3) | 139 (68.1) | 0.209 |
| | 3-4 weeks | 249 (29.9) | 186 (29.6) | 63 (30.9) | |
| | 5-6 weeks | 3 (0.4) | 1 (0.2) | 2 (1.0) | |
| Sex | Male | 415 (49.8) | 311 (49.4) | 104 (51.0) | 0.747 |
| | Female | 418 (50.2) | 318 (50.6) | 100 (49.0) | |
| Mean person-years contributed | | 1.7 (0.6[a]) | 1.9 (0.05[a]) | 1.2 (0.5[a]) | <0.001 |
| Smoking in household | Yes | 249 (70.1) | 188 (29.9) | 61 (29.9) | 0.997 |
| | No | 584 (29.9) | 441 (70.1) | 143 (70.1) | |
| Mean number in household | | 8.7 (4.4[a]) | 8.7 (4.4[a]) | 8.85 (4.2[a]) | 0.337 |
| Mothers with secondary or tertiary education | | 677 (81.3) | 519 (82.6) | 160 (79.2) | 0.192 |
| Fathers with secondary or tertiary education | | 644 (77.3) | 487 (81.3) | 159 (82.4) | 0.878 |

Data are presented as No. (%) unless otherwise indicated.
[a]Standard deviation

*Table 2.3: Incidence of Symptomatic RSV Illness Episodes*

| Characteristic | | RSV cases | Person-years | Incidence rate (95% CI[a]) per 1000 person-years |
|---|---|---|---|---|
| All participants | | 344 | 1386.8 | 248.1 (223.2, 275.7) |
| Age | <3 months | 10 | 149.6 | 66.8 (36.0, 124.2) |
| | 3-5 months | 37 | 208.2 | 177.7 (128.8, 245.3) |
| | 6-11 months | 131 | 362.7 | 361.2 (304.3, 428.6) |
| | 12-23 months | 166 | 666.3 | 249.2 (214.0, 290.1) |
| Sex | Male | 176 | 692.9 | 254.0 (219.1, 294.5) |
| | Female | 168 | 693.9 | 242.1 (208.2, 281.7) |

[a]Confidence intervals calculated using a Poisson distribution.

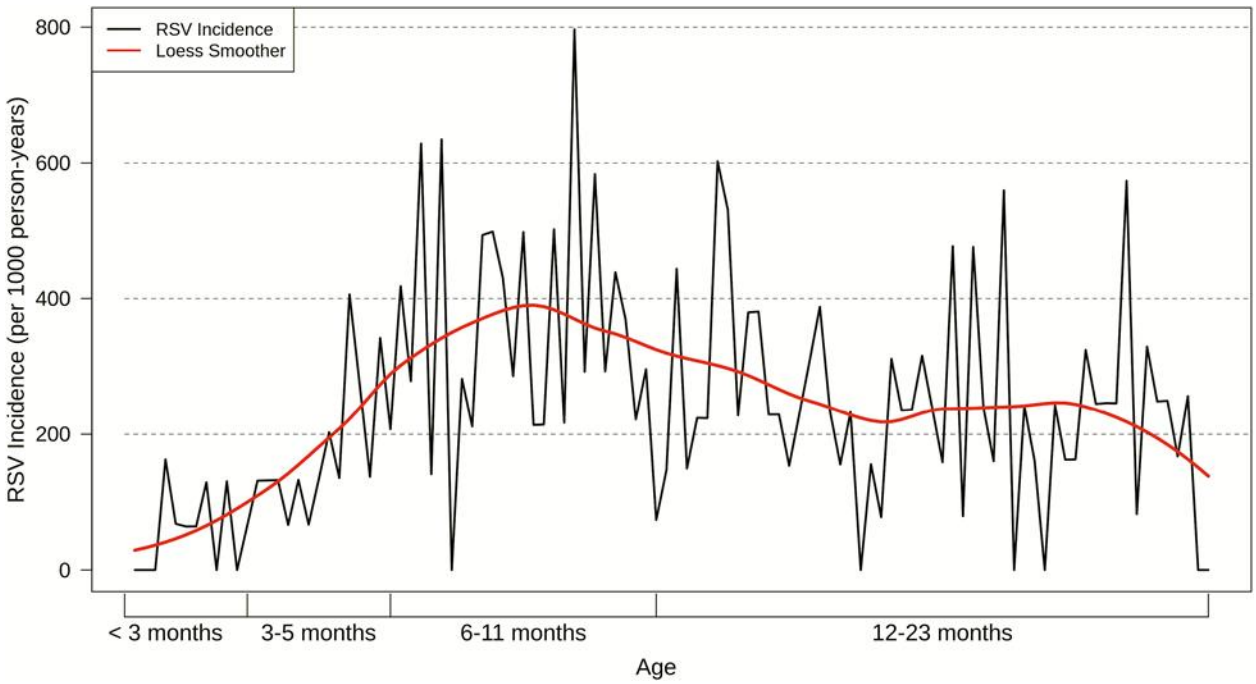


*Figure 2.2: Incidence of Symptomatic RSV Illness Episodes by Age*

The black line reflects the incidence rate of symptomatic RSV illness by week of age, while the red line shows a Loess smoothing function applied to the data to illustrate overall trend.

*Table 2.4: Sensitivity Analysis of RSV Incidence When Measured Fever (≥38°C) Was Used in Testing Criteria*

| Characteristic | | RSV cases | Person-years | Incidence rate (95% CI[a]) per 1000 person-years | % difference[b] |
|---|---|---|---|---|---|
| All participants | | 168 | 1386.8 | 121.2 (104.2, 140.9) | 51.2% lower |
| Age | <3 months | 5 | 149.6 | 33.4 (13.9, 80.3) | 50.0% lower |
| | 3-5 | 12 | 208.2 | 57.6 (232.7, 101.5) | 70.3% lower |
| | 6-11 months | 46 | 362.7 | 126.8 (95.0, 169.3) | 64.1% lower |
| | 12-23 months | 105 | 666.3 | 157.6 (130.2, 190.8) | 36.8% lower |
| Sex | Male | 87 | 692.9 | 125.6 (101.8, 154.9) | 50.1% lower |
| | Female | 81 | 693.9 | 116.7 (93.9, 145.1) | 51.8% lower |

[a]Confidence intervals calculated using a Poisson distribution.
[b]When compared to incidence rates calculated from testing criteria using fever or history of fever (Table 2).

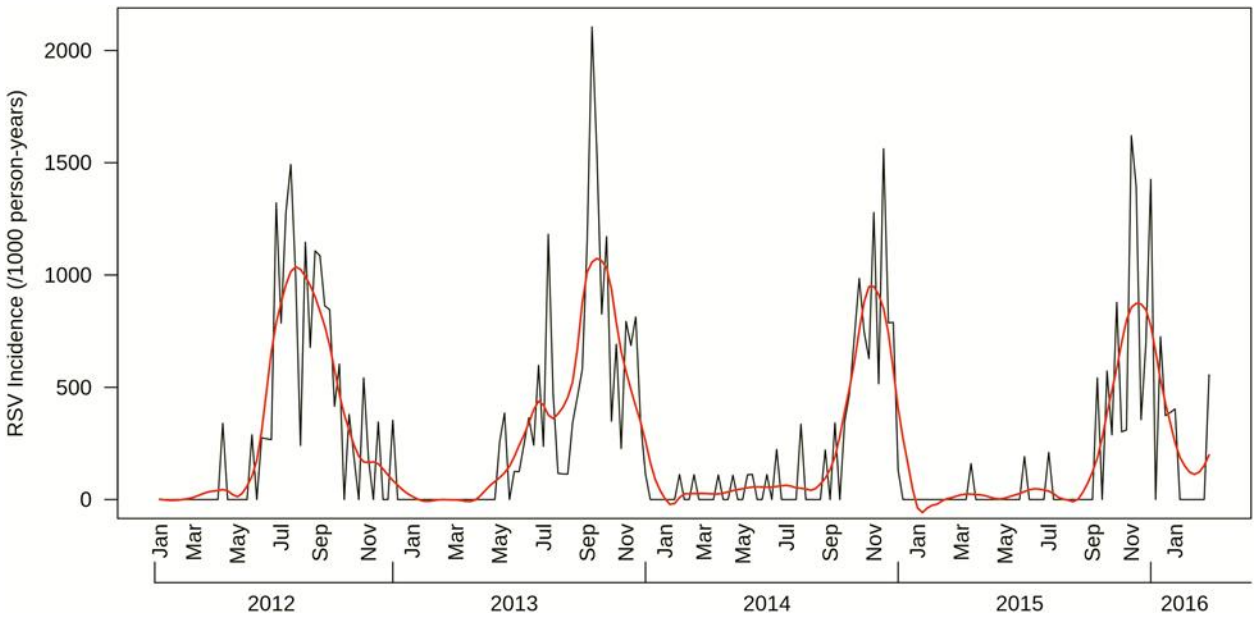


*Figure 2.3: Incidence of Symptomatic RSV Illness by Study Week*

The black line reflects the incidence rate of symptomatic RSV illness by week of study, while the red line shows a Loess smoothing function applied to the data to illustrate the seasonal trend of RSV transmission. Data were truncated at the beginning and end of the study when the total number of participants in the study was below 100.

*Table 2.5: Incidence of RSV-associated ALRI and RSV-associated Severe ALRI by Age and Sex*

| Characteristics | | Person-years | RSV-ALRI | Incidence rate (95% CI) | RSV-Severe ALRI | Incidence rate (95% CI) |
|---|---|---|---|---|---|---|
| All participants | | 1417.4 | 170 | 119.9 (103.2, 139.4) | 21 | 14.8 (9.7, 22.7) |
| Age | <3 months | 159.2 | 8 | 50.3 (25.1, 100.5) | 5 | 31.4 (13.1, 75.5) |
| | 3-5 months | 210.0 | 21 | 100.0 (65.2, 153.4) | 1 | 4.8 (0.7, 33.8) |
| | 6-11 months | 368.6 | 67 | 181.8 (143.1, 231.0) | 8 | 21.7 (10.9, 43.4) |
| | 12-23 months | 679.7 | 74 | 108.9 (86.7, 136.7) | 7 | 10.3 (4.9, 21.6) |
| Sex | Male | 708.3 | 96 | 135.6 (111.0, 165.6) | 12 | 16.9 (9.6, 29.8) |
| | Female | 709.1 | 74 | 104.4 (83.1, 131.1) | 9 | 12.7 (6.6, 24.4) |

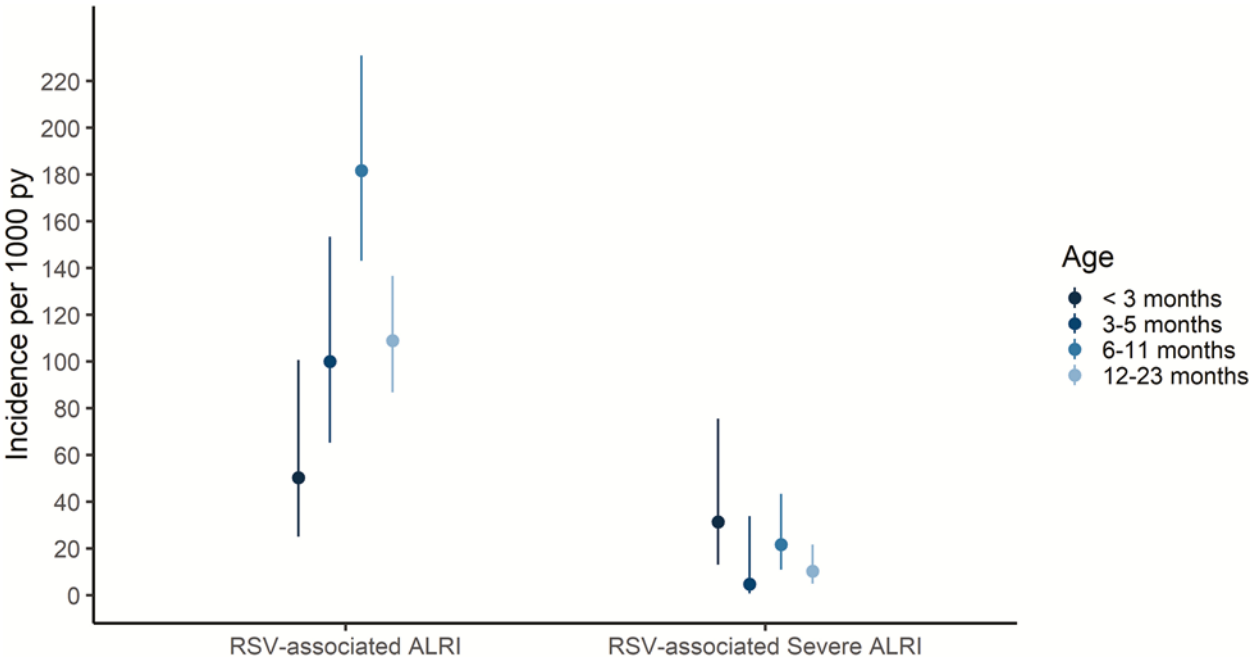


*Figure 2.4: Incidence of RSV-associated ALRI and RSV-associated Severe ALRI by Age*

Plot of incidence rates of RSV-associated acute lower respiratory tract infection (ALRI) and RSV-associated severe ALRI by age category. Lines around point estimates represent 95% confidence intervals estimated using a Poisson distribution.

