Influenza Susceptibility and Transmission

by

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PREFACE

Chapter II (**Neuraminidase antibodies are associated with shortened influenza A(H1N1)pdm viral shedding and illness in naturally infected adults**) has been submitted for publication. The author list is: Hannah E. Maier, Raffael Nachbagauer, Guillermina Kuan, Sophia Ng, Roger Lopez, Nery Sanchez, Daniel Stadlbauer, Lionel Gresh, Amy Schiller, Arvind Rajabhathor, Sergio Ojeda, Andrea F. Guglia, Fatima Amanat, Angel Balmasedae, Florian Krammer, and Aubree Gordon.

Chapter III (**Obesity Increases the Duration of Influenza A Virus Shedding in Adults**) has been published in the Journal of Infectious Disease. The author list is: Hannah E Maier, Roger Lopez, Nery Sanchez, Sophia Ng, Lionel Gresh, Sergio Ojeda, Raquel Burger-Calderon, Guillermina Kuan, Eva Harris, Angel Balmaseda, and Aubree Gordon [52].

Chapter IV (**Obesity and influenza A susceptibility in households in Managua, Nicaragua**) will be submitted for publication. The author list will be Hannah E. Maier, Guillermina Kuan, Roger Lopez, Nery Sanchez, Lionel Gresh, Amy Schiller, Sergio Ojeda, Eva Harris, Angel Balmaseda, and Aubree Gordon.

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LIST OF ABBREVIATIONS

CDC Centers for Disease Control and Prevention

NIAID National Institute of Allergy and Infectious Disease

WHO World Health Organization

NCHS National Center for Health Statistics

LMIC Lower and Middle Income Country

NPICS Nicaraguan Pediatric Influenza Cohort Study

HITS Household Influenza Transmission Study

HICS Household Influenza Cohort Study

HCSFV Health Center Sócrates Flores Vivas

CNDR Centro Nacional de Diagnóstico y Referencia

COPD Chronic Obstructive Pulmonary Disorder

LRTI Lower Respiratory Tract Infection

ALRI Acute Lower Respiratory Tract Infection

ARI2 Acute Respiratory Illness

ARI Acute Respiratory Illness

ILI Influenza-like Illness

A(H1N1)pdm 2009 influenza A(H1N1) swine flu pandemic

HAI hemagglutination inhibiting

ELISA Enzyme-Linked Immunosorbent Assay

HA hemagglutinin

NA neuaraminidase

RT-PCR Reverse Transcription Polymerase Chain Reaction

RNA Ribonucleic Acid

AUC area under the curve

BMI Body Mass Index

AFT Accelerated Failure Time

ETR Event TIme Ratio

CI Confidence Interval

AIC Akaike Information Criterion

MCMC Markov chain Monte Carlo

HR Hazard Ratio

ABSTRACT

Influenza causes substantial burden in terms of illness, lost productivity, hospitalization, and death worldwide, with the highest burden in lower income countries. Vaccines are one of the best protective measures we have against influenza, but current seasonal influenza vaccines offer suboptimal protection, and are strain-specific necessitating yearly vaccine updates and offering no protection against potential pandemic strains. Identifying risk factors for influenza susceptibility and disease severity is important to be able to effectively target prevention and treatments to lower the burden and reduce transmission, particularly in low-resourced settings.

This dissertation looks at immune factors that could improve next generation vaccines in chapter II and obesity as a risk factor for influenza outcomes in chapters III and IV. The work presented here uses data from studies of household influenza transmission in Managua, Nicaragua. I used Accelerated Failure Time (AFT) models (chapters II and III) and logistic regression (chapter IV) to investigate these questions.

In chapter II, we found that pre-existing neuaraminidase (NA) antibodies were associated with decreased viral shedding duration. This suggests that increasing NA immune response could improve future influenza vaccines by decreasing influenza disease duration and thus also decreasing transmission. Chapters III and IV identified obesity as a risk factor for increased influenza A viral shedding duration, which can increase transmission, and increased susceptibility to influenza A(H1N1)pdm, respectively. Identifying obesity as a risk factor for increased influenza susceptibility and transmission may help target future preventive and therapeutic measures.

CHAPTER I

Introduction

1.1 Influenza

1.1.1 Burden

Influenza contributes to a large amount of death, illness, and economic loss worldwide. This burden can be thought of as a pyramid, with a small number of influenza-related deaths at the top, and increasing numbers of hospitalizations, severe and moderate illnesses, and finally a large unknown number of inapparent or asymptomatic infections making up the pyramid base (Figure 1.1).

Estimating the true burden of influenza is complex and challenging for a number of reasons, including co-circulation of other respiratory viruses, variations in symptoms, and non-standardized case definitions [36]. Influenza also contributes to disease burden in indirect (and often undetected) ways: increasing risk of secondary bacterial pneumonia, and causing complications or exacerbations of asthma, COPD, diabetes, cardiovascular disease, and other conditions [36, 93, 41, 20]. And beyond health burden, influenza also causes substantial economic burden in lost school and work days—total economic loss (direct medical costs and indirect costs) in US in 2015 was estimated at $11.2 billion ($6.3-$25.3 billion) [77].

A fraction of the influenza burden can be seen in the global estimates for 2017 of

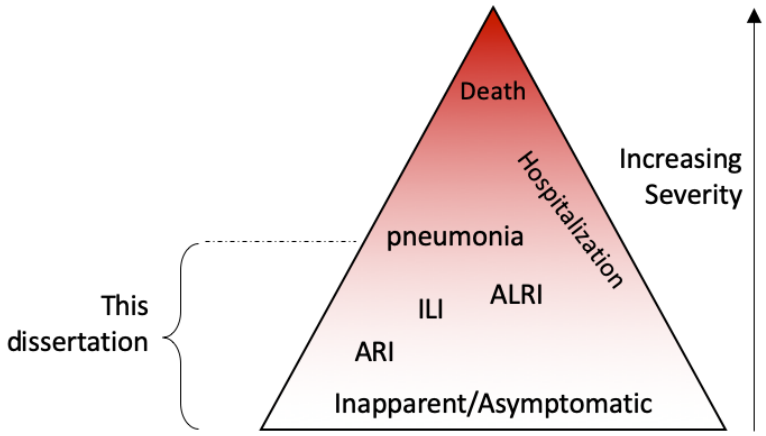

Figure 1.1: Influenza Burden Pyramid and the Focus of this Dissertation

influenza's contribution to Lower Respiratory Tract Infections (LRTIs), one of many measures of influenza burden [87]. This study estimated that influenza caused 145,000 LRTI-related deaths, 9.5 million hospitalizations (2.2 million in children under 5), and 54.5 million episodes of LRTI worldwide in 2017, and that influenza was a cause of 11.5% of all LRTI episodes [87]. For children under 5 worldwide, another study estimated 28,000-111,500 deaths due to influenza-associated Acute Lower Respiratory Tract Infections (ALRIs) in 2008 and 99% of these deaths occurred in developing countries [63]. Respiratory infections in general (upper or lower tract) are among the leading causes of death, especially in lower income countries [19], and pneumonia, which influenza can cause directly and through secondary bacterial infections, is the leading communicable cause of death in children under 5 [51]. Influenza disease burden is made up of much more than just lower respiratory tract infections however, and these burden estimates only scratch the surface of influenza's impact.

The burden described above reflects the seasonal influenza burden, which varies substantially from season to season. Additionally, influenza pandemics happen at unpredictable intervals (past pandemics include those in 1918, 1957, 1968, and 2009 [12]) and can cause a substantially higher burden—the 1918 pandemic is estimated to

have caused 40-50 million deaths globally (with an upper limit of 100 million deaths) [83].

This dissertation will focus on influenza outcomes towards the base of the influenza burden pyramid, an understudied area. In particular, I will focus on infections in a community setting, mostly non-hospitalized, and including asymptomatic infections. Understanding factors that contribute to influenza susceptibility, to both infection and disease, and influenza transmission is critical for the control of influenza. Reducing transmission of less severe influenza is important because it also prevents transmission to those at highest risk of severe outcomes.

1.1.2 Influenza Mutation and Control

Seasonal influenza vaccines are the best tool we have for preventing and limiting the severity of influenza [9], but they are far from perfect, with effectiveness over the past ten influenza seasons estimated at 10 to 60% overall, and lower at times for specific strains [13]. Consequently, the National Institute of Allergy and Infectious Disease (NIAID) recently put out a call to develop a universal influenza vaccine that would offer better and longer lasting protection against more influenza strains [29].

Part of the reason for low seasonal influenza vaccine effectiveness is that the current vaccine elicits protection to the influenza surface glycoprotein hemagglutinin (HA) head region, which has a high mutation rate. The mutations lead to *antigenic drift*, or the accumulation of genetic changes in the HA head, resulting in the need to update influenza virus vaccines twice a year (for northern and southern hemispheres). Predictions are not always accurate, meaning that vaccine strains do not always match the circulating strain, which is a major reason for low vaccine effectiveness. More abrupt genetic changes, called *antigenic shift*, can also occur, when genes from very different influenza viruses combine—often this is when avian or swine influenza combines with human influenza strains—these kind of abrupt changes have the potential

to cause pandemics because the new strains are different enough that a large part or all of the population does not have neutralizing antibodies to the strain [11]. Seasonal influenza vaccines do not protect against pandemic influenza strains.

Influenza immunity is shaped by prior history of exposure to influenza viruses and vaccine antigens [31, 17, 48]. Importantly, vaccines and natural infection elicit different types of immune responses, with current influenza vaccines focusing the antibody response to primarily just one of influenza surface glycoproteins, hemagglutinin (HA), whereas natural infection induces antibody response to both surface glycoproteins, HA and neuaraminidase (NA) [102]. This study population is largely unvaccinated for influenza as the vaccine is not widely available and is cost prohibitive in many Lower and Middle Income Countries (LMICs) [73], so immunity is a result of natural infection. Chapter II of this dissertation addresses questions of the effectiveness of pre-existing antibodies targeted to influenza virus surface glycoproteins at reducing viral shedding duration, with the goal of improving our understanding of the role of antibodies targeted to each surface glycoprotein in order to inform next-generation influenza vaccines.

In addition to vaccines, antivirals are also available to control and prevent influenza infections and are recommended for people at high risk of complications. Identifying risk factors for influenza infection and complications is essential for controlling influenza, allowing preventive and therapeutic measures to be focused on higher risk groups.

Chapters III and IV both focus on identifying risk factors for influenza disease—obesity in particular, a novel risk factor for less severe influenza outcomes—and look at two different influenza outcomes in response to obesity: viral shedding duration, and transmission.

1.2 Obesity

1.2.1 Global Trends

The global prevalence of obesity has nearly tripled since 1975. In 2016, about 13% of the world's adult population was obese (39% was overweight), and just over 18% of children age 5-19, and an estimated 41 million children under 5 were overweight or obese [94]. The prevalence of obesity has in the past been higher in higher income settings, but it is present and rising rapidly in every region, and in some places with conflict or food insecurity, it may exist as a dual burden of under- and over-nutrition, sometimes even in the same home [30].