*Table 2.6: Incidence of RSV-associated Hospitalization and RSV-associated Pneumonia by Age and Sex*

| Characteristics | | Person-years | RSV-associated hospitalizations | Incidence rate (95% CI) | RSV-associated pneumonia | Incidence rate (95% CI) |
|---|---|---|---|---|---|---|
| All participants | | 1417.4 | 32 | 22.6 (16.0, 31.9) | 100 | 70.6 (58.0, 85.8) |
| Age | <3 months | 159.2 | 6 | 37.7 (16.9, 83.9) | 6 | 37.7 (16.9, 83.9) |
| | 3-5 months | 210.0 | 1 | 4.8 (0.7, 33.8) | 9 | 42.9 (22.3, 82.4) |
| | 6-11 months | 368.6 | 12 | 32.6 (18.5, 57.3) | 42 | 114.0 (84.2, 154.2) |
| | 12-23 months | 679.7 | 13 | 19.1 (11.1, 32.9) | 43 | 63.3 (46.9, 85.3) |
| Sex | Male | 708.3 | 18 | 25.4 (16.0, 40.3) | 55 | 77.7 (59.6, 101.2) |
| | Female | 709.1 | 14 | 19.7 (11.7, 33.3) | 45 | 63.5 (47.4, 85.0) |

*Table 2.7: Comparison of Incidence of RSV-ALRI Across Studies*

| Location/ reference | Incidence rate of RSV-ALRI per 1000 person-years (95% CI) | | | Minimum criteria for sample collection/testing | Study design and population |
|---|---|---|---|---|---|
| | 0-5 months | 6-11 months | 12-23 months | | |
| **Nicaragua [this study]** | **67** | **160** | **93** | **Fever (≥37.8°C), reported fever, or severe respiratory symptoms** | **Prospective birth cohort study of children 0-2 years, enrolled from home or during well baby visits** |
| Peru[91] | 343.8 | 338.1 | 304.3 | Reported fever or cough in last 7 days | Prospective cohort study including children aged <3 years, recruited from home |
| Kenya[13, 14] | 147.0 | 63.0 | 71.0 | Difficulty breathing, rhinorrhea and/or nasal congestion, or cough in last 7 days | Prospective birth cohort over 4 calendar years, recruited from maternity ward or maternal child clinic |
| Dhaka, Bangladesh [67] | 150.0 | 110.0 | 120.0 | Fever (≥38°C), difficulty breathing, cough, or rhinorrhea | Prospective birth cohort of children 0-2 years, recruited from area surrounding clinic |
| Ballabgarh, India [90] | 60.2 | 18.0 | 52.0 | Cough or difficulty breathing | Prospective birth cohort of children 0-3 years, recruited from 2 towns following identification from medical records |
| San Marcos, Guatemala [35] | 107.7 | 172.4 | 124.5 | Children diagnosed with pneumonia | Randomized control trial of children ≤18 months comparing homes with traditional or intervention cook stove |

Table adapted from review paper by Shi et al.[2]

# Chapter 3 Individual-level Association of Influenza Infection With Subsequent Pneumonia: A Case-control and Prospective Cohort Study

## 3.1 Author summary

We observed children with symptomatic influenza had substantially greater odds of developing pneumonia in the subsequent 30 days when compared to children without influenza. This elevated risk of pneumonia was also not consistent over this period, but was higher in the first and third weeks following influenza infection. These distinct periods of elevated risk suggest the existence of two etiologic pathways between influenza and pneumonia.

## 3.2 Abstract

Pneumonia is a leading cause of mortality worldwide. Influenza may result in primary pneumonia or be associated with secondary bacterial pneumonia. While the association with secondary pneumonia has been established ecologically, individual-level evidence remains sparse and the risk period for pneumonia following influenza poorly defined.

We conducted a matched case-control study and a prospective cohort study among Nicaraguan children aged 0-14 years from 2011-2018. Physicians diagnosed pneumonia cases based on Integrated Management for Childhood Illness (IMCI) guidelines. Cases were matched with up to 4 controls on age (months) and study week. We fit conditional logistic regression models to assess the association between influenza subtype and subsequent pneumonia development, and a Bayesian non-linear survival model to estimate pneumonia hazard following influenza.

Participants with influenza had greater risk of developing pneumonia in the 30 days following onset compared to those without influenza (matched odds ratio [mOR]: 2.7, 95% CI: 1.9, 3.9). Odds of developing pneumonia were highest for participants following A(H1N1)pdm09 illness (mOR: 3.7, 95% CI: 2.0, 6.9), followed by influenza B, and A(H3N2). Participants' odds of pneumonia following influenza were not constant, showing distinct peaks 0-6 days (mOR: 8.3, 95% CI: 4.8, 14.5) and 14-20 (mOR: 2.5, 95% CI: 1.1, 5.5) days post influenza infection.

Influenza is a significant driver of both primary and secondary pneumonia among children. Distinct periods of elevated pneumonia risk in the 30 days following influenza supports multiple etiological pathways.

## 3.3 Introduction

Despite progress in reducing morbidity and mortality, the global burden of pneumonia remains substantial, particularly among children in low- and middle-income countries (LMICs).[101] Influenza is an important contributor to pneumonia burden.[77] This may occur directly, as primary viral pneumonia[61], or indirectly through secondary bacterial pneumonia.[70] Seasonal influenza peaks coincide with, or are followed by, peaks of pneumonia, suggesting population-level association.[49, 102] Additionally, investigations of the 1918 and 2009 influenza pandemics, make the case for secondary bacterial infections being drivers of mortality during influenza pandemics.[68, 70, 103-106] Laboratory studies have established plausible biological mechanisms through which influenza infection may lead to increased susceptibility to secondary bacterial pneumonia.[45, 107, 108] However, substantial gaps in the literature remain that can only be addressed through large participant-level epidemiologic studies.[109, 110]

Previous large-scale studies focused on individuals hospitalized for pneumonia. Though community comparisons strengthen some studies' findings, respiratory samples were often collected concurrently with pneumonia diagnosis (cross-sectional), limiting causal inference. Studies which addressed the limitations of cross-sectional analysis faced different challenges, specifically small sample size[50] and seasonal confounding.[51]

We used a nested, matched case-control study and Bayesian time-to-event modeling to explore the risk of developing pneumonia following symptomatic influenza infection in a prospective cohort of Nicaraguan children aged 0-14.

## 3.4 Methods

### 3.4.1 Ethics statement

The study was approved by the Institutional Review Boards of the Nicaraguan Ministry of Health, University of Michigan, and University of California, Berkeley. Written informed consent was obtained from a parent/guardian of all participants. Verbal assent was obtained from children aged ≥6 years.

### 3.4.2 Study population and sample collection

Study participants were from two prospective cohorts of Nicaraguan children, the Nicaraguan Influenza Birth Cohort and the Nicaraguan Pediatric Influenza Cohort. Participants were pooled as they were enrolled from the same population and shared the same data collection methods. The resulting cohort included children aged 0-14 years who participated in the study between 2011 and 2018. The methods employed in these studies have been described in detail previously.[111, 112] Briefly, healthy children were enrolled when brought to Health Center

Sócrates Flores Vivas (HCSFV), or were recruited through home visits. A detailed clinical history and sociodemographic survey were collected on enrollment and yearly thereafter. Nicaragua introduced the pneumococcal vaccine (PCV13) in 2010 with a three-dose schedule (2, 4 and 6 months) and a catch-up dose for children aged 12-24 months. By the end of 2012, nearly 100% of infants were appropriately vaccinated for their age.[75]

Nasal and oropharyngeal swabs were collected from all children meeting the testing definition. Study nurses and physicians are available at HCSFV 24 h/day, 365 days/year, and parents agreed to bring their child to HCSFV at the first sign of fever. The criteria for sample collection and testing was illness onset within 4 days, fever or reported fever, and rhinorrhea and/or cough for children aged ≥ 2 years, or fever or reported fever for children aged < 2 years.[111] Respiratory samples were also collected/tested for influenza for any child presenting with clinical pneumonia or severe respiratory illness (i.e. requiring transfer to hospital).

### 3.4.3 Laboratory methods

RNA was extracted from swabs (QIAamp Viral RNA Mini Kit, Qiagen) and tested for influenza A and B using validated CDC RT-PCR protocols.[86] Influenza A positive samples were subtyped according to CDC protocols.[111] Samples were not tested for bacterial pathogens and influenza B lineage was not considered in this analysis.

### 3.4.4 Data collection and case definitions

Yearly surveys assessing household and participant-level risk factors were completed in March/April, before the typical start of seasonal influenza transmission in June.[111] With each visit to the study health center, a comprehensive medical consult form was completed. These data were also collected at follow-up visits, which were scheduled until the participant's illness clears, with frequency of visits depending on severity.

Study physicians identified cases of clinical pneumonia among those presenting to the clinic using age-specific guidelines for rapid breathing from the Nicaraguan Ministry of Health based on the Integrated Management of Childhood Illness (IMCI) (Table 3.1).[113] Parents reported the onset date of symptoms which was used as the start of the influenza-episode in all subsequent analyses. Diagnosis date was used to define pneumonia onset. Pneumonia episodes occurring within 0-6 days of influenza illness onset were considered cases of likely primary viral pneumonia, while those occurring ≥7 days after influenza onset were considered likely secondary bacterial pneumonias.[50]

### 3.4.5 Statistical analysis

Two study designs were employed: a nested, matched case-control study, and a prospective cohort study. Conditional logistic regression models were fit using the *survival* package, survival models were fit with the *brms* package for Bayesian regression modeling with *Stan*, and figures were generated with *ggplot2* and the *tidybayes* packages for R 3.6.1.[114-117]

#### Matched case-control

Pneumonia cases were matched to up to 4 controls on age (months) and study week ensuring the appropriate risk set was used when assigning controls. Controls were selected from the cohort at large after excluding those with a pneumonia diagnosis in the previous 45 days (Figure 3.1). Children were able to serve as a case (if distinct episode) or control multiple times if they met the previously described criteria.

To explore the relationship between influenza subtype and risk of pneumonia in the 30 days following onset, a conditional logistic regression model was fit (Model 1) with categorical variable ($s_{ik}$) indicating no influenza, H3N2, H1N1pdm09, or influenza B for case $i$ in pair $k$. To assess the risk period for pneumonia following symptomatic influenza infection, a separate

conditional logistic regression model was fit (Model 2), with categorical variable ($w_{ik}$) assessing the risk of pneumonia in 0-6, 7-13, 14-20, and 21-30 days following influenza infection. Those without influenza in the 30 days prior to the case's pneumonia diagnosis were the reference group.

Model 1:

$$logit(y_{ik}) = \alpha_k + \beta_1 sex_{ik} + \beta_2 s_{ik}$$

Model 2:

$$logit(y_{ik}) = \alpha_k + \beta_1 sex_{ik} + \beta_2 w_{ik}$$

**Bayesian survival model**

To estimate the daily rate of pneumonia during the 30 days following influenza onset in the entire cohort, we used a discrete time survival model, in which the outcome $y_{it}$= 1 denotes that individual *i* was diagnosed with pneumonia on day *t* of the study period, and $y_{it} = 0$ indicates that the individual was not. We fit a model with two penalized spline terms: 1) for the month of study (1:95), denoted as $\lambda_0(t)$, where $\lambda_0(t)$ is a function mapping days to the baseline log-hazard of pneumonia for the month containing day *t*. To represent the log-hazard ratio of pneumonia risk on each day post-influenza, we defined a second smoothed term, $f(t - \zeta_i, s_i)$, where $\zeta_i$is the day of influenza onset for individual $i$, and $s_i$ indicates the infecting influenza subtype (H3N2, H1N1pdm09, or B). This allowed for the modeling of time-varying log-hazard of pneumonia by influenza subtype. Finally, we defined $\beta$ to be a vector of hazard ratios, corresponding to their respective combination of age and sex, $x_i$. We then defined the rate of pneumonia for individual *i* on day *t* as:

Model 3:

$$\log\big(\lambda_i(t)\big) = \lambda_0(t) + x_i'\beta + I(s_i > 0)f(t - \zeta_i, s_i)$$

Where $I(s_i > 0)$ is an indicator variable evaluating to 1 if the individual was infected by any influenza subtype in the last 30 days, and 0 otherwise. We can then express this rate as the probability of pneumonia on any given day using the conditional log-log link function, i.e. $\Pr(y_{it} = 1) = 1 - \exp(-\lambda_i(t))$, which allows the values of $\lambda_0$(t) and $\beta$ to be interpreted as a baseline hazard and hazard ratios respectively.[118] Additional details on the statistical analysis can be found in Appendix A

### 3.5 Results

Between January 1, 2011 and December 31, 2018, 3234 children participated in the study (Table 3.2). The mean age at enrollment was 3.2 years (SD: 3.8), and mean follow-up time was 3.7 years (SD: 2.5). The proportion of study participants withdrawn or lost to follow-up was low at 3.1% per year. The most common reasons for early withdrawal/removal from the study were not meeting the requirements of the annual sampling routine (54.8%) and inability to locate the participant's home (23.2%). A total of 12 (0.4%) participants died during the study. Pneumonia was listed on the death certificate as a cause of death for 8 (66.7%). While seasonal influenza vaccination in the cohort was low averaging 3.2% (range: 0.7, 7.7%) of participants per year, Oseltamivir was relatively common, being used in 41.0% of influenza episodes. Antibiotics were provided in 27.1% of total clinic visits and 26.1% of clinic visits associated with an influenza episode.

There were 1199 cases of clinical pneumonia (Table 3.2, Figure 3.2), of these 226 (18.9%) required hospitalization. Pneumonia cases were more likely to occur in children who were younger and male, with nearly 60% of pneumonia cases in males and over 70% in children

aged <2 years (Table 3.2). Pneumonia cases among infants (< 1 year) more frequently required hospitalization than those among participants aged 5-14 years (28.6% vs. 4.4%).

Among clinical pneumonia cases, 62 (5.2%) had RT-PCR confirmed influenza infection in the 30 days preceding pneumonia diagnosis. Of these, 21 (33.9%) were A/H3N2, 24 were A/H1N1pdm09 (38.7%), and 17 (27.4%) were influenza B. Pneumonia episodes following A/H1N1pdm09 were more often severe, with 25.0% requiring hospitalization compared with 4.8% and 5.9% for H3N2 and influenza B respectively (p =0.08). Primary pneumonia was more common than secondary pneumonia following influenza with 40 (64.5%) pneumonia cases occurring within 0-6 days following influenza vs. 22 (35.5%) cases occurring ≥7 days following influenza (p=0.01). Primary pneumonia occurred an average of 2.1 (s.d.: 1.5) days after influenza symptom onset compared to 18.8 (s.d.: 7.4) days for secondary pneumonia. We observed no difference in the severity of primary and secondary pneumonias with 12.8% and 13.0% of each group respectively (p=0.98) being hospitalized.

For participants with a laboratory confirmed influenza infection the matched odds ratio (mOR) of developing clinical pneumonia in the 30 days post influenza onset were 2.7 (95% confidence interval [CI]: 1.9, 3.9) times that of children without influenza (Table 3.3). Sex-specific effects were also observed, with male participants' odds of developing pneumonia 1.6 (95% CI: 1.4, 1.8) times that of females. For every 1000 infants with symptomatic influenza there were 36.3 (95%CI: 17.9, 60.0) excess pneumonia cases among males, and 26.2 (95% CI: 11.9, 43.8) excess cases among females (Table 3.4). The overall number of excess cases per 1000 symptomatic influenza infections among children under 5 years of age was 17.4 (95% CI: 8.4, 28.7) for males, and 12.7 (95% CI: 6.0, 21.4) for females.

### 3.5.1 Influenza subtype and subsequent pneumonia

Examined by subtype (Model 1), those with symptomatic H1N1pdm09 infections had the highest odds of developing clinical pneumonia in the subsequent 30 days, 3.7 (95% CI: 2.0, 6.9) times that of participants without influenza illness. Those with symptomatic H3N2 or influenza B infection also had greater odds of developing pneumonia, specifically 2.1 (95% CI: 1.2, 3.7) and 2.7 (95% CI: 1.5, 5.2) times that of participants without influenza respectively.

Similarly, the time-to-event model (Model 3), indicated an increased hazard of clinical pneumonia in the 30 days following symptomatic influenza infection (Figure 3.3). Hazard ratios were highest for H1N1pdm09, followed by Influenza B, and then H3N2 (Figure 3.3). The relationship between symptomatic influenza and clinical pneumonia was largely consistent across participants aged 0-5 (regardless of subtype) but became difficult to distinguish among older participants (> 7 years) because of sparse data. As such, results reported from model 3 are limited to those aged ≤7 years (Figures 3.4 and 3.5). While the HRs remained relatively consistent from age 0-5, incidence decreased sharply as age increased, particularly beyond 2 years (Figures 3.4 and 3.6).

### 3.5.2 Risk period for clinical pneumonia following influenza

Model 2 assessed the risk period for clinical pneumonia following any symptomatic influenza infection in the matched case control study. Participants with symptomatic influenza infection had substantially higher odds of developing pneumonia in the 30 days post-influenza when compared to participants without symptomatic influenza. Specifically, the relative odds of pneumonia were highest in the first (0-6 days), and third weeks (14-20 days) following symptomatic influenza infection. Participants with symptomatic influenza had 8.3 (95% CI: 4.8, 14.5) times higher odds of developing pneumonia in the week following infection, and 2.5 (95%

CI: 1.1, 5.5) times higher odds of developing pneumonia in the third week following infection (Table 3.5) compared to participants without symptomatic influenza, a pattern similar to that observed in the survival model (Model 3). For each subtype, an initial peak in relative hazard of clinical pneumonia was observed during the first week following influenza illness, though its magnitude varied by subtype. Additionally, H1N1pdm09 displayed a secondary peak of pneumonia hazard beginning around the 3$^{rd}$ week post infection. While the confidence region did include the null value, the magnitude closely matches the OR for the corresponding period in model 2 (Figure 3.3 and Table 3.5).

## 3.6 Discussion

We show that among children, symptomatic influenza infection is associated at the individual level with increased risk of pneumonia in the 30 days following illness onset. This association was observed across influenza subtypes (H1N1pdm09, H3N2, and B), and was stronger among young children. We also observed that pneumonia risk was not constant throughout the 30-days following symptomatic influenza infection, with distinct periods of elevated pneumonia risk 0-6, and 14-20 days following influenza illness onset. This suggests differing pathologies causing pneumonia, with primary pneumonias nearly concurrent with influenza, and secondary pneumonias after a 2-3 week lag.

Multiple studies have suggested that secondary bacterial pneumonia was a primary driver of mortality in influenza pandemics including those in 1918[68, 69] and 2009.[49, 70, 72] However, it is unclear whether this extends to inter-pandemic periods, or non-fatal secondary bacterial pneumonia. A 2000 matched case-control study found that cases of pneumococcal pneumonia were more likely to have reported influenza-like-illness (ILI) in the 7-28 days preceding hospital admission (mOR 12.4, 95% CI: 1.7, 306) than age-matched controls.[50] The

magnitude of this association decreased when influenza infection was determined using H1N1 serology, but remained substantial (mOR 3.7, 95% CI: 1.0, 18.1). This is very similar to our estimate for H1N1pdm09 over a comparable timeframe of 30 days (mOR: 3.7, 95% CI: 2.0, 6.9). A South African study from 2016 reported prevalence of influenza-associated severe pneumonia and influenza-associated pneumonia requiring hospitalization as 20% and 33% respectively among children aged ≤ 2 years. [119] While our estimates for this age group were lower (14% severe, 19% hospitalized), this difference reflects a small variation in the absolute number of cases.

The proportion of Nicaraguan children who are age-appropriately vaccinated with PCV13 is nearly 100%.[75] The burden of influenza-associated pneumonia observed in our study may be different from populations with lower PCV coverage where a greater number of secondary bacterial pneumonias would be expected. Additionally, pneumococcus is not the only cause of secondary bacterial pneumonias and given our use of clinical pneumonia we cannot therefore exclude the possibility that some secondary pneumonia cases resulted from other bacteria or even (non-influenza) viral infections.

Our observation that H1N1pdm09 was associated with greater risk of subsequent pneumonia compared to H3N2 may seem to contradict the widely accepted convention of more severe disease during H3N2-predominant seasons. However, the differences that we found were not statistically significant beyond the first 3 days following infection. Also, our models assessed the expected severity of illness given infection, which is different from disease frequency.

Exploring the relationship between viral respiratory infections and subsequent pneumonia is notoriously difficult to do at a participant level. A recent review of studies examining the

relationship between viral respiratory infection and subsequent pneumococcal disease found that nearly 90% of relevant studies were ecologic, substantially limiting causal inference.[109] Among the two participant-level studies of influenza and pneumonia, one was limited by seasonal confounding[51], while the other[50] was hampered by its small sample size (13 cases). Several large-scale studies exploring pneumonia etiology have recently published their results including PERCH[7], GABRIEL[120], EPIC[121], and the Drakenstein Cohort.[119] These studies have focused on assessing pathogens that are detectable upon diagnosis with pneumonia compared to non-pneumonia controls. However, none of these studies have yet examined the temporal dynamics of pneumonia following influenza.

This study has several strengths. First, data were obtained from a community-based prospective cohort, limiting the potential for reverse causation bias. Second, this study was conducted on a participant-level, allowing us to calculate individual-level hazard rather than population-level correlation. Third, this analysis involved a larger sample size than previous studies, improving power and precision of effect estimates. Fourth, seasonality of the exposure and outcome were accounted for in both the matched case-control and the prospective cohort. The consistency of trends observed in both the conditional logistic regression and survival models lends further support to the primary conclusions of this analysis.

This analysis did have some limitations. Influenza-associated pneumonia is a rare outcome, and categorization by subtype and lag-time between influenza and pneumonia only resulted in fewer cases per strata. This limited statistical power to assess variation in risk period for pneumonia by influenza subtype, as well as the number of covariates included in the model. As such, the existence of residual confounding is a possibility. However, we would not expect such residual confounding to affect the temporal relationship between pneumonia and influenza

as confounders would likely be constant over such a short time scale (30 days). While we did not examine other respiratory pathogens as potential causes of pneumonia, by accounting for calendar time the model captures such associations. This doesn't account for co-infections between influenza and other respiratory pathogens (particularly RSV), but we would anticipate the effect of co-infections to be minimal as previous analyses have shown influenza/RSV co-infections rare in this population.[112] Perhaps the biggest limitation of this study is our use of clinical pneumonia to define cases rather than imaging and molecular diagnostics. While we were unable to definitively state whether a pneumonia case was viral or bacterial in origin, the importance of clinical pneumonia diagnosis shouldn't be forgotten. Pneumonia diagnosis using IMCI criteria remains a widely utilized approach, particularly in LMICs. Even with more sophisticated diagnostics like RT-PCR, bacterial culture, and chest radiography, distinguishing between primary viral and secondary bacterial pneumonias is challenging.[61] Lastly, we were unable to determine exact date of pneumonia onset and instead used diagnosis date, however, as this corresponds with when the symptoms were severe enough to seek treatment, it is a reasonable measure of pneumonia.

Many important questions remain regarding the biological, social, and environmental factors that affect the relationship between influenza and pneumonia. We hope the results of this study highlight the importance of understanding the temporal dynamics between influenza and pneumonia. Further, we hope that increased collaboration and data sharing may facilitate the exploration of aspects of this relationship which studies to date have been underpowered to address. Regardless, it is clear that influenza remains an important driver of the global pneumonia burden, through both primary and secondary pneumonias. More effective tools to

prevent and treat influenza present promising mechanisms by which the burden of pneumonia can be reduced.