Childhood obesity is associated with adult obesity and disability later in life, and premature death [94]. Obesity is a well-known as a risk factor for cardiovascular disease, diabetes, musculoskeletal disorders, and some cancers. These associations alone, given the dramatic increases in obesity prevalence, are cause for concern regarding obesity. But since the 2009 influenza A(H1N1) swine flu pandemic, A(H1N1)pdm, obesity has become recognized as a risk factor for severe influenza H1N1pdm outcomes and death, linking it now also with infectious diseases, and increasing its impact [92]. However, the evidence for obesity as a risk factor for influenza is still limited.

1.2.2 Height- and Weight-based Measures

Obesity was originally defined as the amount of fat associated with increased health risks [103], but as Body Mass Index (BMI) (kg/m^2) has become the more widely used measure of over-nutrition, the term obesity has also been applied to extreme measures of high weight-for-height BMI to classify obesity [70]. BMI tends to correlate well with fatness at the extreme values, but less well for middle values, as it cannot distinguish between muscle, fat, water, and bone. As such, BMI is a reasonably good measure of adiposity at the population level, but less well suited to

classify individual adiposity.

1.2.2.1 Children under 5

Children's heights and weights are compared to a reference growth chart to determine the child's weight status, because normal body fatness changes throughout childhood. While there is great variation in child growth around the globe, it is now understood that children, regardless of race or ethnicity, have the same potential for growth, at least until age 7, and that deviations from normal growth are driven primarily by environmental factors [24, 38, 98]. This understanding prompted the construction of the current WHO global childhood growth *standard* for ages 0-59 months, which comprises children from diverse geographic locations where at least 20% of the sample was raised in settings that promoted growth and lacked factors that restricted growth (i.e., they were healthy, breastfed for at least 12 months, their mothers did not smoke, and they came from advantaged homes within their communities). The data comes from children in in Brazil, Ghana, India, Norway, Oman, and USA and was collected from 1997-2003. The use of a *standard*, rather than a reference, theoretically allows judgements to be made on the nutritional status of a child or population (are the children growing as much as they *should*, if they had access proper nutrition and healthy environments) [23, 98, 95]?

With the use of the new World Health Organization (WHO) standard, it is important to note that breastfed infants have growth patterns that are quite different from those of primarily formula-fed infants; they have increased height and weight measures compared to formula-fed infants shortly after birth, but their height and weight measures fall below those of formula-fed infants during the latter half of infancy [95]. Breastfeeding exposure is typically categorized in a number of ways—studies have looked at any vs no breastfeeding, exclusive breastfeeding, and arbitrarily-chosen cut points of length of (exclusive) breastfeeding; in some cases, it may affect the child's

growth what other beverages are given (sugary juices, etc). In spite of the variability in the classification of breastfeeding exposure, a systematic review of the existing studies found that breastfeeding reduces the risk of obesity in childhood and that breastfeeding substantially decreases the risk of death from infection in the first two years of life [2]. While this dissertation did not adjust for breastfeeding, it may be an important confounder of obesity and influenza outcomes and future work should consider this.

1.2.2.2 Children 5-19

The current WHO growth reference for school-aged children (ages 5-19) still uses data collected for the 1977 NCHS/WHO reference, from non-obese children and adolescents in the United Stated surveyed between 1963-1974, but the curves were reconstructed in order to provide smooth transitions between the current WHO growth *standard* for children under 5 and the adult BMI cutoffs for overweight and obesity [25]. A limitation of this reference is that the US reference population is skewed overweight, leading to an underestimation of obesity when using the current reference. A *standard* population for this age group could provide a more accurate measure of obesity if it was based on a population reflecting healthy, unconstrained yet not excessive growth, but creating one would come with some additional difficulty as there are larger differences in growth across ethnic groups in these older children [4].

1.2.3 Cutoff Values

In adults, BMI values are used directly, and are categorized into the weight status classes underweight (BMI $<$ 18.5), normal weight (BMI 18.5 to $<$25), overweight (BMI 25.0 to $<$30), and obese (BMI $\geq$ 30). Cutoffs for the overweight and obese categories were originally established based on the association of BMI value and all-cause mortality, but the WHO report that established these cutoffs acknowledged

that the method used to determine cutoff points were largely arbitrary and based on data from mostly Europe and the USA, and mostly middle-age, middle class men [103]. Others have noted that meaningful BMI cutoffs vary by ethnicity; Asians, for example, have increased health risks at lower BMI values [80] and so cutoffs should be interpreted cautiously.

Cut points for defining weight status in children are statistically based, as a number of standard deviations away from the WHO standard or reference median value [94]. The cut points match well with adult BMI cut points for overweight and obesity: at 19 years, a BMI Z score ≥ 2 is equivalent to a BMI of 30. Childhood BMIs $\geq$ 95 percentile have also been shown to be associated with increased numbers of cardiovascular risk factors [Bogalusa]; a BMI Z score of 2 corresponds to the 97.7 percentile. As has been shown with adults [80], the relevant BMI or BMI Z score cutoffs for children may also vary by ethnicity.

1.3 Study Setting

As the burden of influenza is greatest among Lower and Middle Income Countries (LMICs), these are important places to study influenza and its risk factors. Nicaragua is a lower middle-income country in Central America, and Managua, its capital city, is the location of several completed and ongoing studies of influenza and other viruses. The studies our group has established in this region make use of the Health Center Sócrates Flores Vivas (HCSFV), the study health center that provides care to study participants. A great strength of these studies is that they have been established in the region for more than a decade and built up trust with the community, resulting in high participation levels [35, 34].

While the prevalence of obesity is lower in our study setting than in the US and other high income countries, roughly a quarter of adults were obese in Latin America and the Caribbean in 2017 [30], and our study setting had even higher adult prevalence

of about 40% [52]. Of note, the influenza vaccination rate is very low in this setting, and most of the study participants have not been vaccinated. Thus, this is a unique setting in which to study factors affecting influenza susceptibility and transmission independent of vaccine effects. Our population is an ideal population in which to investigate how naturally acquired immunity offers protection from influenza virus infection and disease and and to study immune responses to prior natural infection. While influenza vaccines are becoming more available, the cost is still prohibitive for lower- and middle-income countries [73], and our identification of obese individuals, a population that is prevalent and growing in many LMICs, as a high risk population can help influence policy and target vaccination.

This dissertation uses data from several of the studies in Managua, Nicaragua which are described in more detail in the subsequent chapters. The Household Influenza Transmission Study (HITS) is a case-ascertained study of household influenza transmission that was conducted from 2012-2017. Data from HITS is used in chapters II, III, and IV. The Household Influenza Cohort Study (HICS) is an ongoing (2017-current) study similar to HITS but nested within a cohort of households and is used in chapters III, IV, 5.3 and appendix A. After identification of an influenza positive index case and enrollment of their household members, both HITS and HICS have intensive monitoring periods lasting approximately two weeks where study staff visit the households every 2-3 days to collect nasal/oropharyngeal swabs to test for virus and symptom diaries that participants record daily. The Nicaraguan Pediatric Influenza Cohort Study (NPICS) is an ongoing (2011-current) cohort study of children aged 0-14 years and data from this study are used in chapter 5.3 and appendix A as a measure of the risk of influenza from the community [34].

1.4 Aims

The overall goal of this dissertation is to identify factors affecting influenza transmission and susceptibility in a tropical, low-resourced setting. In chapters II and III I focus on *transmission*, using influenza viral shedding duration as the outcome of interest. Viral shedding duration is directly related to transmission, as an indirect measure of how long a person can infect others. Chapter II examines pre-existing immunity resulting from prior influenza infections and chapter III examines obesity as a risk factors for influenza viral shedding duration. In chapter IV I examine the effect of obesity on *susceptibility* to influenza infection and disease.

CHAPTER II

Neuraminidase antibodies are associated with shortened influenza A(H1N1)pdm viral shedding and illness in naturally infected adults

2.1 Significance Statement

Influenza causes a large burden worldwide and seasonal influenza virus vaccines have limited effectiveness. Our goal was to inform future vaccine development by characterizing the protection associated with antibodies directed at the influenza virus surface glycoproteins. We identified influenza cases at the study health clinic in Managua, Nicaragua, invited their households to participate in the study, and monitored closely for virus transmission within the household. We then studied the association of specific types of pre-existing antibodies and influenza viral shedding and illness duration. We found that antibodies against neuraminidase were associated with significantly shortened viral shedding, and among adults they were also associated with shortened symptom duration. These results support neuraminidase as a potential target of next-generation influenza virus vaccines.

2.2 Abstract

Influenza causes a substantial burden worldwide, and current seasonal influenza vaccine has suboptimal effectiveness. To develop better, more broadly protective vaccines, a more thorough understanding is needed of how antibodies that target the influenza virus surface antigens, HA (HA; including head and stalk regions), and neuaraminidase (NA), impact influenza illness and virus transmission. We used a case-ascertained, community-based study of household influenza virus transmission set in Managua, Nicaragua. Using data from 170 RT-PCR-confirmed influenza virus A(H1N1)pdm infections and 45 household members with serologically-confirmed infection, we examined the association of pre-existing NA, hemagglutination inhibiting (HAI), and HA stalk antibody levels and influenza viral shedding and disease duration using Accelerated Failure Time (AFT) models. Among Reverse Transcription Polymerase Chain Reaction (RT-PCR)-confirmed infections in adults, pre-existing NA antibody levels of $\geq$40 were associated with a 69% (95%CI: 34%, 85%) shortened shedding duration (mean: 1.0 vs 3.2 days). NA antibody levels of $\geq$80 were associated with further shortened shedding and significantly shortened symptom duration (ILI: 82%, 95%CI: 39%, 95%). Among RT-PCR-confirmed infections in children, HAI titers of $\geq$1:20 were associated with a 35% (95%CI: 16%, 50%) shortened shedding duration (mean: 3.9 vs 6.0 days). Our results suggest that NA antibodies play a large role in reducing influenza illness duration in adults and may impact transmission, most clearly among adults. Neuraminidase should be considered as an additional target in next-generation influenza virus vaccine development.

2.3 Introduction

The burden of influenza is large, with an estimated annual global burden of 3-5 million severe cases and 290,000-650,000 deaths [97]. Current vaccine effectiveness is

only 10-60% [10] and new formulations are needed each year, prompting a new push to develop a more effective and longer lasting influenza vaccine suitable for all age groups [29, 72]. In addition to preventing disease, next-generation vaccines might also aim to reduce influenza symptoms and transmission. Thus, a better understanding of how antibodies affect both illness duration and viral shedding is needed. Influenza virus has two surface glycoproteins, hemagglutinin (HA) and neuaraminidase (NA). Current seasonal vaccines are designed to elicit antibody responses to the HA head but not specifically to NA or the HA stalk [75]. NA evolves more slowly than the HA head, which could allow NA-based immunity to protect against otherwise drifted strains, making it an attractive vaccine target [28]. NA antibodies are also suggested to limit influenza disease once a person has been infected [28], potentially lessening severity and decreasing transmission, but evidence of this association is limited [75, 49].