*Table 3.1: Clinical Pneumonia Case Definition Used by the Nicaraguan Ministry of Health*

| Age | |
|---|---|
| < 2 months | ≥ 60 breaths per minute |
| 2-11 months | ≥ 50 breaths per minute |
| 12-59 months | ≥ 40 breaths per minute |
| ≥ 60 months | ≥ 25 breaths per minute |

*Based on the Integrated Management for Childhood Illness (IMCI) criteria

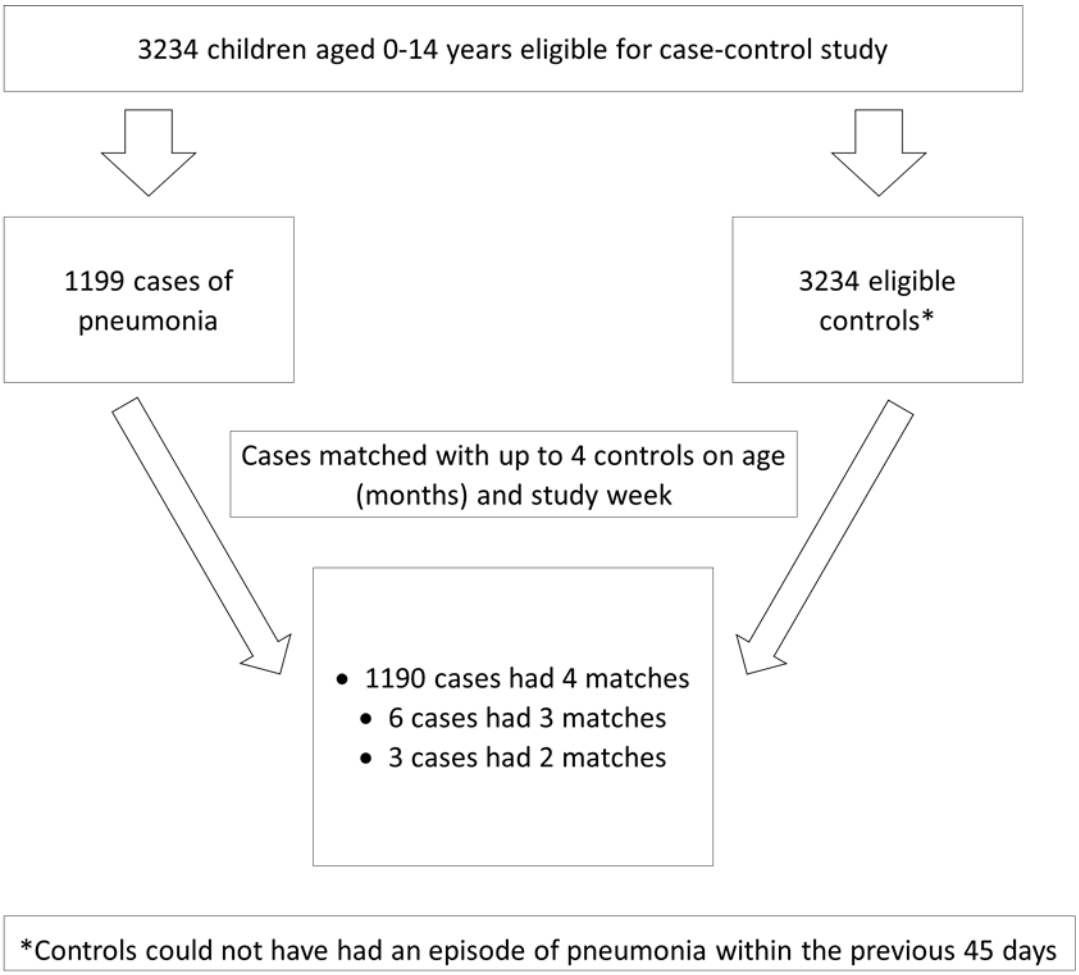


*Figure 3.1: Matching Scheme for Nested Matched Case-control Study*

*Table 3.2: Characteristics of Participants and Pneumonia Cases*

| | | All participants | Clinical pneumonia cases | Influenza-associated pneumonia cases |
|---|---|---|---|---|
| | | N = 3234 | N = 1199 (683 participants) | N = 62 (59 participants) |
| Age at enrollment in years[b] | | 3.6 (3.6) | 0.5 (1.7) | 0.6 (2.1) |
| Person-years contributed[b] | | 3.7 (2.5) | 4.5 (2.6) | 4.8 (2.6) |
| Male | | 1602 (49.5%) | 702 (58.5%)[c] | 34 (54.8%) [c] |
| Smoking in household | | 998 (31.3%) | 239 (35.0%) [c] | 25 (42.4%) [c] |
| Share a bed | | 1964 (63.2%) | 475 (71.6%) [c] | 44 (74.6%) [c] |
| Mothers with secondary or tertiary education | | 2312 (76.5%) | 506 (74.1%) [c] | 46 (91.5%) [c] |
| Fathers with secondary or tertiary education | | 2082 (73.1%) | 464 (67.9%) [c] | 38 (64.4%) [c] |
| Age | <12 months | .. | 496 (41.4%) | 22 (35.5%) |
| | 12-23 months | .. | 389 (32.4%) | 20 (32.2%) |
| | 24-59 months | .. | 224 (18.7%) | 14 (22.6%) |
| | ≥60 months | .. | 90 (7.5%) | 6 (9.7%) |
| Required hospitalization | | .. | 226 (18.9%) | 8 (12.9%) |
| Deemed severe pneumonia | | .. | 176 (14.7%) | 6 (9.7%) |
| Primary pneumonia (0-6 days following influenza) | | .. | .. | 40 (64.5%) |
| Secondary pneumonia (7-30 days following influenza) | | .. | .. | 22 (35.5%) |
| Influenza type | H3N2 | .. | .. | 21 (33.9%) |
| | H1N1pdm09 | .. | .. | 24 (38.7%) |
| | Influenza B | .. | .. | 17 (27.4%) |

[a]Data reflect number(column %) unless otherwise specified
[b]Mean(SD)
[c]Column % reflects the number of participants

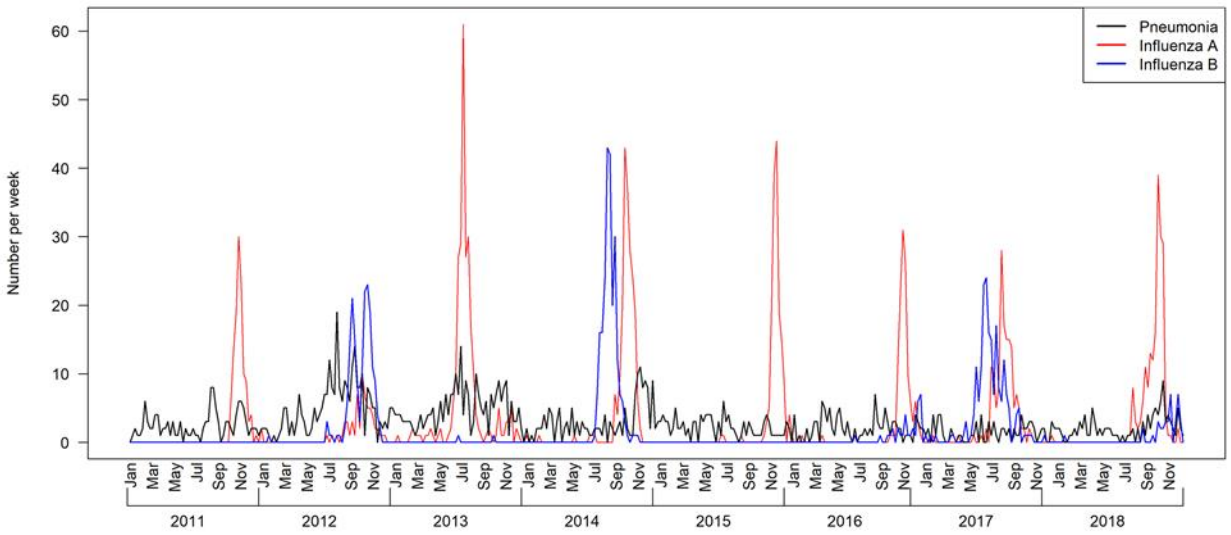


*Figure 3.2: Influenza and Clinical Pneumonia among Cohort Members 0-14 years, Nicaragua 2011-2018*

The lines represent the number of cases of influenza A, influenza B, and clinical pneumonia per week over the course of the study.

*Table 3.3: Matched Odds Ratios of Developing Clinical Pneumonia Within 30 Days Following Symptomatic Influenza Infection by Gender and Influenza Subtype*

| | Matched Odds Ratio (mOR) | 95% Confidence Interval | p-value |
|---|---|---|---|
| Male | 1.6 | (1.4, 1.8) | <0.001 |
| Influenza (overall) | 2.7 | (1.9, 3.9) | <0.001 |
| Influenza A | 2.7 | (1.8, 4.1) | <0.001 |
| A/H3N2 | 2.1 | (1.2, 3.7) | 0.008 |
| A/H1N1pdm09 | 3.7 | (2.0, 6.9) | <0.001 |
| Influenza B | 2.7 | (1.5, 5.2) | 0.0018 |

[a]Results obtained from model 1

*Table 3.4: Excess Cases of Pneumonia in Next 30 Days per 1000 Symptomatic Influenza Infections*

| Age (years) | Male | Female |
|---|---|---|
| < 5 | 17.4 (8.4, 28.7) | 12.7 (6.0, 21.4) |
| < 1 | 36.3 (17.9, 60.0) | 26.2 (11.9, 43.8) |
| 1 | 24.0 (11.3, 40.3) | 16.9 (8.1, 29.1) |
| 2 | 15.3 (7.3, 26.4) | 10.9 (4.8, 18.5) |
| 3 | 9.8 (4.3, 17.3) | 7.0 (2.4, 12.6) |
| 4 | 6.5 (2.6, 11.4) | 4.5 (1.3, 8.2) |

*Obtained using the conditional survival probabilities from the posterior distribution

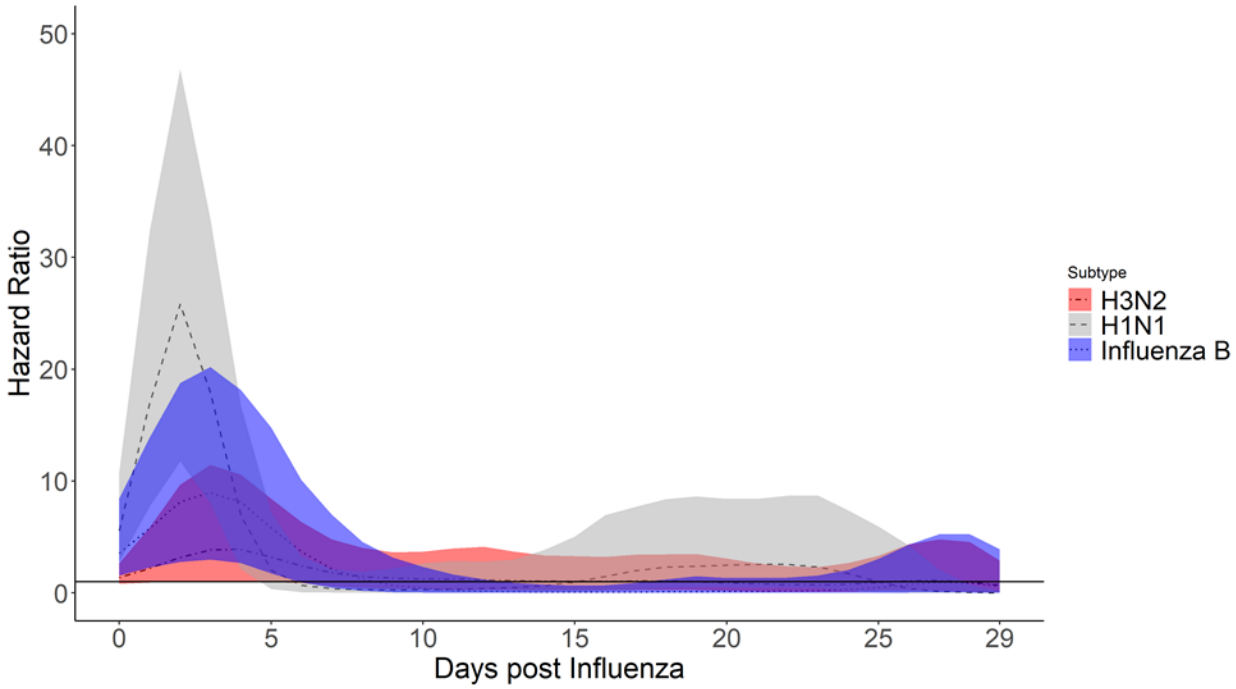


*Figure 3.3: Hazard Ratios for Pneumonia in the 30 days Following Influenza Infection Among Participants Aged < 5 Years*

The lines represent the relative hazard of pneumonia (model 3) in the 30 days following an influenza infection compared to those who had no influenza infection. The relative hazard of pneumonia for each influenza subtype can be distinguished by line type provided in the legend. The shaded areas reflect the 95% confidence intervals with those that are overlapping being indicative of differences that were not statistically significant at $\alpha=0.05$.