Human transmission studies in natural settings offer a unique opportunity to study the effects of pre-existing antibody titers on influenza infection and symptoms. The Household Influenza Transmission Study (HITS) is a case-ascertained study of natural influenza transmission in households in Managua, Nicaragua [37]. Here we assess the effects of pre-existing influenza A(H1N1)pdm hemagglutination inhibiting (HAI), HA stalk, and NA antibodies on viral shedding and symptom duration in children and adults.

2.4 Results

2.4.1 Study Population

Seven hundred and seventy-seven people in 161 households were enrolled in the HITS over the 2013 and 2015 influenza seasons. In 2013, both influenza A(H3N2) and A(H1N1)pdm circulated, and in 2015, A(H1N1)pdm predominated (Fig. 2.6). An average of 4.3 swabs and median 10 days of symptom diaries were collected

Table 2.1: Participant characteristics by analysis group and HAI titer

			RT-PCR+ only				RT-PCR+ & 4-fold HAI increase			
			HAI titer ≥40				HAI titer ≥40			
		level	no	yes	p	Total	no	yes	p	Total
Age	Total		148 (87.1)	22 (12.9)		170	184 (85.6)	31 (14.4)		215
0-14y	n (row %)		100 (84.7)	18 (15.2)		118	114 (83.8)	22 (16.2)		136
	Age (mean (sd))		5.6 (3.8)	8.0 (4.7)	0.02	5.9 (4.0)	5.8 (4.0)	8.2 (4.6)	0.01	6.2 (4.2)
	Sex (%)	F	46 (46.0)	9 (50.0)	0.95	55 (46.6)	53 (46.5)	10 (45.5)	1	63 (46.3)
		M	54 (54.0)	9 (50.0)		63 (53.4)	61 (53.5)	12 (54.5)		73 (53.7)
	Type of case (%)	index	61 (61.0)	12 (66.7)	0.85	73 (61.9)	65 (57.0)	13 (59.1)	1	78 (57.4)
		secondary	39 (39.0)	6 (33.3)		45 (38.1)	49 (43.0)	9 (40.9)		58 (42.6)
15+y	n (row %)		48 (92.3)	4 (7.7)		52	70 (88.6)	9 (11.4)		79
	Age (mean (sd))		38.0 (17.6)	22.5 (8.7)	0.09	36.8 (17.5)	38.3 (18.0)	31.6 (11.2)	0.28	37.5 (17.4)
	Sex (%)	F	36 (75.0)	2 (50.0)	0.62	38 (73.1)	52 (74.3)	7 (77.8)	1	59 (74.7)
		M	12 (25.0)	2 (50.0)		14 (26.9)	18 (25.7)	2 (22.2)		20 (25.3)
	Type of case (%)	index	3 (6.2)	1 (25.0)	0.71	4 (7.7)	3 (4.3)	1 (11.1)	0.94	4 (5.1)
		secondary	45 (93.8)	3 (75.0)		48 (92.3)	67 (95.7)	8 (88.9)		75 (94.9)

per participant. Index cases presented to the study health clinic on average 0.9 days from symptom onset. There were 91 A(H1N1)pdm index cases, all except 6 of whom were children aged 0-14 years, and a total of 196 RT-PCR-confirmed influenza A(H1N1)pdm infections. Two of the participants had co-infections with A(H3N2) and were excluded. Of the remaining, 170 had HAI results. An additional 45 had serologically-confirmed infections, for a total of 215 influenza virus infections included in these analyses (Table 2.1, Fig. 2.7).

2.4.2 Pre-existing Antibody Levels

In general, pre-existing antibody levels were low in participants with influenza A(H1N1)pdm infections. Only 15% of children and 8% of adults had HAI titers of $\geq$1:40, 9% of children and 51% of adults had HA stalk levels of $\geq$40, and 5% of children and 6% of adults had NA levels of $\geq$40 (Fig. 2.1A-C). Serologically-confirmed RT-PCR-negative infections (shown in dark purple—Fig. 2.1 and 2.4A-C) had higher antibody levels than RT-PCR-positive infections—geometric mean HAI titers: 1:12.5 vs 1:11.2, HA stalk and NA ELISA area under the curves (AUCs): 23.5 vs 13.2 and 12.63 vs 10.87, respectively (Table 2.1. Children with HAI antibody titers $\geq$1:40 were significantly older than those with lower titers (mean age of 8 vs 6 years), while adults with HAI antibody titers $\geq$1:40 were younger (mean age 23 vs 38 years, not significant, Table 2.1).

2.4.3 Pre-existing Antibodies and Shedding Duration in Adults with RT-PCR-Confirmed Infection

NA antibodies in adults were associated with decreased influenza shedding duration (Fig. 2.1C and F). An NA antibody level $\geq$40 (vs $<$40) was associated with a 69% decrease in shedding duration (adjusted ETR: 0.31, 95%CI: 0.15, 0.66, Table S1), with predicted mean shedding times of 1.0 vs 3.2 days (Fig. 2.2). Further, the

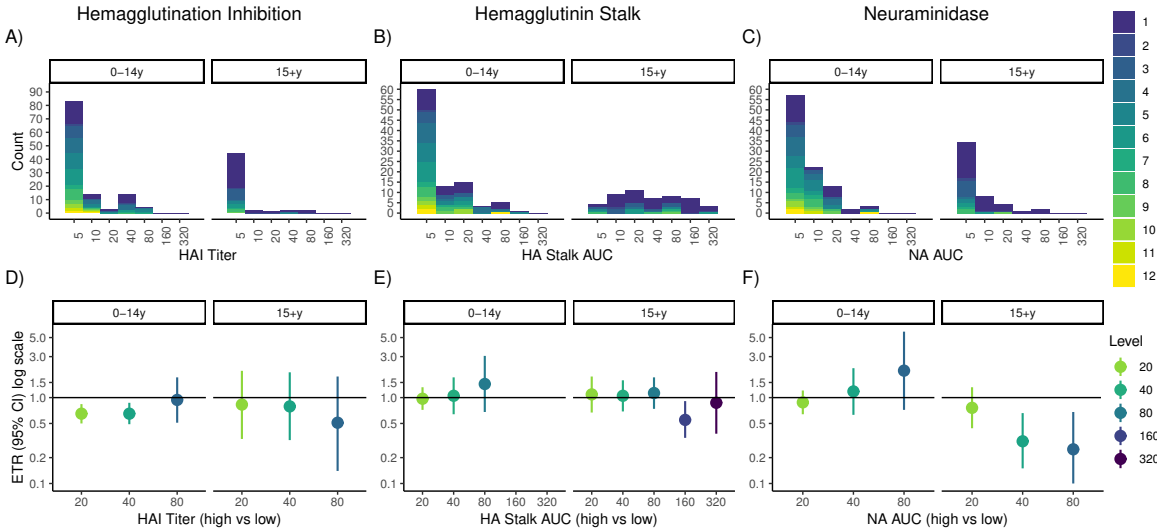

Figure 2.1: Pre-existing antibodies and influenza virus shedding duration among RT-PCR-positive infections. Top (panels A, B, C): histograms of hemagglutination inhibition (HAI), hemagglutinin (HA) Stalk, and neuraminidase (NA) antibody levels by age. AUC = area under the curve. Histograms are colored according to influenza shedding duration. Bottom (panels D, E, F): Event Time Ratios (ETRs) from accelerated failure time (AFT) models, adjusted for age and sex, compare the shedding duration in those with high ($\geq$threshold) vs low ($<$threshold) antibody levels. ETR <1 corresponds to a shorter duration in high antibody group.

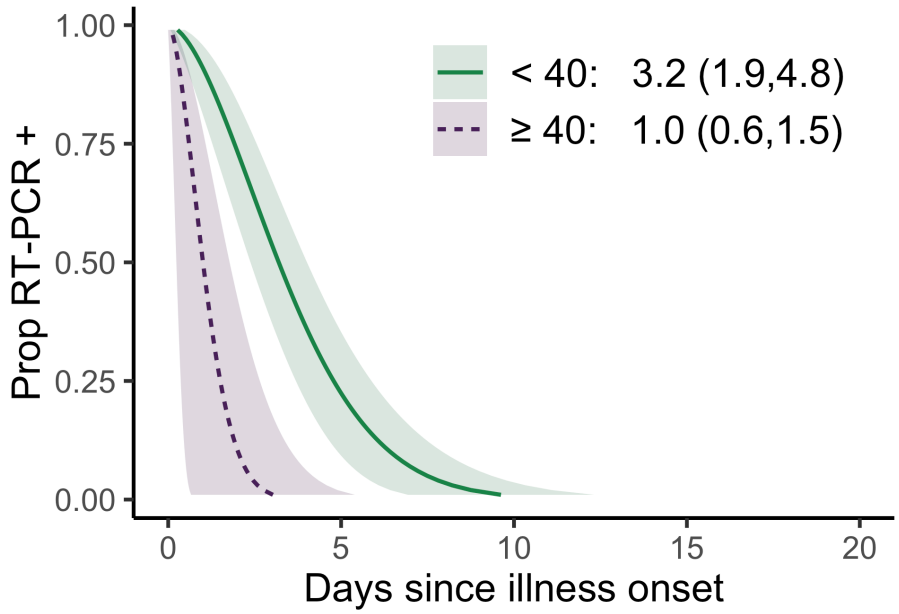

Figure 2.2: Predicted influenza virus shedding duration by high and low pre-existing NA antibody levels. Curves show the proportion of people still RT-PCR-positive (y-axis) each day since the start of shedding (x-axis), for an adult female of average age (36y). Mean and interquartile range (IQR) shedding times are presented in the legend.

relationship between NA antibody level and shedding had a dose-response pattern, with antibody levels ≥80 associated with further shortening of shedding duration. We observed no associations with HAI or HA stalk antibodies and shedding duration in adults (Fig. 2.1).

To assess whether NA antibodies in adults were independently associated with shortened shedding, the same models were adjusted for HAI and HA stalk antibodies (Fig. 2.3). After adjusting, the same dose-response relationship was seen in adults and an NA antibody level of ≥80 was still associated with significantly shortened shedding duration (ETR 0.22, 95% CI 0.05, 0.91, Fig. 2.3, Table S3).

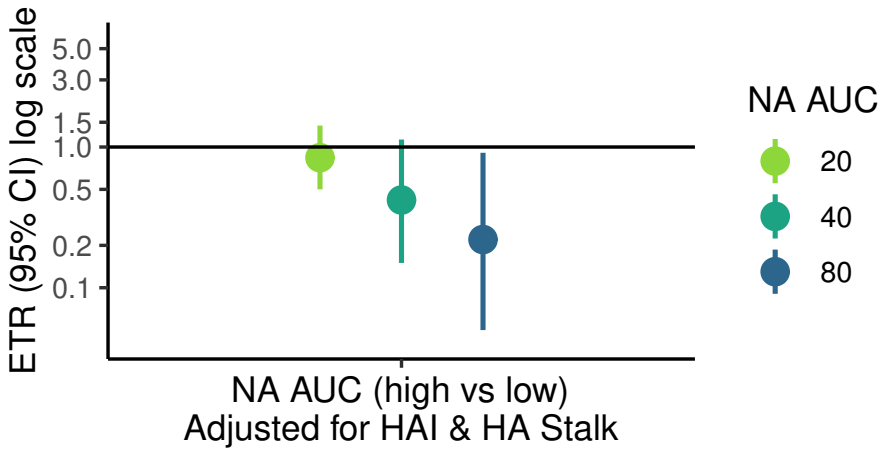

Figure 2.3: Pre-existing neuraminidase (NA) antibodies and influenza virus shedding duration among RT-PCR-confirmed infections, adjusted for HAI and anti-HA stem antibodies. Event Time Ratios (ETRs) from accelerated failure time (AFT) models compare the shedding duration in those with high (≥threshold) vs low (<threshold) NA antibody levels. All models are adjusted for (x-axis) age and sex, and antibody-adjusted models adjusted for HAI (titer ≥1:80) and HA stalk antibodies (area under the curve (AUC) ≥160).

2.4.4 Pre-existing Antibodies and Shedding Duration in Adults with RT-PCR- or Serologically-Confirmed Infection

When serologically-confirmed infections were included, all tested NA antibody levels (≥AUC 20) were associated with significantly shortened shedding duration and showed a strong dose-response relationship (Fig. 2.4F); an NA level of ≥20 was associated with a 49% decrease in shedding (adjusted ETR: 0.51, 95% CI 0.30, 0.87, Table S2), while an NA antibody level of ≥80 was associated with a 74% decrease in shedding duration (adjusted ETR: 0.26, 95% CI 0.1, 0.7). On including serologically-confirmed infections, an HAI titer of ≥1:160 was associated with significantly shortened shedding (Fig. 2.4D).

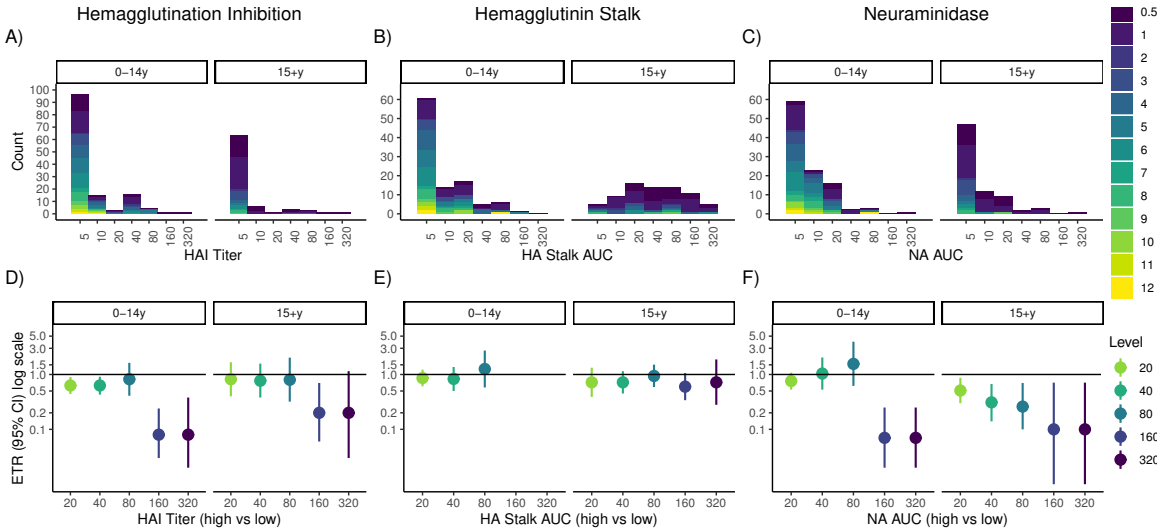

Figure 2.4: Pre-existing antibody levels and influenza shedding duration among RT-PCR-positive and serologically-confirmed infections. Top (panels A, B, C): histograms of hemagglutination inhibition (HAI), hemagglutinin (HA) Stalk, and neuraminidase (NA) antibody levels by age. Histograms are colored according to influenza virus shedding duration. Shedding duration was set to 0.5 days for serologically-confirmed, RT-PCR-negative individuals. Bottom (panels D, E, F): Event Time Ratios (ETRs) from accelerated failure time (AFT) models, adjusted for age and sex, compare the shedding duration in those with high ($\geq$threshold) vs low ($<$threshold) antibody levels. . ETR <1 corresponds to a shorter duration in high antibody group.

2.4.5 Pre-existing Antibodies and Shedding Duration in Children with RT-PCR-Confirmed Infection

In RT-PCR positive children, we did not observe an association between NA antibody level and viral shedding duration. However, HAI antibody titers of $\geq$1:20 and $\geq$1:40 decreased influenza shedding duration (Figure 2.1D). Indeed, shedding was reduced by 35% for children with HAI titers of $\geq$1:20 (adjusted ETR: 0.65, 95% CI: 0.50, 0.84, Table S1), with predicted mean shedding of 3.9 vs 6.0 days (Table S1). As with adults, we observed no associations with HA stalk and shedding duration in children.

2.4.6 Pre-existing Antibodies and Shedding Duration in Children with RT-PCR or Serologically-Confirmed Infection

When we included both RT-PCR and serologically-confirmed infections, NA antibody levels of ≥160 in children were associated with shortened shedding (Fig. 2.4F). Further, HAI titers of ≥1:160 were associated with significantly shortened shedding duration (no detectable shedding, Fig. 2.4D).

2.4.7 Excluding One Child with High Pre-existing Antibodies and 12 Days of Viral Shedding

A single child who had high pre-existing HA stalk and NA antibody levels and a shedding duration of 12 days was identified in the study (see Fig. 2.1B, C). In order to examine the effect of this one child on the association between antibody levels and viral shedding, we reran the analysis excluding the child. On exclusion, in RT-PCR confirmed infections, an NA antibody level ≥40 was significantly associated with shortened shedding (adjusted ETR: 0.50, 95% CI: 0.26, 0.96, Fig. 2.8).

When serologically-confirmed infections were included (Fig. 2.9), NA levels of ≥ 20 and ≥40 were significantly associated with shortened shedding duration in children (adjusted ETRs: ≥40 HA stalk 0.53, 95% CI: 0.32, 0.86, ≥20 NA: 0.63, 95% CI: 0.45, 0.89, Fig S4).

2.4.8 Pre-existing Antibody Levels and Symptom Duration among RT-PCR-Confirmed Infections

Among adult RT-PCR positive influenza cases, NA levels of ≥80 were associated with a shorter duration of ILI, cough, and runny nose (Fig. 2.5). An NA level of ≥80 was associated with shortening of symptom duration by 82% for ILI (ETR 0.18, 95% CI 0.05, 0.61), 92% for cough (ETR 0.08, 95%CI 0.01, 0.58), and 89% for runny nose (ETR 0.11, 95% CI: 0.02, 0.60). NA antibody levels were not significantly associated

with symptom duration in children.

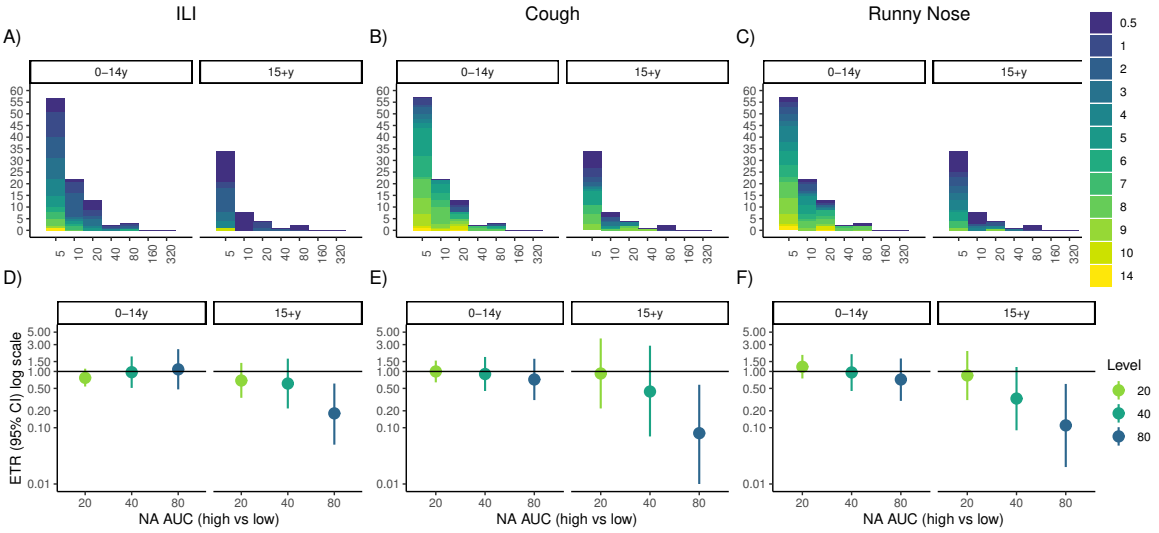

Figure 2.5: Pre-existing neuraminidase (NA) antibodies and symptom duration among RT-PCR-positive infections. Top (panels A, B, C): histograms of NA antibody levels by age. Histograms are colored according to duration of symptoms for influenza-like illness (ILI) (A), cough (B), and runny nose (C). Symptom duration was set to 0.5 days for individuals with no recorded symptoms. Bottom (panels D, E, F): Event Time Ratios (ETRs) from accelerated failure time (AFT) models, adjusted for age and sex, compare duration of ILI (D), cough (E), and runny nose (F) in those with high ($\geq$threshold) vs low ($<$threshold) antibody levels. ETR <1 corresponds to a shorter duration in high antibody group.

2.5 Discussion

We found that pre-existing NA antibodies were associated with shorter viral shedding duration in natural influenza A(H1N1)pdm infections in a community-based household transmission study. We also found that the association differed by age: in adults, the association was stronger and had a dose-dependent relationship with increasing NA antibody levels associated with even shorter shedding, while in children there was a threshold effect with only very high NA levels ($\geq$160) associated with shortened shedding. After adjusting for HAI and HA stalk antibodies, the relationship of NA antibodies and shedding duration in adults persisted. Further, NA

antibodies were also associated with shorter symptom duration in adults.

We observed that HAI antibodies were associated with shortened shedding duration in adults to a much lesser extent than NA antibodies, and with no dose-response relationship. However, it is important to note that there were very few influenza A(H1N1)pdm-infected adults with high HAI titers. In children, there was a threshold effect for HAI antibodies, with a small decrease in shedding duration for low titers, and no observed viral shedding for high HAI titers. We saw no association with HA stalk antibodies and shedding duration. Recently, in this study population, we identified HAI and HA stalk, but not NA antibodies, as independent correlates of protection from infection from influenza A H1N1pdm [66]. Taken together with our findings here, these results fit nicely with the mechanism of action of these antibodies laid out by molecular studies. HA antibodies prevent viral attachment and entry into host cells, while NA antibodies prevent viral particles from budding and spreading from host cells, and to some degree also prevent virus from leaving the mucosal entryways (reviewed in [49]). This last mechanism of action suggests that the importance of NA antibodies might vary by the route of infection.