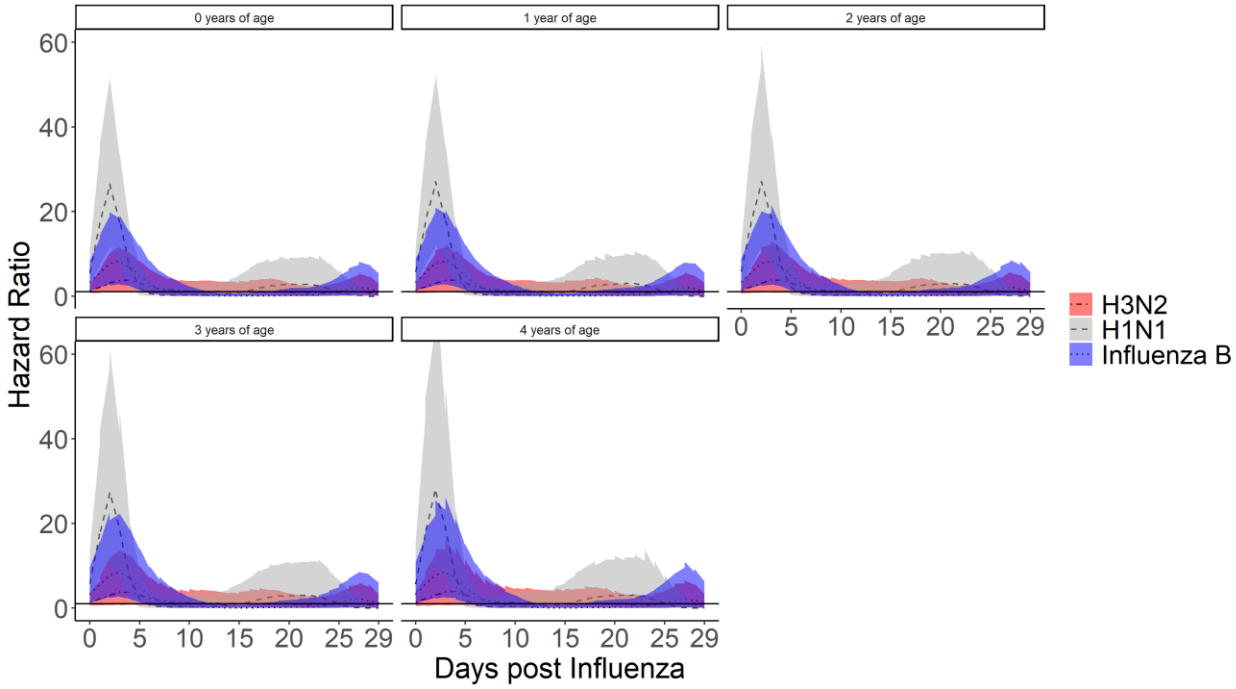


*Figure 3.4: Hazard Ratios for Pneumonia in the 30 Days Following Influenza and Subtype Among Participants Aged 0-4 Years*

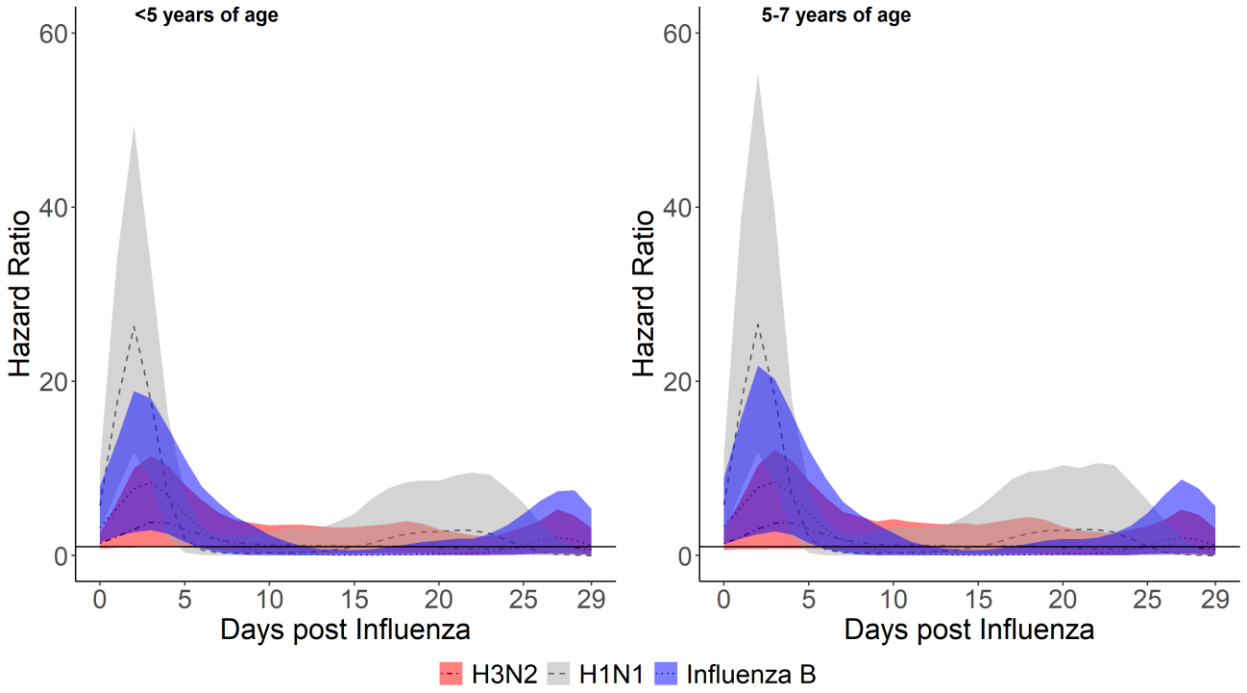


*Figure 3.5: Hazard Ratios for Pneumonia in the 30 Days Following Influenza by Subtype Among Participants Aged 0-4 vs. 5-7 Years*

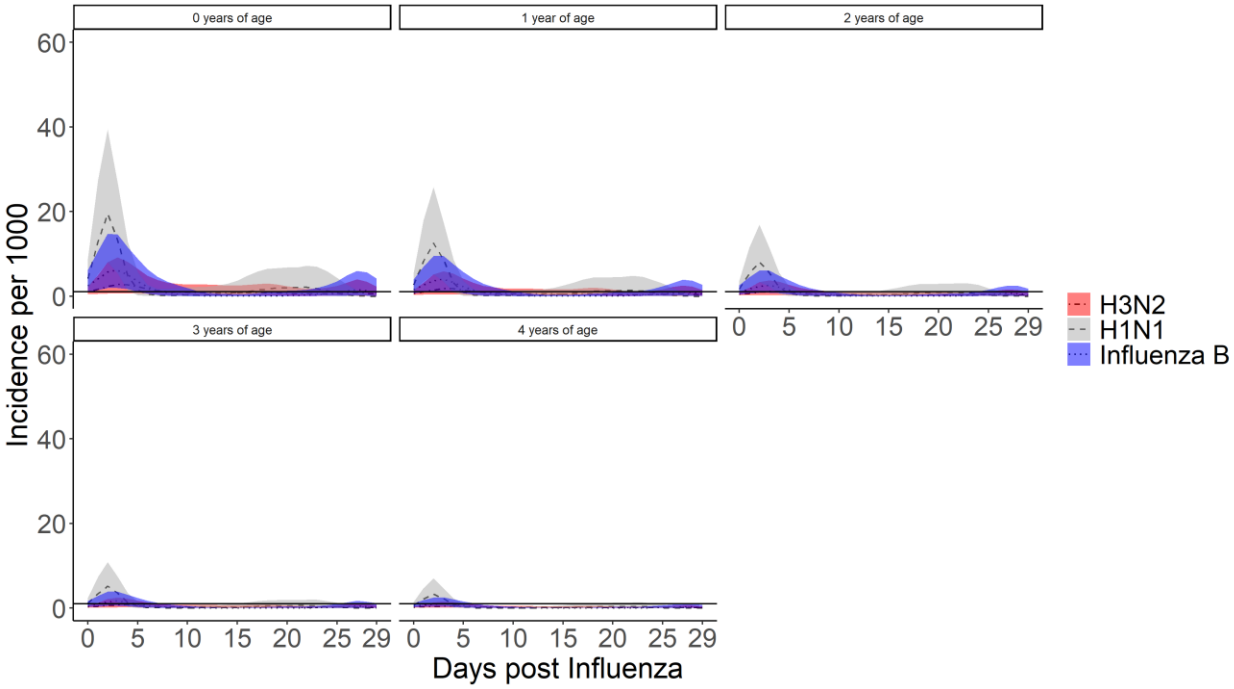


*Figure 3.6: Incidence Rate of Pneumonia per 1000 in the 30 Days Following Influenza Infection Among Participants Aged 0-5 Years*

*Table 3.5: Matched Odds Ratios of Developing Pneumonia in the 30 Days Following Influenza Infection by Week*

| | Matched Odds Ratio (mOR) | 95% Confidence Interval | p-value |
|---|---|---|---|
| Male | **1.6** | **(1.4, 1.8)** | **<0.001** |
| Influenza 0-6 days prior | **8.3** | **(4.8, 14.5)** | **<0.001** |
| Influenza 7-13 days prior | 0.9 | (0.4, 2.3) | 0.8 |
| Influenza 14-20 days prior | **2.5** | **(1.1, 5.5)** | **0.03** |
| Influenza 21-30 days prior | 0.9 | (0.4, 2.0) | 0.7 |

[a]Results obtained from model 2

# Chapter 4 Exploring the Risk Period for Pneumonia Following Symptomatic Influenza Infection Among Nicaraguan Children Before and After the Introduction of the Pneumococcal Conjugate Vaccine

## 4.1 Author summary

Participants with symptomatic influenza had greater odds of developing subsequent pneumonia in the subsequent 30 days when compared to participants without influenza. This was true for all influenza types/subtypes except the pre-pandemic strain of H1N1. The odds of developing pneumonia following infection with a specific influenza subtype were largely consistent across the pre- and post-PCV cohorts. The same was true of the risk period for pneumonia, with the odds of developing pneumonia highest in the first two weeks following influenza infection. The risk period was different than that described in Chapter 3 which may suggest an age effect we were underpowered to detect.

## 4.2 Abstract

Influenza is associated with primary viral and secondary bacterial pneumonias; however, the dynamics of this relationship in populations with varied levels of pneumococcal vaccination remain unclear. We conducted nested matched case-control studies in two prospective cohorts of Nicaraguan children aged 2-14 years: one before PCV introduction (2008-2010) and one following its introduction and near universal adoption (2011-2018). The association between influenza and pneumonia was similar in both cohorts. Participants with influenza (across types/subtypes) had higher odds of developing pneumonia in the month following influenza

infection. These findings underscore the importance of considering influenza in interventions to reduce global pneumonia burden.

## 4.3 Introduction

Influenza is an important cause of pneumonia, with evidence suggesting that this occurs both directly via primary viral pneumonia and indirectly via secondary bacterial pneumonias caused by pathogens including *Streptococcus pneumoniae* and *Staphylococcus aureus*.[68, 69, 77, 104] Globally, the introduction of pneumococcal conjugate vaccines has been associated with decreased pneumonia morbidity and mortality.[74, 75] However, the effect this might have on influenza-associated pneumonias requires examination.