Our findings advance those of previous human studies to establish the relationship of HA and NA antibodies and protection against influenza. A previous human challenge study among prisoners of an NA-containing vaccine found similar numbers of infections but less illness among the vaccinated [22], while several studies of natural infections in humans additionally found that NA antibodies correlated with protection from serologically-confirmed infection [60], and PCR-confirmed symptomatic infection [21, 61]. While our results that NA antibodies affect shedding duration but not infection risk appear to be discordant, it is important to note that studies of PCR-confirmed symptomatic influenza infections likely may have missed milder or asymptomatic influenza infections and would not have actually been able to differentiate between shortened shedding duration and protection from infection. It is

also important to note that because NA and HA stalk antibodies correlate, NA antibodies would appear to independently correlate with protection from infection in serologically-confirmed infections not adjusted for HA stalk antibody [66]. A recent human challenge study also found that NA antibodies were associated with shorter shedding and symptom duration, but limitations of this model include restricting to healthy adults, infecting intra-nasally with a high dose, and only seeing mild disease, less than in a community setting [57]. Another important difference between this study and previous studies [60, 57] is that antibodies in this study were measured in a binding assay using recombinant NA while previous studies used viruses expressing an N1 in combination with an H6 HA. While the H6 HA is not recognized by H1 HA head antibodies, stalk antibodies can bind to it and can also inhibit the NA by steric hindrance [101, 79]. It is therefore possible, that anti-HA stalk antibodies were also detected in neuraminidase inhibiting (NI) assays leading a composite correlate of protection driven by antibodies to the HA stalk and the NA. Since HA stalk antibodies were recently identified to correlate with protection from infection, it is reasonable that the NI titer could also correlate with protection from infection.

This study is the first to look at the effect of bona fide NA antibodies on shedding duration in children and in our main analyses we found a threshold effect with high NA associated with shorter influenza virus shedding duration. Because there were so few influenza A(H1N1)pdm-infected children with high NA levels, the one outlying child with high NA levels and very long viral shedding strongly influenced the results. On exclusion of this child, lower NA levels were also significantly associated with shorter shedding, and there was a dose-response relationship similar to that in adults. Thus, it is possible that children and adults might have the same association with NA and shedding duration, but the outlier combined with the small number of children with high NA levels prevented us from observing it. In addition it is possible that the difference in the association of NA antibody levels and shedding in children and

adults could be due to 1) glycoproteins evolving over time and epitopes of the first exposure shaping future immune responses—adults may have developed antibodies to different/more (and potentially more important) NA epitopes [17], and 2) adults might additionally have non-antibody-mediated immune responses that are important along with the NA antibodies [76].

We were limited by the number of infected participants we could observe with high levels of pre-existing antibodies because such levels can prevent infection. By including serologically-confirmed infections, we increased the number of observed infections and were able to capture individuals who may have shed virus too briefly for us to detect, although since we collected samples every two to three days, we could not distinguish between those who shed very briefly and those who did not shed virus.

The NA antibodies measured in this study resulted primarily from natural infections, because the vaccination rate is low in this setting, but we also know that current influenza vaccines containing both HA and NA favor an HA response and that natural infections have been shown to produce more NA antibodies [16]. Following the early human studies of NA as a correlate of protection [60], an NA-only vaccine was developed [46] which was then not pursued in part because the clinical endpoints used to evaluate the vaccine did not allow the development of symptoms [27], but interest in NA as a vaccine antigen is growing again with the rising need for improved seasonal influenza vaccine effectiveness [29, 72, 75, 28, 49].

Our results suggest that NA antibodies play a large and independent role in shortening influenza disease duration, in both children and adults. Increasing NA immunity could reduce influenza illness, severity, and transmission, and should be a priority for future influenza vaccines.

2.6 Methods

2.6.1 Ethics Statement

This study was approved by the institutional review boards at the Nicaraguan Ministry of Health and the University of Michigan. Informed consent or parental permission for minors was obtained from all participants. Assent was obtained for children aged 6 years and older.

2.6.2 Study Design and Population

The HITS study is a case-ascertained study of households in the catchment area of the Health Center Sócrates Flores Vivas (HCSFV) in District II of Managua, the capital of Nicaragua. Briefly, influenza index cases were recruited at the HCSFV and through the ongoing Nicaraguan Pediatric Influenza Cohort Study (NPICS) [34] and their households were invited to enroll. Inclusion criteria for index cases in HITS were 1) a positive influenza QuickVue A+B rapid test, 2) experienced onset of Acute Respiratory Illness (ARI) within the previous 48 hours, and 3) live with at least one other household member. Two seasons with influenza A(H1N1)pdm activity were included for this analysis: May-October 2013 and November-December 2015.

2.6.3 Data Collection

Demographic information and clinical history were collected at enrollment. Household members and index cases were monitored through home visits. Nasal and oropharyngeal swabs were collected at enrollment and every 2-3 days thereafter for a total of 5 visits over a period of 10-14 days. Daily symptom data was collected by study staff. Blood samples were collected from participants aged 6 months or older at enrollment and 30-45 days later. If participants sought care at the HCSFV while enrolled, data from the visit was recorded [34].

2.6.4 Laboratory Testing and Influenza Infection Definitions

Pooled nasal and oropharyngeal swabs maintained in viral transport media at 4ÂřC and blood samples were sent within 48 hours to the Nicaraguan National Virology Laboratory at the Nicaraguan Ministry of Health. Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed on RNA extracted from the swab samples per validated CDC protocols for influenza A(H1N1)pdm detection [96, 68].

Antibody levels were measured as HAI titers (reciprocals) and ELISA area under the curves (AUCs). HAI assays were performed following standard World Health Organization (WHO) protocols, using A/California/07/09 as an antigen [104]. An initial sample dilution of 1:10 was used and serial twofold dilutions were made in 96-well plates. HAI titers were determined by visual detection of red blood cell agglutination in wells.

ELISAs were performed to determine the quantity of HA stalk and NA antibodies on the subset of participants that had sufficient sample volume for both baseline and convalescent draws [40]. For HA stalk, a chimeric HA was used, consisting of the stalk domain of A(H1N1)pdm A/California/04/09 and the head domain from an H6N1 virus (which has not infected humans and to which no antibodies should be present in samples). For NA, the NA of A(H1N1)pdm A/California/04/09 was used. Both HA and NA were expressed as soluble proteins, maintaining correct protein folding, conformational epitopes, and enzymatic activity, as previously described [54, 102]. Areas under the curve (AUCs) were calculated with GraphPad Prism. All assays were performed by personnel who were blinded to the influenza status of samples. Geometric mean antibody levels were calculated from log2-transformed variables.

Influenza infections were defined in two ways: 1) as those RT-PCR-positive for influenza A(H1N1)pdm, and 2) as those RT-PCR-positive or serologically-confirmed for A(H1N1)pdm infection. Serologically-confirmed infections were defined as a $\geq$4-

fold rise in HAI titer [8]. Since household contacts were tested for influenza every 2-3 days, it is possible that individuals may have shed influenza virus that was not detected. The second definition allowed us to examine the potential effects of the limit of viral shedding detection due to sampling frequency. Outcome values for non-detected shedding and symptoms were set to a non-zero value of 0.5 days in the analysis.

2.6.5 Influenza Shedding and Symptom Duration

Influenza viral shedding duration was defined using the RT-PCR results of up to 6 samples, as the time from first RT-PCR-positive sample to shedding cessation. Because shedding cessation occurred in the interval between the last RT-PCR-positive and subsequent negative sample (interval censoring), it was defined by using the upper and lower bounds of this interval, as previously described [65, 52]. Symptom duration was defined as time from symptom onset to resolution of influenza-like illness (ILI), cough, and runny nose. ILI was defined as having fever with a cough or sore throat.

2.6.6 Statistical Methods

Parametric Accelerated Failure Time (AFT) models were used to calculate Event TIme Ratios (ETRs), as done previously [65, 52], comparing disease duration in influenza infections with high vs low pre-existing antibody levels. Weibull distributions were selected to account for censoring [86, 42]. Analyses were stratified by age, as children aged 0-14 years and adults aged 15-85 years and were adjusted for age and sex. A series of antibody cutoffs was used to categorize high and low HAI titers and HA stalk and NA ELISA AUCs: $\geq$1:20/20, 1:40/40, 1:80/80, 1:160/160, and 1:320/320 vs lower. Statistical analyses were conducted in R version 3.5.2 (), using the package "Survival" version 2.43.3 () to run the AFT models and to predict disease duration accounting for age and sex, "SurvRegCensCov" version 1.4 () to convert the

model output to ETRs, and "ggplot2" version 3.1.0 () for plotting.

2.7 Acknowledgements

We thank the many dedicated study personnel in Nicaragua at the Centro Nacional de Diagnóstico y Referencia and the Health Center Sócrates Flores Vivas. This work was supported by the National Institute for Allergy and Infectious Disease [U01 AI088654, R01 AI120997, contract numbers HHSN272201400006C and HHSN272201400008C].

2.8 Supplemental Information

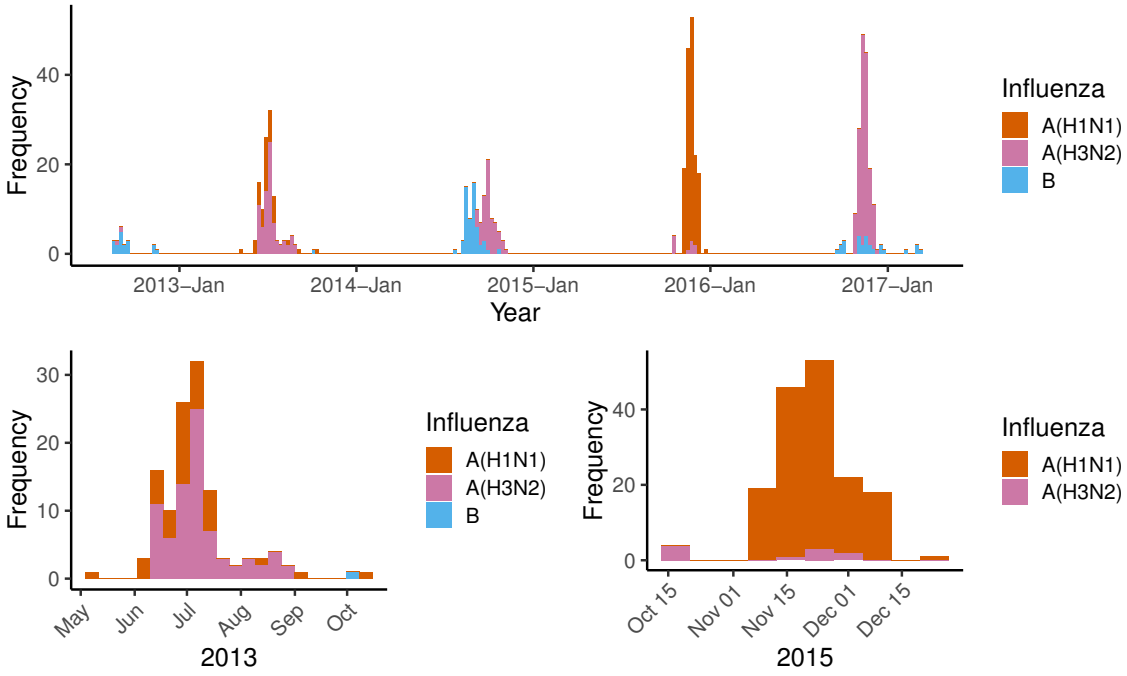

Figure 2.6: Influenza in the Household Influenza Transmission Study (HITS)

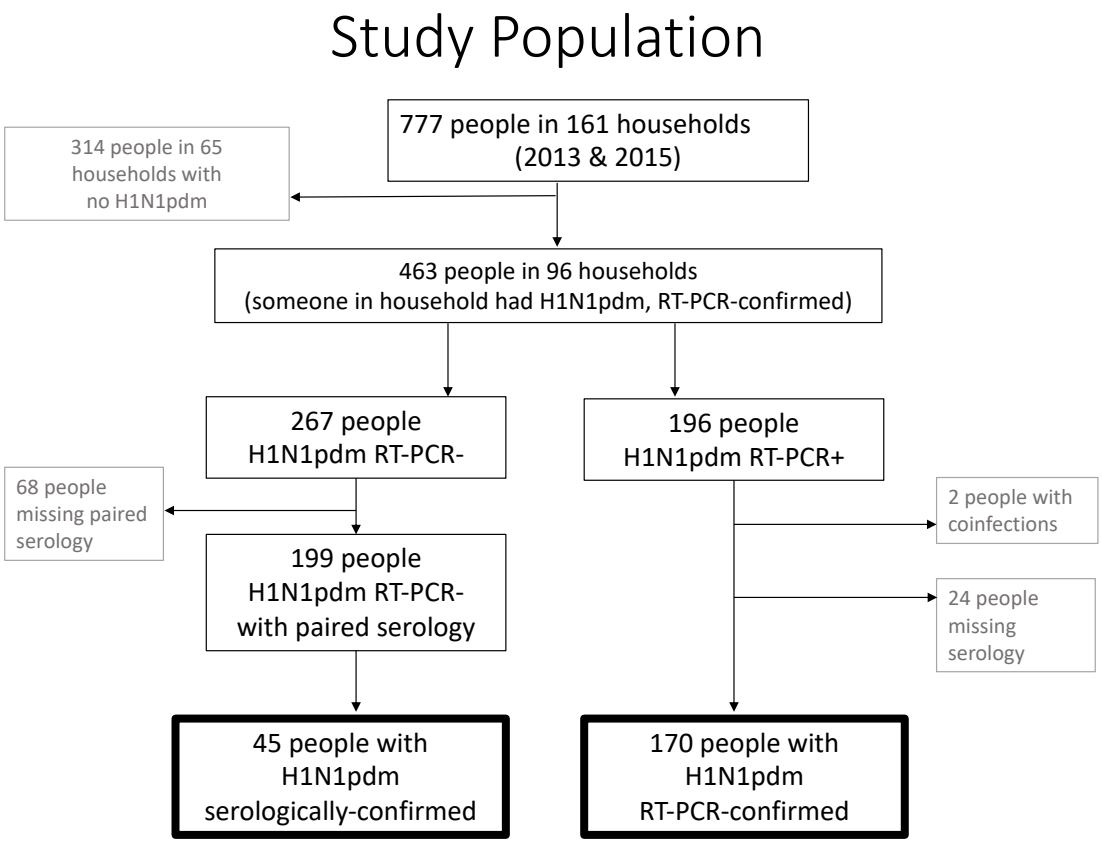

Figure 2.7: Flowchart of who was included in the study

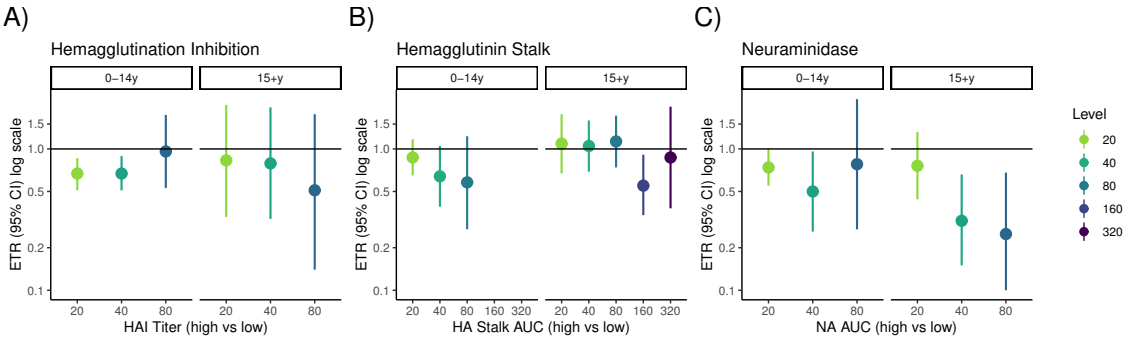

Figure 2.8: Pre-existing antibodies and influenza shedding duration among RT-PCR-positive infections, excluding one child outlier. This figure is the same as Fig. 2.1, except that one child with high pre-existing antibodies and 12 days of viral shedding (yellow, in Fig. 2.1B and C) was excluded from AFT models.

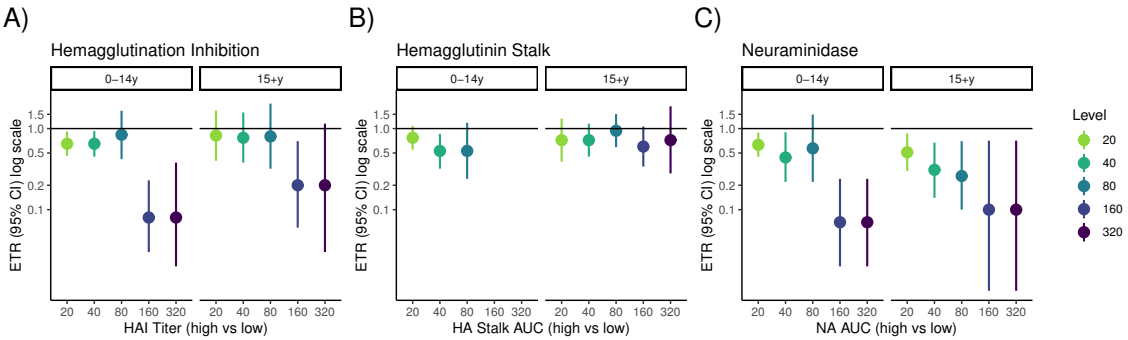

Figure 2.9: Pre-existing antibody levels and influenza shedding duration among RT-PCR-positive and serologically-confirmed infections, excluding one child outlier. This figure is the same as Fig. 4, except that one child with high pre-existing antibodies and 12 days of viral shedding (yellow, in Fig. 4B and C) was excluded from AFT models.

Table 2.2: Pre-existing antibodies and influenza shedding duration among RT-PCR-positive infections. (from Figure 2.1)

Age	Antibody	Level	crudeETR	crude 95% CI	sig	ETR	95% CI	sig	high	low
0-14y	hai	20	0.66	(0.51, 0.85)	*	0.65	(0.5, 0.84)	*	21	97
		40	0.67	(0.51, 0.88)	*	0.65	(0.49, 0.87)	*	18	100
		80	0.95	(0.52, 1.74)		0.94	(0.51, 1.72)		4	114
	ha_stalk	20	1	(0.75, 1.34)		0.97	(0.72, 1.32)		24	73
		40	1.06	(0.65, 1.72)		1.05	(0.64, 1.72)		9	88
		80	1.44	(0.68, 3.04)		1.44	(0.68, 3.06)		6	91
	na	20	0.89	(0.65, 1.22)		0.88	(0.64, 1.21)		18	79
		40	1.17	(0.63, 2.16)		1.18	(0.63, 2.2)		5	92
		80	2.02	(0.71, 5.74)		2.06	(0.72, 5.86)		3	94
15+y	hai	20	0.91	(0.37, 2.25)		0.83	(0.33, 2.05)		5	47
		40	0.87	(0.35, 2.15)		0.79	(0.32, 1.97)		4	48
		80	0.47	(0.13, 1.68)		0.51	(0.14, 1.76)		2	50
	ha_stalk	20	1.04	(0.65, 1.64)		1.09	(0.67, 1.76)		36	13
		40	1.08	(0.72, 1.63)		1.05	(0.69, 1.59)		25	24
		80	1.14	(0.74, 1.75)		1.13	(0.74, 1.72)		18	31
		160	0.61	(0.37, 1.01)		0.55	(0.34, 0.91)	*	10	39
		320	1.03	(0.46, 2.28)		0.87	(0.38, 1.99)		3	46
	na	20	0.76	(0.44, 1.31)		0.76	(0.44, 1.32)		7	42
		40	0.34	(0.16, 0.71)	*	0.31	(0.15, 0.66)	*	3	46
		80	0.34	(0.14, 0.86)	*	0.25	(0.1, 0.68)	*	2	47

Table 2.3: Pre-existing antibodies and influenza shedding duration among RT-PCR-positive & serologically-confirmed infections. (from Figure 4)

Age	Antibody	Level	crudeETR	crude 95% CI	sig	ETR	95% CI	sig	high	low
0-14y	hai	20	0.63	(0.44, 0.89)	*	0.63	(0.44, 0.9)	*	25	111
		40	0.62	(0.43, 0.9)	*	0.63	(0.43, 0.91)	*	22	114
		80	0.8	(0.4, 1.59)		0.82	(0.41, 1.63)		6	130
		160	0.08	(0.03, 0.24)	*	0.08	(0.03, 0.24)	*	2	134
		320	0.08	(0.02, 0.37)	*	0.08	(0.02, 0.38)	*	1	135
	ha_stalk	20	0.87	(0.63, 1.2)		0.86	(0.61, 1.22)		29	75
		40	0.82	(0.51, 1.33)		0.83	(0.5, 1.37)		12	92
		80	1.23	(0.57, 2.63)		1.26	(0.58, 2.73)		7	97
	na	20	0.76	(0.53, 1.08)		0.76	(0.53, 1.08)		22	82
		40	1.01	(0.52, 1.97)		1.04	(0.53, 2.04)		6	98
		80	1.5	(0.59, 3.78)		1.56	(0.62, 3.96)		4	100
		160	0.08	(0.02, 0.27)	*	0.07	(0.02, 0.25)	*	1	103
		320	0.08	(0.02, 0.27)	*	0.07	(0.02, 0.25)	*	1	103
15+y	hai	20	0.82	(0.4, 1.68)		0.82	(0.4, 1.67)		10	69
		40	0.77	(0.37, 1.58)		0.77	(0.38, 1.58)		9	70
		80	0.75	(0.29, 1.9)		0.8	(0.32, 2.03)		5	74
		160	0.18	(0.05, 0.64)	*	0.2	(0.06, 0.7)	*	2	77
		320	0.19	(0.03, 1.12)		0.2	(0.03, 1.15)		1	78
	ha_stalk	20	0.72	(0.4, 1.3)		0.72	(0.39, 1.33)		60	14
		40	0.76	(0.47, 1.2)		0.72	(0.45, 1.15)		44	30
		80	0.96	(0.6, 1.54)		0.94	(0.59, 1.51)		30	44
		160	0.67	(0.39, 1.17)		0.6	(0.34, 1.06)		16	58
		320	0.9	(0.37, 2.18)		0.72	(0.28, 1.88)		5	69
	na	20	0.5	(0.29, 0.86)	*	0.51	(0.3, 0.87)	*	15	59
		40	0.33	(0.16, 0.71)	*	0.31	(0.14, 0.67)	*	6	68
		80	0.34	(0.13, 0.87)	*	0.26	(0.1, 0.7)	*	4	70
		160	0.18	(0.03, 1.1)		0.1	(0.01, 0.71)	*	1	73
		320	0.18	(0.03, 1.1)		0.1	(0.01, 0.71)	*	1	73