The pneumococcal conjugate vaccine (PCV13) was introduced to Nicaragua in December 2010, and by the end of 2012, nearly 100% of children were appropriately vaccinated for their age.[75] In this analysis, we conducted a nested, matched case-control study within a cohort of Nicaraguan children aged 2-14 years followed from June 2007-December 2010 to assess the risk period for influenza-associated pneumonias in a population largely unvaccinated for pneumococcus. We repeated these analyses within another cohort of children from the same community from January 2011-December 2018 (where PCV13 coverage was ~100% for children in the target age-groups) to describe the association between influenza and pneumonia before and after PCV13 was introduced.

## 4.4 Methods

### 4.4.1 Ethics statement

This study is a collaboration between the Sustainable Sciences Institute, the Nicaraguan Ministry of Health, the University of California, Berkeley (UCB), and the University of

Michigan (UM). The study was approved by the Institutional Review Boards (IRBs) of the Nicaraguan Ministry of Health, UCB, and UM. Written informed consent was obtained from a parent/guardian of all participants. Verbal assent was obtained from children aged ≥6 years.

### 4.4.2 Study population and sample collection

This analysis used data from two prospective cohort studies of Nicaraguan children aged 2-14 years.[111, 122] The first, conducted from June 2007 through 2010, is hereafter referred to as the pre-PCV cohort, and the second, conducted from January 2011 through 2018, is hereafter referred to as the post-PCV cohort. The methods of both studies have been described in detail previously.[111, 122] Briefly, participants were enrolled from District II of Managua, Nicaragua, at Health Center Sócrates Flores Vivas (HCSFV). A detailed sociodemographic survey was collected upon enrollment and yearly thereafter for the duration of the child's participation. A clinical history was also collected on enrollment and was updated any time the child came to the HCSFV. Healthcare was provided to all study participants, and study nurses and physicians were available at the HCSFV 24 hours/day, 365 days/year. Parents agreed to bring their child to the clinic any time they were sick, and in particular, when they had a recorded fever or were "feverish."

Upon presenting at the study clinic, participants were assessed for influenza-like illness (ILI) defined as: fever or reported fever and rhinorrhea, cough, or sore throat. In the pre-PCV cohort, respiratory samples for influenza testing were obtained from a random sample of 25% of participants presenting with these symptoms using nasal and oropharyngeal polyester-tipped plastic swabs.[122] In the post-PCV cohort, samples were obtained from all participants meeting the testing criteria.[111] Episodes of clinical pneumonia were diagnosed by study physicians using guidelines based on the Integrated Management of Childhood Illness (IMCI).[113]

Pneumonia onset was considered to be date of diagnosis, while the start of symptoms reported with an influenza-positive episode was considered to be the date of influenza onset.

### 4.4.3 Laboratory methods

The QIAamp Viral RNA Mini Kit (Qiagen) was used to extract RNA from swabs, which was then tested for influenza A and B by RT-PCR following the Centers for Disease Control and Prevention (CDC) protocol. Samples positive for influenza A were subtyped (H3N2, H1N1, or H1N1pdm09). Samples were not routinely tested for other pathogens.

### 4.4.4 Statistical analysis

Cases of clinical pneumonia were matched on age (months) and week of study to up to four controls in their respective cohorts. Participants with no episodes of clinical pneumonia in the previous 45 days were eligible to serve as controls.

Conditional logistic regression models were fit to assess the relative odds of clinical pneumonia in the 30 days post-influenza. Separate models were fit to examine the relationship between influenza subtype and subsequent pneumonia (model 1), and the time-lag (0-6 days, 7-13 days, 14-29 days) between influenza and pneumonia (model 2) to ensure sufficient power.

Since only a random sample of pre-PCV cohort participants were tested for influenza, some influenza-positive participants may have been misclassified as influenza-negative. To achieve unbiased estimates, we repeated the previously described models with 100 complete datasets using multiple imputation methods described by Keogh et al.[123] Influenza subtype was imputed within each matched set for those who met the testing criteria and weren't sampled. Imputation models were fit using logistic or multinomial regression (if >1 subtype circulated) with sex and ILI as predictors. Age and seasonality were accounted for by conducting the

imputation within each matched set. All analyses were conducted using R version 3.6.3. Additional information regarding the statistical methods can be found in Appendix B.

### 4.5 Results

A total of 4517 and 2731 children participated in the pre-PCV and post-PCV cohorts, respectively. No participants were vaccinated for pneumococcus in the pre-PCV cohort, while nearly 100% of participants enrolled before their 2nd birthday into the post-PCV cohort were vaccinated with PCV13. Influenza vaccination also occurred in the post-PCV cohort, but at very low levels (<1% per year).

There were 1117 episodes of clinical pneumonia that occurred in the pre-PCV cohort and 314 in the post-PCV cohort (Tables 4.1, 4.2 and Figure 4.1). This disparity remained after standardizing the age distributions for comparison, with the incidence rate of all-cause pneumonia in the pre-PCV cohort 3.4 times that of the post-PCV cohort (Table 4.5). A greater proportion of all-cause pneumonias in the post-PCV cohort required hospitalization compared to the pre-PCV cohort (13.1% vs. 1.7%, p = <0.0001). However, the opposite was true for pneumonias occurring within 30 days of laboratory-confirmed influenza. In the pre-PCV cohort, 10.3% of influenza-associated pneumonias required hospitalization, while none in the post-PCV cohort required hospitalization (p=0.3).

Of the 1117 episodes of clinical pneumonia in the pre-PCV cohort, 39 (3.5%) were considered influenza-associated. There were 3 influenza A subtypes that circulated in the population during the study period (H3N2, H1N1, and H1N1pdm09), along with influenza B. H1N1pdm09 was most frequently associated with clinical pneumonia with 15 episodes (38.5%), followed by influenza B with 12 episodes (30.8%), H3N2 (9 episodes, 23.1%), and H1N1 (3 episodes, 7.7%).

There were 21 (6.7%) episodes of influenza-associated pneumonia in the post-PCV cohort. Influenza B was the most commonly observed with 9 (42.9%) episodes, followed by H3N2 and H1N1pdm09, each with 6 (28.6%) episodes.

### 4.5.1 Influenza subtype and subsequent pneumonia

In both the pre- and post-PCV cohorts, participants with symptomatic H3N2, influenza B, or H1N1pdm09 infections displayed higher odds of pneumonia in the 30 days following influenza onset compared to participants without influenza illness (Table 4.3). Odds of subsequent pneumonia associated with specific influenza types/subtypes were similar in both the pre- and post-PCV cohorts. The importance of male sex differed between the cohorts, as it was associated with 30% higher odds of developing pneumonia in the post-PCV cohort, but showed no difference in the pre-PCV cohort.

### 4.5.2 Characterizing the risk period for pneumonia following influenza

We also examined how the odds of pneumonia following symptomatic influenza changed over time, specifically in the periods 0-6, 7-13, and 14-29 days following influenza onset. In the pre- and post-PCV cohorts, we observed the greatest odds of pneumonia in the first 0-6 days after influenza (Table 4.4). The odds that participants with any symptomatic influenza infection would develop pneumonia in the first 0-6 days were 11.3 times (95% CI: 5.0, 25.4), and 63.8 times (95% CI: 8.2, 498.5) that of participants without influenza in the pre- and post-PCV cohorts respectively. We observed similarly elevated odds of pneumonia in the 7-13 days following influenza in both cohorts, specifically 4.1 times (95% CI: 1.3, 13.1) and 9.6 times (95% CI: 1.2, 75.1) that of participants without influenza in the pre- and post-PCV cohorts. Finally, for the period 14-29 days post-influenza, which we hypothesize to be the risk period for

bacterial pneumonia, the mOR in each cohort were similar, and slightly but non-significantly greater than 1 (Table 4.4).

## 4.6 Discussion

In this study, we described the risk period for developing clinical pneumonia following symptomatic influenza infection within cohorts of Nicaraguan children both before and after the introduction of pneumococcal conjugate vaccines (PCV). Notably, we did not observe a difference in the odds of developing pneumonia in the 30 days post-influenza following the introduction of PCV13. There were, however, substantially more episodes of pneumonia in the pre-PCV cohort. Influenza-associated pneumonia episodes also tended to be more severe in the pre-PCV cohort, with 10.3% requiring hospitalization compared to 0% in the post-PCV cohort.

The literature exploring the risk period for pneumonia following influenza at an individual level remains limited. A 2018 review by Li et al. found only two individual-level studies exploring pneumococcal disease following influenza, and both had substantial limitations.[50, 51, 109] A matched case-control study from 2000 reported that patients hospitalized with severe pneumonia were more likely to have positive convalescent serology (titer $\geq$ 1:40) for H1N1 than non-ill controls (mOR: 3.7, 95% CI:1.0, 18.1).[50] While we did not see a significant association between H1N1 and subsequent pneumonia, this difference may be attributable to our use of RT-PCR for influenza diagnosis, a more reliable indicator of acute infection than convalescent serology.

We recently published an analysis assessing the risk period for pneumonia following influenza in a broader cohort of Nicaraguan children from which the post-PCV cohort in this analysis was drawn.[124] The only difference is that in this analysis, children aged <2 years were excluded to make the pre- and post-PCV cohorts comparable. In that analysis, we similarly

observed that influenza, regardless of type/subtype, was associated with increased risk of developing pneumonia in the next 30 days. There were some (non-significant) differences in which subtype was associated with the highest risk of pneumonia, and in the apparent time-trend of pneumonia risk. This may suggest that the risk period differs for children aged <2, as the majority of influenza-pneumonia cases in our previous analysis occurred in this age group. However, the wide and overlapping confidence intervals also indicate we are underpowered to conclusively identify such differences.

This study has several strengths. First, as a case-control study nested within a prospective cohort, we were better able to ensure temporality between exposure and outcome. Second, while power limitations remained a challenge given the rarity of the outcome, this analysis had a larger sample size than much of the literature exploring the risk period for pneumonia following influenza, particularly at an individual level. Third, both cohorts were enrolled from the same Nicaraguan community (i.e., base population). This provided a unique opportunity to describe the nature of the influenza/pneumonia association both before and after the vaccine was introduced. Fourth, our use of multiple imputation to account for incomplete exposure ascertainment provides a reasonable approximation of the unbiased association between influenza and pneumonia in the pre-PCV cohort.

This study also has some limitations. First, in the pre-PCV cohort, not all participants presenting to the study clinic and meeting the sampling criteria were tested for influenza. Instead, a 25% random sample was obtained. However, through multiple imputation and additional bias analysis, we were able to confirm that our imputed values fell within the bounds of what we would expect for the unbiased estimate (Table 4.6). Second, as pneumonia is a rare outcome, sparse data limited the covariates we were able to include in our models. Third, our analysis did

not include children aged <2 years, the pediatric age group with the greatest burden of pneumonia. Finally, use of symptom-based criteria for clinical pneumonia to define cases limited our ability to make definitive distinctions between viral and bacterial etiologies. However, this is a common problem in pneumonia diagnosis, even with diagnostics like chest radiography or PCR.[61, 69]

In this analysis, we observed that influenza substantially increased the odds of pneumonia in the subsequent 30 days, and that the risk period for pneumonia following symptomatic influenza infection among children was similar before and after PCV13 was introduced to Nicaragua. This further underscores the importance of influenza in considering how best to reduce the global burden of pneumonia.

*Table 4.1: Characteristics of Participants and Pneumonia Cases in the Pre-PCV Cohort*

| | | All participants | Clinical pneumonia cases[a] | Influenza-associated pneumonia cases[a] |
|---|---|---|---|---|
| | | 4517 | 1117 | 39 |
| Age at enrollment (years)[b] | | 6.0 (2.9) | 4.3 (2.5) | 4.5 (2.6) |
| Person-years contributed[b] | | 3.0 (0.9) | 3.0 (0.8) | 3.2 (0.6) |
| Male | | 2274 (50.3) | 392 (51.9) | 13 (33.3) |
| Age | 24-59 months | -- | 545 (48.7) | 14 (35.9) |
| | ≥ 60 months | -- | 574 (51.3) | 25 (64.1) |
| Required hospitalization | | -- | 19 (1.7) | 4 (10.3) |
| Primary pneumonia[c] | | -- | -- | 25 (64.1) |
| Secondary pneumonia[d] | | -- | -- | 14 (35.9) |
| Influenza type | H3N2 | -- | -- | 9 (23.1) |
| | H1N1 | -- | -- | 3 (7.7) |
| | H1N1pdm09 | -- | -- | 15 (38.5) |
| | Influenza B | -- | -- | 12 (30.8) |

Data reflect number (column %) unless otherwise specified
[a]Column % reflects the number of participants
[b]Mean (SD)
[c]Within 7 days of influenza onset
[d]>7 days following influenza onset

*Table 4.2: Characteristics of Participants and Pneumonia Cases in the Post-PCV Cohort*

| | | All participants | Clinical pneumonia cases[a] | Influenza-associated pneumonia cases[a] |
|---|---|---|---|---|
| | | 2731 | 314 | 21 |
| Age at enrollment (years)[b] | | 4.6 (3.2) | 2.0 (2.8) | 2.0 (3.2) |
| Person-years contributed[b] | | 4.2 (2.7) | 5.3 (2.3) | 5.7 (2.3) |
| Male | | | 126 (54.1%) | 8 (42.1%) |
| Age | 24-59 months | -- | 224 (71.3%) | 15 (71.4%) |
| | ≥ 60 months | -- | 90 (28.7%) | 6 (28.6%) |
| Required hospitalization | | -- | 41 (13.1%) | 0 (0%) |
| Primary pneumonia[c] | | -- | -- | 14 (66.7%) |
| Secondary pneumonia[d] | | -- | -- | 7 (33.3%) |
| Influenza type | H3N2 | -- | -- | 6 (28.6%) |
| | H1N1pdm09 | -- | -- | 6 (28.6%) |
| | Influenza B | -- | -- | 9 (42.9%) |

Data reflect number (column %) unless otherwise specified
[a]Column % reflects the number of participants
[b]Mean (SD)
[c]Within 7 days of influenza onset
[d]>7 days following influenza onset

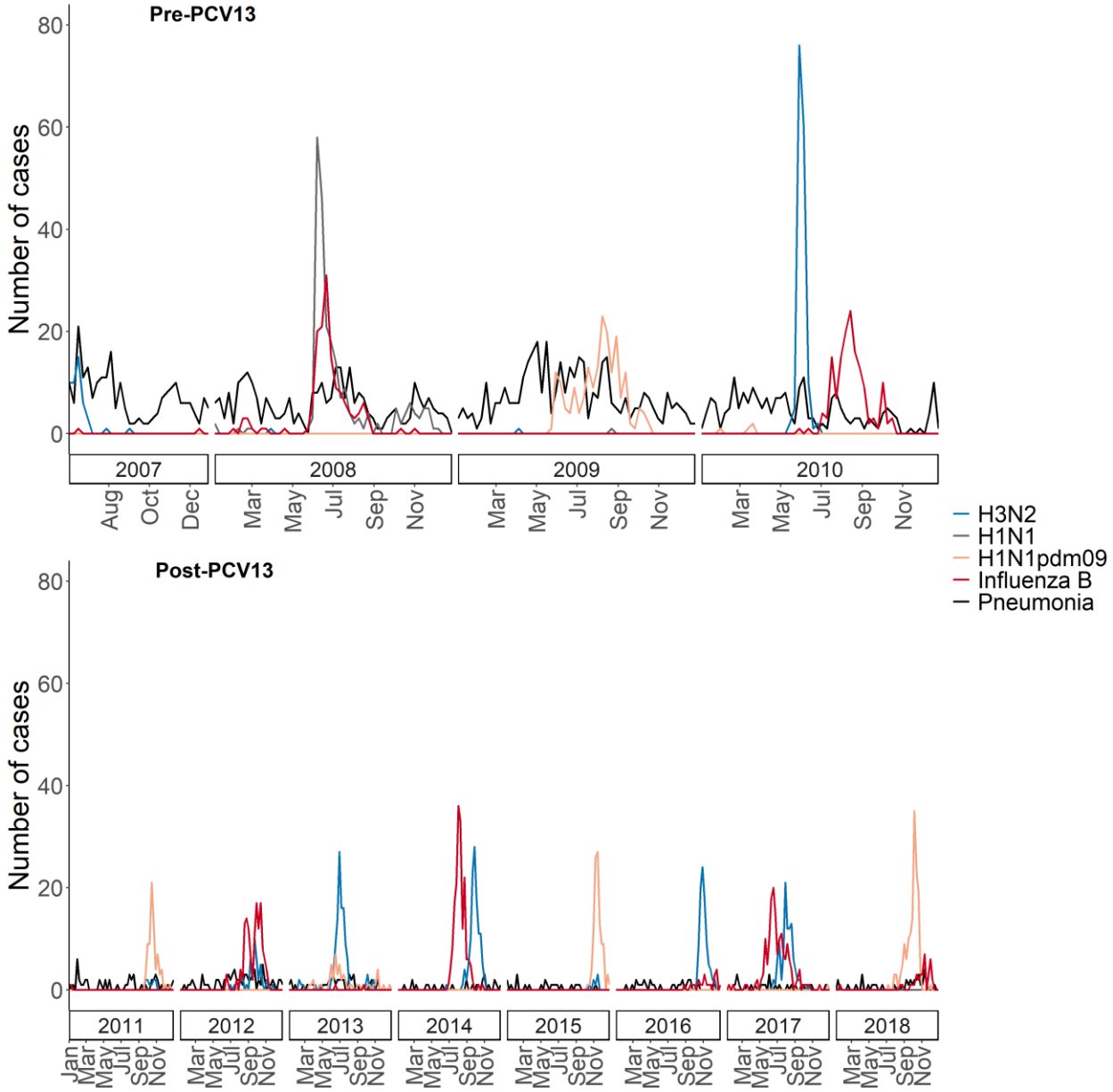


*Figure 4.1: Influenza Seasonality in the Pre-PCV and Post-PCV Cohorts*

*Table 4.3: Comparing Matched Odds Ratios (mOR) of Developing Pneumonia in the 30 Days Following Symptomatic Influenza Infection Among Nicaraguan Children Aged 2-14 Years*

| | Pre-PCV [a] | | | | Post-PCV | |
|---|---|---|---|---|---|---|
| | Crude analysis | | Imputation analysis | | | |
| | **mOR** | **95% CI** | **mOR** | **95% CI** | **mOR** | **95% CI** |
| **Male** | 1.0 | 0.8, 1.1 | 1.0 | 0.98,1.0 | 1.3 | 1.0, 1.7 |
| **Influenza (overall)** | 4.2 | 2.6, 6.7 | 6.3 | 5.4,7.3 | 8.5 | 3.9, 18.9 |
| **Influenza A** | 3.5 | 2.0, 6.1 | 5.0 | 4.1,6.1 | 8.1 | 2.8, 23.7 |
| **A/H3N2** | 7.3 | 1.8, 29.6 | 9.3 | 3.4, 34.0 | 23.6 | 2.7, 204.7 |
| **A/H1N1pdm09** | 5.3 | 2.4, 11.5 | 6.4 | 5.2, 13.5 | 5.2 | 1.5, 18.1 |
| **A/H1N1** | 1.0 | 0.3, 3.7 | 1.3 | 0.6 2.7 | --[b] | --[b] |
| **Influenza B** | 5.3 | 2.2, 12.7 | 11.7 | 6.7, 20.6 | 9.0 | 2.8, 29.4 |

[a]2007 excluded from analysis, see Appendix B

[b]The pre pandemic (2009) H1N1 strain has not circulated in Nicaragua beyond 2009

*Table 4.4: Assessing the Risk Period for Pneumonia in the 30 Days Following Symptomatic Influenza Illness Among Children Aged 2-14 Years*

| | Pre-PCV [a] | | | | Post-PCV | |
|---|---|---|---|---|---|---|
| | Crude analysis | | Imputation analysis | | | |
| | **mOR** | **95% CI** | **mOR** | **95% CI** | **mOR** | **95% CI** |
| **Male** | 1.0 | 0.9, 1.2 | 1.0 | 0.99,1.01 | 1.4 | 1.0, 1.7 |
| **0-6 days** | 11.3 | 5.0, 25.4 | 13.6 | 10.0, 18.4 | 63.8 | 8.2, 498.5 |
| **7-13 days** | 4.1 | 1.3, 13.1 | 5.9 | 3.2, 11.1 | 9.6 | 1.2, 75.1 |
| **14-29 days** | 1.5 | 0.7, 3.4 | 1.2 | 0.7,1.9 | 1.9 | 0.5, 6.6 |

[a]2007 excluded from analysis, see Appendix B

*Table 4.5: Age Standardized Incidence Rates of Clinical Pneumonia*

| | Pre-PCV cohort | | Post-PCV cohort | |
|---|---|---|---|---|
| | **Incidence Rate** | **95% CI**[b] | **Incidence Rate** | **95% CI**[b] |
| **Pneumonia** | 9.3 | 8.6, 9.7 | 2.7 | 2.5, 3.1 |

[a]Age distribution of post-PCV cohort used as reference for standardization
[b]95% confidence intervals obtained using a Poisson distribution

*Table 4.6: Sensitivity Analysis to Assess the Bounds of Effect Estimate in Pre-PCV Cohort*

| | | Lower bound[a] | | Multiple imputation | | Upper bound[b] | |
|---|---|---|---|---|---|---|---|
| | | **mOR** | **95% CI** | **mOR** | **95% CI** | **mOR** | **95% CI** |
| **Model 1** | **Male** | 0.99 | 0.9, 1.1 | 1.0 | 0.98, 1.0 | 0.99 | 0.8, 1.2 |
| | **Influenza (overall)** | 4.2 | 2.6, 6.7 | 6.3 | 5.4, 7.3 | 22.1 | 17.6, 27.7 |
| **Model 2** | **Male** | 1.0 | 0.9, 1.2 | 1.0 | 0.99, 1.01 | 0.99 | 0.8, 1.2 |
| | **0-6 days** | 11.3 | 5.0, 25.4 | 13.6 | 10.0, 18.4 | 56.8 | 40.3, 80.0 |
| | **7-13 days** | 4.1 | 1.3, 13.1 | 5.9 | 3.2, 11.1 | 20.3 | 13.7, 30.1 |
| | **14-29 days** | 1.5 | 0.7, 3.4 | 1.2 | 0.7,1.9 | 3.4 | 2.3, 5.0 |

[a]Assumed those with ILI who were not sampled/tested were influenza negative
[b]Assumed those with ILI who were not sampled/tested were influenza positive

# Chapter 5 Knowledge Added and Future Directions

A key principle of responsible research is to pursue questions that add to the collective knowledge in a given area. This is particularly important in epidemiology where research advances have the potential to dramatically improve health and well-being in society. In this chapter I will discuss what knowledge was contributed by my dissertation research and discuss avenues that may be pursued in future research.

## 5.1 Aim 1

In chapter 2 we aimed to answer some of the most basic questions concerning the burden of RSV illness among children in Nicaragua. While RSV's important role in driving morbidity and mortality among young children has been repeatedly demonstrated [2, 7], substantial knowledge gaps remain.[27, 125, 126] RSV studies have largely been centered in hospital settings and high-income countries. Accurately characterizing the burden within communities and addressing geographic disparities is crucial to understanding the true scale of RSV's effects.[2] Prior to this study, our understanding of RSV in Nicaragua was largely anecdotal, and quite limited in scope. In describing the burden of RSV within a prospective birth cohort we were able to address three fundamental questions related to RSV in Nicaragua.

First, we were able to describe the relationship between age and incidence rates of RSV and RSV-associated severe illnesses like pneumonia and bronchiolitis. While children aged 6-11 months had the highest incidence of symptomatic RSV, the incidence of severe RSV peaked among those aged < 3 months and declined as age increased (Tables 2.3 and 2.5, Figures 2.2 and

2.4). This differed from estimates reported in other LMICs where the highest incidence of both RSV and severe RSV were generally among children aged < 6 months (Table 2.7). It was, however, consistent with other estimates from Latin America—particularly Guatemala.[35] A second key question we were able to explore was that of RSV seasonality in Nicaragua. Though transmission occurred year-round, there were regular, yearly periods of epidemic transmission (Figure 2.3). This has critical implications for the design of future vaccination campaigns—once a vaccine is developed. It can also help identify which children are at increased risk for severe RSV illness because of the timing of their birth. Third, and perhaps most striking, was RSV's role in driving infant mortality in the cohort. While the specific criteria used to attribute deaths to RSV remains a subject of debate [92, 96], at least 25% of illness deaths among cohort participants were associated with RSV infection. Though this represents a small proportion of the cohort, it is suggestive of a substantial burden in the base population from which the study was sampled.

The results of this analysis provide a strong foundation on which to continue building our knowledge of RSV in Nicaragua and other LMICs. Further, it emphasizes the importance of considering RSV prevention and mitigation in the continued efforts to reduce infant mortality. These efforts will hopefully soon be aided by the introduction of new preventatives, therapeutics, and even a vaccine. Still, there remain important questions regarding RSV in Nicaragua that have yet to be explored.