CHAPTER III

Obesity Increases the Duration of Influenza A Virus Shedding in Adults

3.1 Abstract

Epidemiologic studies indicate that obesity increases the risk of severe complications and death from influenza virus infections, especially in elderly individuals. This work investigates the effect of obesity on the duration of viral shedding within household transmission studies in Managua, Nicaragua, over 3 seasons (2015-2017). Symptomatic obese adults were shown to shed influenza A virus 42% longer than nonobese adults (adjusted ETR, 1.42; 95% Confidence Interval (CI), 1.06-1.89); no association was observed with influenza B virus shedding duration. Even among paucisymptomatic and asymptomatic adults, obesity increased the influenza A shedding duration by 104% (adjusted ETR, 2.04; 95% CI, 1.35-3.09). These findings suggest that obesity may play an important role in influenza transmission.

3.2 Introduction

Epidemiologic studies indicate that obesity increases the risk of severe complications and death from influenza virus infections, especially in elderly individuals [14, 92]. The global prevalence of obesity has increased dramatically over the last few

decades. The regional burden varies widely—in 2014, the prevalence of adult obesity in the United States was 35.5%, compared with 17.4% in Nicaragua and 4.4% in other low-income economies-but in every region, adult obesity is increasing, and the pace of increase is accelerating [30].

Both the duration and quantity of viral shedding influence influenza transmission [26, 89]. Children are known to be important for influenza transmission, and young age has been associated with longer duration of shedding [65]. Shedding has also been shown in paucisymptomatic and asymptomatic influenza cases, highlighting the transmission potential of less severe cases [39].

Obesity leads to altered immune function and chronic inflammation, which increases with age, in addition to mechanical difficulties in breathing and increased oxygen requirements [53, 58, 56]; these are plausible mechanisms by which obesity could alter influenza risk, severity, and transmission potential. We hypothesize that this immune dysfunction could lead to a longer duration of influenza virus shedding, possibly increasing the transmission potential of infected individuals. While obesity is associated with severe influenza outcomes [14, 92], the effect of obesity on less severe influenza infections and transmission dynamics has not been as well studied. Here, we use household influenza transmission studies to examine the association between obesity and influenza virus shedding duration.

3.3 Methods

3.3.1 Study Population and Procedures

This work uses data from 2 studies of households in the catchment area of the Health Center Sócrates Flores Vivas (HCSFV) in District II of Managua, the capital of Nicaragua. Three influenza seasons are included: late 2015, 2016/2017, and mid/late 2017. The Household Influenza Transmission Study (HITS) has a case-

ascertained design, in which cases were identified from the HCSFV study clinic and their households enrolled; it provided data for the first 2 seasons. The Household Influenza Cohort Study (HICS) has the same design features as the HITS; it is nested within a prospective cohort study, in which enrollment occurs before the introduction of influenza to the households. The HICS started in 2017 and provided data from the 2017 influenza season. Height and weight measurements were collected at enrollment. Each measurement was taken twice; if there was a difference of >5% between the 2 measurements, a third was taken. Measurements were averaged.

For both studies, participating household members were intensively monitored for 10-13 days once a symptomatic influenza case was identified in the household. A full description of the inclusion criteria has previously been published [65]. Daily symptom diaries were recorded for all participants and up to 5 combined nasal/oropharyngeal swab specimens, and temperatures were measured for each household contact during follow-up, regardless of symptoms. As with our other studies, if a participant visited the HCSFV study clinic while enrolled, data from the visit were collected and were available for study use [34]. As described previously, all respiratory swab samples are tested by RT-PCR analysis following validated Centers for Disease Control and Prevention (CDC) protocols for influenza A and B virus detection [65]. Subtype and lineage are obtained for all influenza A and B virus-positive samples, respectively, through additional RT-PCR assays.

3.3.2 Ethics Statement

These studies were approved by the institutional review boards at the Nicaraguan Ministry of Health, the University of Michigan, and the University of California, Berkeley. Informed consent or parental permission for minors was obtained from all participants. Assent was obtained for children aged ≥6 years.

3.3.3 Weight Status

Body Mass Index (BMI) Z scores were calculated for children <18 years old, based on the World Health Organization (WHO) child growth standard for children aged <5 years and reference for children aged 5-17 years [98, 25]. BMI was calculated for adults as the weight in kilograms divided by the height in meters squared. Obesity was defined as a BMI of ≥30 in adults and as a BMI Z score of >3 or >2 for children aged <5 or 5-17 years, respectively. Underweight was defined as a BMI of <18.5 in adults and as a BMI Z score of ≥2 in children. The nonobese reference group was defined as those who were not underweight or obese.

3.3.4 Shedding Duration

Shedding duration was defined as the time from illness onset to viral shedding cessation, as described previously [65]. Symptom data were obtained from daily symptom diaries and clinic visits, and illness was defined as Acute Respiratory Illness (ARI2) with at least 2 of the following symptoms: measured fever (temperature >37.8°C) or reported fever, sore throat, cough, or runny nose on any day. Illness onset was defined as the earlier of the day that symptoms first appeared or that RT-PCR results were positive; if there were gaps >2 days without symptoms, illness onset was defined as the first day of the symptomatic period closest to the RT-PCR-positive event. Shedding cessation was defined either as occurring in the interval between the last positive RT-PCR result and subsequent negative RT-PCR result (interval censoring) or as right censored if the participant‘s last sample was RT-PCR positive. To minimize left censoring, we restricted this analysis to secondary cases.

In sensitivity analyses, illness was also defined as RT-PCR positivity (regardless of symptoms) and as Influenza-like Illness (ILI; defined as measured or reported fever with cough or sore throat).

3.3.5 Statistical Methods

Parametric Accelerated Failure Time (AFT) models with Weibull distributions, which can handle censored data, adjusted for age and sex, were used to calculate Event TIme Ratios (ETRs) to compare shedding duration in obese versus nonobese participants [26, 89]. Statistical analyses were conducted in R, version 3.4.3 (`https://www.R-project.org/`), using the package "Survival" (`https://CRAN.R-project.org/package=survival`), to run the AFT models and to predict mean shedding duration accounting for age and sex; "SurvRegCensCov" (`https://CRAN.R-project.org/package=SurvRegCensCov`), to convert the model output to ETRs; and "ggplot2" (`http://ggplot2.org`), for plotting.

3.4 Results

3.4.1 Study Population

In total, 1783 people in 320 households participated in intensive monitoring periods. The HITS contributed 800 participants from the first 2 seasons, and the HICS contributed 983 participants in 2017 who were enrolled in intensive monitoring periods. These individuals provided 7,066 swab samples for testing, with a mean number of 4.0 swabs/participant. Symptoms were reported for 15,905 days, with a median symptom diary duration of 10 days. There were 340, 631, and 812 participants in age groups 0-4, 5-17, and 18-92 years, respectively. Sex ratios were approximately equal in children but not equally distributed in adults, among whom 74% were women. The obesity prevalence varied significantly by age, with 2%, 9%, and 42% aged 0-4, 5-17, and 18-92 years, respectively.

Secondary cases aged 5-92 years made up 287 of 694 RT-PCR-positive influenza cases (41.3%); of these, 4 (1.4%) were missing height and weight data, and 9 (3.1%) were underweight and were excluded from analysis, leaving 276 secondary cases.

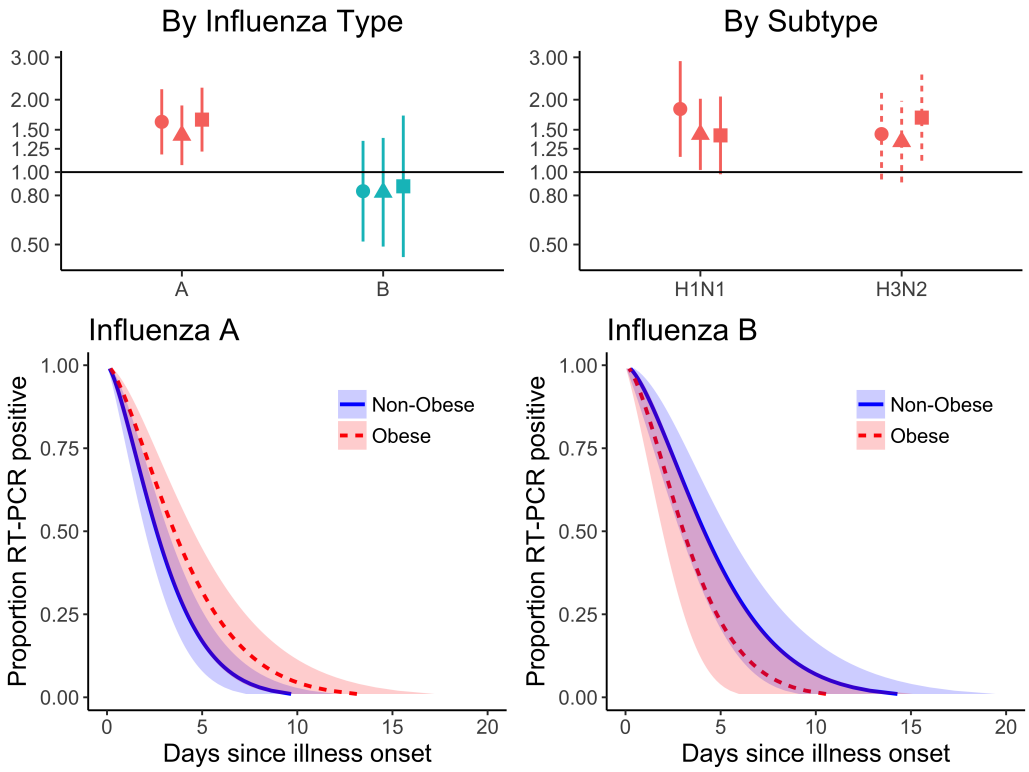

Figure 3.1: Association of obesity and influenza virus shedding in adults. A and B, Event time ratios adjusted for age and sex, by influenza virus type (A) and subtype (B), among obese relative to nonobese adults. Red indicates ratios for influenza A virus, and blue indicates ratios for influenza B virus. Shapes refer to illness onset definition, with circles indicating reverse transcription (RT-PCR)-based illness; triangles, acute respiratory illness (see Methods for definition); and squares, influenza-like illness. C and D, Predicted shedding duration of influenza A virus (C) and influenza B virus (D), using the RT-PCR-based illness definition.