While Aim 1 characterized the burden of RSV and its severe manifestations, it did so for a relatively narrow age group—children under 2. This age group is of crucial importance given their greater frequency of severe RSV-associated illness, but it is far from the only group

affected. An important next step will be to describe the burden of RSV among older children and the elderly, providing a more comprehensive picture of the RSV burden in Nicaragua.

Our understanding of RSV seasonality is also incomplete as it has only been assessed over a small number of years. While we confirmed yearly periods of epidemic RSV transmission, assessing this trend over longer time scales will provide important data on how these seasonal epidemics may vary from year to year. Fortunately, continued RSV testing through other cohort studies in the same population will provide this additional data and will help more accurately characterize the seasonal patterns of RSV transmission in Nicaragua and Latin America as a whole.

There are also more specific questions that our general exploration of RSV burden did not address, particularly the burden of RSV subtypes and the dynamics of repeat infection. Future RSV testing to distinguish between RSV-A and -B could provide important insights related to subtype variability in transmission, illness severity, and even immunity, all of which would be helpful in informing the implementation of a future vaccine. Additionally, while we know that immunity to RSV is generally transient and allows for re-infection, we know very little about the frequency and risk factors associated with repeated RSV infection. By understanding what biological, social, and environmental factors may affect one's risk of re-infection we may be able to tailor prevention and mitigation efforts to those most at risk. Understanding such mechanisms may also inform implementation of a future vaccine by informing the need for (or timing of) booster doses.

### 5.2 Aim 2

In aim 2 of this dissertation we assessed the individual-level association between influenza and subsequent pneumonia and explored the temporal dynamics between them. While

the association between influenza and pneumonia has long been assumed, the evidence on which this assumption has been based largely on population-level correlations. Using a large prospective cohort of Nicaraguan children aged 0-14 years we were able to characterize the risk of developing pneumonia following symptomatic influenza infection at an individual level with precision not previously achieved.

Through this analysis we observed that children with symptomatic influenza infection were at substantially higher risk of developing pneumonia in the subsequent 30 days, compared to those without influenza. This was true across subtypes although the magnitude of the risk differed (Table 3.3, Figure 3.3). We also observed two distinct periods of elevated pneumonia risk following influenza, specifically in the first and third weeks following influenza infection (Table 3.5). This suggests the presence of distinct etiologic pathways connecting influenza and pneumonia. Further, it fits well with the hypothesis of influenza being associated with both primary viral and secondary bacterial pneumonias, as we would expect viral pneumonias to occur soon after influenza, followed by secondary bacterial pneumonias after a delay.

This analysis provides robust individual-level evidence of the association between influenza and pneumonia. This is important in its own right and could also help focus future studies and allow for greater precision in calculating effect estimates. Given the dearth of previous evidence we used relatively uninformed priors in our Bayesian survival model. Future studies of the relationship between influenza and pneumonia in new populations, can use more informed priors based on our results which may help to improve inferences.

The results in aim 2 were an important first step in assessing the individual-level association and temporal dynamics between influenza and pneumonia. There are, however, several avenues through which this research can and should be continued and expanded. First,

the cohort study used in this analysis has continued, so there are years of additional data that can be analyzed. Though Aim 2 represents the largest individual-level study of this question to date, pneumonia remained a rare outcome which limited statistical power. Assessing the relationship between influenza and pneumonia within larger datasets will help to increase statistical power and improve precision. Aim 2 was also largely intended to provide a descriptive baseline upon which future research can be built. Assessing potential risk factors for pneumonia following influenza may help create more informed public health interventions. Similarly, identifying factors that modify a child's risk period for pneumonia following influenza may help us better understand the mechanisms driving such an association. Finally, influenza is far from the only respiratory virus that has been linked with pneumonia. Characterizing the risk and temporal trends of pneumonia following viruses like RSV, human metapneumovirus, and now SARS-COV-2, is essential if we are to continue to reduce the burden of pneumonia worldwide.

### 5.3 Aim 3

In Aim 3 of this dissertation, we described the individual-level association between influenza and pneumonia within two cohorts of Nicaraguan children aged 2-14 years. One cohort was from before pneumonia conjugate vaccines were introduced to the country (pre-PCV), and the other after their introduction and near-universal adoption (post-PCV). By exploring this relationship in additional populations we continued to expand the published evidence in this important area and added further support to the findings from aim 2.

Much like aim 2 we conducted a nested matched case-control study in each cohort matching on age (months) and calendar time (study week) to account for confounding by age and seasonality. Conditional logistic regression models were fit to assess the odds of developing subsequent pneumonia after infection with specific influenza subtypes and how the odds of

pneumonia changed over time following influenza infection. In the pre-PCV cohort we also contended with incomplete exposure ascertainment as only a 25% random sample of children meeting the testing criteria were sampled/tested for influenza. However, using multiple imputation and additional sensitivity analyses we were able to estimate a reasonable approximation of the unbiased effect measures along with their upper and lower bounds (Table 4.5).

We again observed that influenza was associated with increased odds of developing pneumonia in the subsequent 30 days. This was true for all subtypes except for seasonal influenza A/H1N1. We also assessed the risk period for pneumonia following influenza, however, three time periods (0-6 days, 7-13 days, >13days post influenza) were used to assess the risk-period (instead of the four used in chapter 3) because of sparse data in the post-PCV cohort. Even so we can see some important differences in the results we observed in aim 2. Namely we saw significantly higher odds of developing pneumonia in the second week following influenza while we observed no such increased risk in this period in aim 2 (Tables 3.5 and 4.4). This elevated risk of pneumonia in the 2$^{nd}$ week following influenza illness was observed in both the pre- and post-PCV cohorts suggesting that the difference in observed risk periods may result from an age effect that we were underpowered to detect. The post-PCV cohort in aim 3 is after all simply a subset of the aim 2 cohort excluding those aged < 2 years to allow comparability to the pre-PCV cohort.

Aim 3 also explored the relationship between influenza and subsequent pneumonia, so many of the future research directions described for Aim 2 also apply. Identifying key risk factors for pneumonia following influenza, or those that affect the risk period could be particularly useful. Improving statistical power by increasing sample size and characterizing the

risk period for pneumonia following other viral infections is also important but would require use of data beyond the pre-PCV cohort. Still, the results of this analysis raise questions that should be explored further. While the risk period for pneumonia following influenza appeared largely similar in both the pre- and post-PCV cohorts we cannot unequivocally state that potential differences do not exist. It also remains unclear whether age may affect the risk period for pneumonia following influenza. Comparisons between the results from Aim 2 and the post-PCV cohort of Aim 3 would seem to suggest this, but we are underpowered to say so definitively. Considering the many populations with relatively low coverage of pneumococcal vaccines, it will be important to consider the relationship between influenza and pneumonia within populations that have different levels of vaccination.

### 5.4 Conclusions

In this dissertation we aimed to address some fundamental questions relating to RSV burden among Nicaraguan children, and to begin characterizing the relationship between influenza and subsequent pneumonia. By describing the incidence of RSV and the severe illnesses associated with it we added knowledge regarding its effects on a population particularly susceptible to it. In doing so we highlighted its importance in considerations of how to continue to reducing morbidity and mortality associated with acute respiratory infections. By describing the association between influenza and pneumonia within multiple prospective cohorts of Nicaraguan children we provided much needed individual-level data on the both the magnitude of the association and its timing. Such knowledge helps improve our understanding of the complex mechanisms that lead to severe respiratory illnesses like pneumonia. Each of these aims has opened new avenues of research to be pursued and it is our hope and belief that these results, and those that follow, will continue to reduce the toll exacted by acute respiratory infections.

# Appendices

## Appendix A: Supplemental Methods for Chapter 3

All statistical analyses were conducted using R version 3.6.1(R Foundation for Statistical Computing). Specifically the *brms* and *stan* packages were used for the Bayesian analysis, the *survival* package was used to run the conditional logistic regression models, and *ggplot2* and *tidybayes* were used to create figures.[114-117]

### Statistical Model

Here, we will describe a model to predict the risk of clinical pneumonia following influenza as a function of an observed time-varying background log-odds of pneumonia, denoted as $\gamma(t)$, log odds ratios $\beta$. We can then define the hazard of influenza-associated pneumonia for individual $i$ at time $t$ as: $log(\lambda_{it}) = log(h(\mathbf{x}_{it}; t)) = \gamma(t) + \mathbf{x}_{it}'\beta$.

### Preparing the data

Each individual *i* has a vector of covariates associated with his/her observation. These include sex, age at the time of observation, number of days following influenza infection, etc. To simplify fitting of the model without increasing the computational burden, we can take advantage of the fact that each vector of covariates is not unique, i.e. that it may be shared by multiple individuals. So, for every possible combination of covariates, denoted by the set **X**, we count up the number of matching vectors $x_i$ in the original dataset. We denote $\mathbf{x_j}$ to be the vector corresponding to covariate combination $j$, $N_j$ to be the number of observations $x_i = x_j$, and $y_j$ to be the number of cases of clinical pneumonia among individuals with parameters $x_j$. This is essentially fitting a discrete time model like those commonly employed in environmental and occupational epidemiology.

**Model likelihood**

To fit the model to the data prepared as described in the section above, we define the probability of pneumonia for individuals with covariate combination $j$ as $p = 1 - exp(-\lambda_{it})$ where $\lambda_{it} = exp(\gamma(t_j) + \mathbf{x}'_j\beta_j)$, and then model the probability of observing $y_j$ clinical pneumonia among the $N_j$ individuals with parameters $\theta_j$ as $y_j \sim \text{Binomial}(N_j, p_j)$. This is the equivalent of using a complementary log-log (cloglog) link function. Using the cloglog link allows for the calculation of hazard ratios from the model.

**Defining the model in brms**

To estimate the model defined above in brms, we first expand each participant interval in the original data into a long-form dataset showing days of participation in study. Using dplyr, we group by unique combinations of covariates and time periods (calendar time and time exposed), and then sum up the total number of individuals in each group, as well as the total number of clinical pneumonia cases.

Using the cloglog link we can employ the linear syntax in brms as follows:
we can define the *likelihood* in terms of the covariates impacting the log-hazard (in this case just denoted lambda), and then translate this into the value of $p_{it}$ needed to estimate the probability of observing $y_{jt}$ pneumonia cases among the $N_{jt}$ susceptible individuals in the cohort on that day with parameter combination $j$:

```
model_f3 <- bf(pneumo_tot|trials(tot_exposed) ~
          offset(lde) + sexo + age_y + s(days_pflu_1, by  = o_sub) + s(study_month))
```

In the code above, s(study_month) s(days_pflu_1, by = o_sub) instructs brms to use a spline to model smooth variation in clinical pneumonia rates as a function of study month and days post influenza. The inclusion of by = o_sub in the spline term for days post influenza tells

brms to condition fitting the spline term on an ordered factor variable, o_sub, for influenza type. This allows the smoothed rate of clinical pneumonia to both vary by subtype and avoid being applied to instances where participants did not have an influenza infection. Finally, offset (lde) functions as a measure of the log person time at risk per year, *log(days_exposed/365)*.

```
m3e <- brm(model_f3,
           data = d_day,
           chains = 4,
           iter = 6000,
           warmup = 3000,
           family = binomial("cloglog"),
           set_prior("normal(0, 1)", class = "b"),
           control = control_pars1
)
```

## Appendix B: Supplemental Methods for Chapter 4

Multiple imputation was performed within each matched set using the *mice* package in R.[127] These methods and example code have been described in detail in Keogh et al. 2018.[123] Influenza subtype was imputed separately for each year to restrict possible imputed values to those circulating that year. Subtypes that occurred rarely in a given season (<5% of influenza positives) were excluded as possibilities from the imputation due to insufficient data. The 2007 influenza season was dropped from the imputed analysis as the imputation failed to converge for that year. In 2008 and 2010 when multiple influenza subtypes circulated in the population multinomial logistic regression was used as the imputation model. In 2009, when only H1N1pdm09 circulated, logistic regression was used as the imputation model.

As an additional sensitivity analysis, we fit models where we assumed that any participant presenting with influenza-like illness was flu positive. This provided an upper bound for what the unbiased effect estimates could be, while the models where we assumed those presenting with ILI who were not sampled/tested provide the lower bound (Table 4.5).

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