Prevalence of obesity was similar among these secondary cases as compared to the overall study population that participated in intensive monitoring periods (Tables 3.2 and 3.3).

Of the 276 secondary cases included in the analysis, 19.9% were infected with 2009 pandemic influenza A(H1N1)pdm virus, 38% were infected with influenza A(H3N2) virus, and 42% were infected with influenza B virus. Figure 3.2 shows the epidemic curves by influenza virus type for each season (numbers are provided in Table 3.4).

3.4.2 Shedding Duration

Children aged 0-4 years shed influenza virus 40% longer (crude ETR, 1.40; 95% CI, 1.22-1.60) and children aged 5-17 years shed influenza virus 30% longer (crude ETR, 1.30; 95% CI, 1.15-1.48) than adults aged 18-92 years. These trends were similar for both influenza A and B viruses (Table 3.5). However, influenza B virus shedding was longer and displayed larger variance than influenza A virus shedding for all ages. Mean predicted influenza virus shedding duration was 7.7 days, 7.2 days, and 5.5 days for ages 0-4, 5-17, and 18-92 years, respectively. Mean predicted shedding duration among individuals aged 0-4, 5-17, and 18-92 years was 7.0, 6.4, and 5.1 days, respectively, for influenza A virus and 9.3, 8.8, and 6.4 days, respectively, for influenza B virus.

3.4.3 Obesity and Shedding Duration

Symptomatic obese adults shed influenza A virus 42% longer (adjusted ETR, 1.42; 95% CI, 1.06-1.89) than nonobese adults, with predicted mean shedding times of 5.23 days versus 3.68 days. They also shed influenza A(H1N1) virus 43% longer than nonobese adults (adjusted ETR, 1.43; 95% CI, 1.02-2.02). No association was observed between obesity and shedding duration for influenza B virus (Table 3.1 and Figure 3.1). Obesity was not associated with shedding duration in children aged 5-17

years (Table 3.6). There were not enough obese secondary cases aged <5 years old to include in this analysis.

Varying the shedding definition in sensitivity analyses did not substantially influence our findings, although for influenza A(H3N2) virus, the association increased when using the Influenza-like Illness (ILI) definition, and all associations increased when using the definition based on RT-PCR positivity regardless of symptoms (Table 3.7).

Obese individuals with influenza tended to have more symptomatic/severe illness, and fewer obese individuals had asymptomatic influenza, although the differences were not significant (Table 3.8). To examine whether the association of obesity and increased shedding duration existed among less symptomatic adults, the same analyses were performed among cases who had ≤1 symptom, not including fever. Of the 147 adult influenza cases, 40.8% had ILI, 69.4% had ARI2, 11.6% were paucisymptomatic (not including fever), and 17.7% were asymptomatic; among nonobese cases, 16.5% were paucisymptomatic, and 18.7% were asymptomatic, compared with 3.6% and 16.1% of obese cases, respectively. Among cases with ≤1 symptom not including fever, obese adults shed influenza A virus 104% longer than nonobese adults (adjusted ETR, 2.04; 95% CI, 1.35-3.09).

3.5 Discussion

While previous studies identified obesity as a risk factor for severe influenza outcomes [14, 92], we showed that obesity also affects less severe outcomes by significantly increasing the duration of influenza A virus shedding in adults. Further, we found that, even in asymptomatic or mildly ill individuals, obese adults shed influenza A virus for a longer duration than nonobese adults. This has important implications for influenza transmission.

No association was found with obesity and duration of influenza B virus shedding.

It is unclear why this association is specific to influenza A virus, but it is consistent with previous findings of obesity and severe influenza outcomes primarily for influenza A(H1N1) virus [14, 92]. Human challenge studies also found more variability in influenza B virus shedding, compared with influenza A(H1N1) virus shedding [5]. Obesity was not associated with shedding duration in children 5-17 years old. This is in agreement with the hypothesis that obesity increases the shedding duration through chronic inflammation. The study was underpowered to assess this association in children aged <5 years.

In addition to studies of obesity and influenza severity, a recent study that sampled exhaled breath from college students with influenza for virus found an association between obesity and how much virus is shed. The authors noted that most participants had nasal shedding and that obesity was associated with increased aerosol-based shedding; however, they did not assess whether obese individuals shed virus for a longer duration than nonobese individuals [105].

Here we focus on shedding duration, but virus quantity is also important; both are assumed to be positively associated with transmission, although the relationship was not found to be directly proportional within households and needs more investigation [89]. Owing to differences in contact patterns and types, transmission occurs earlier within households than in the wider community, which may reduce the impact of shedding duration on transmission in the household setting [26, 107]. Further analyses are underway to examine the effect of obesity on influenza transmission in Nicaraguan households.

This study had several limitations. Nose and throat samples were only collected every 2-3 days (interval censoring), and cases for whom final samples were RT-PCR positive were right censored, preventing observation of the precise shedding cessation time. Shedding was measured by RT-PCR, which only measures the presence of viral RNA but does not indicate whether the virus is infectious. In addition, quantitative

data could have provided additional evidence on viral shedding, however, quantification standards were not available at the time of testing.

This work has identified obesity as an important predictor of influenza A virus shedding duration in adults. Obesity may play an important role in influenza transmission, especially as the prevalence of obesity rises, and may be an important target for intervention and prevention strategies. Further, these results add to existing evidence linking obesity to infectious diseases, making it now even more important to work toward controlling and preventing the obesity epidemic.

3.6 Acknowledgments

We thank the many dedicated study personnel in Nicaragua at the Centro Nacional de Diagnóstico y Referencia and the Health Center Sócrates Flores Vivas.

3.7 Supplemental Information

Supplemental information here is numbered for this dissertation and the numbering from the publication is also presented.

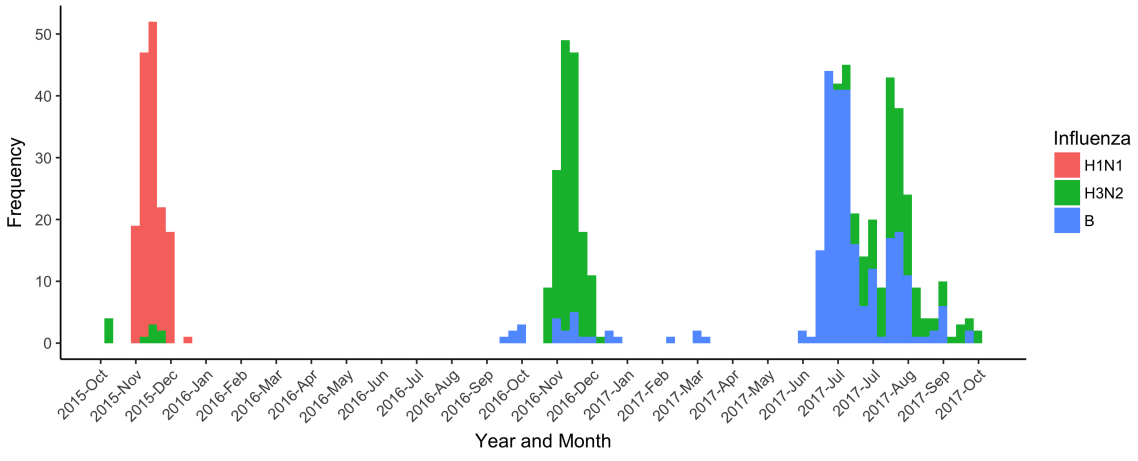

Figure 3.2: Supplementary Figure 1. Influenza seasonality during the study period from 2015-2017

Table 3.1: Accelerated Failure Time (AFT) Models of Obesity and Shedding Duration Among Adult Secondary Influenza Cases Aged 18-92 Years, by Influenza Virus Type and Strain

Variable		Crude ETR (95% CI)	Adjusted ETR (95% CI)	Predicted Shedding Duration, Mean Days (IQR)
Symptomatic illness (ARI2)				
All influenza viruses (n = 102)	Nonobese	Reference	Reference	4.67 (2.82-6.93)
	Obese	1.14 (.86-1.50)	1.14 (.87-1.50)	5.32 (3.22-7.90)
By influenza virus type				
A (n = 62)	Nonobese	Reference	Reference	3.68 (2.41-5.13)
	Obese	1.42 (1.05-1.92)	1.42 (1.06-1.89)	5.23 (3.42-7.30)
B (n = 40)	Nonobese	Reference	Reference	6.55 (3.79-10.07)
	Obese	0.80 (.48-1.35)	0.82 (.49-1.39)	5.40 (3.13-8.30)
By influenza A virus subtype				
H1N1 (n = 23)	Nonobese	Reference	Reference	3.56 (2.65-4.49)
	Obese	1.75 (1.12-2.71)	1.43 (1.02-2.02)	5.10 (3.80-6.44)
H3N2 (n = 39)	Nonobese	Reference	Reference	3.77 (2.40-5.41)
	Obese	1.22 (.81-1.84)	1.23 (.91-1.98)	5.05 (3.21-7.24)
Paucisymptomatic /asymptomatic illness				
All influenza viruses (n = 43)	Nonobese	Reference	Reference	1.97 (1.31-2.72)
	Obese	1.55 (.91-2.65)	1.43 (.85-2.41)	2.81 (1.87-3.89)
By influenza virus type				
A (n = 25)	Nonobese	Reference	Reference	1.57 (1.26-1.87)
	Obese	2.35 (1.62-3.42)	2.04 (1.35-3.09)	3.21 (2.57-3.82)
B (n = 18)	Nonobese	Reference	Reference	2.37 (1.49-3.41)
	Obese	1.35 (.39-4.61)	1.07 (.30-3.81)	2.54 (1.60-3.65)

Abbreviations: ARI2, acute respiratory illness (see Methods for def); CI, confidence interval; ETR, event time ratio; IQR, interquartile range.
Models are adjusted for age and sex.
Paucisymptomatic cases have 1 symptom, not including fever

Table 3.2: Participant Characteristics

Supplementary Table 1. Participant Characteristics						
All participants with intensive monitoring periods						
		Underweight	Non-Obese	Obese	p	Total
Age 0-4y						
n (row %)b		5 (1.6)	300 (93.8)	7 (2.2)		320
Sex (col %)	F	3 (60.0)	152 (50.7)	3 (42.9)	0.842	162 (50.6)
	M	2 (40.0)	148 (49.3)	4 (57.1)		158 (49.4)
Case Status (col %)	no influenza	0 (0.0)	104 (34.7)	3 (42.9)	0.523	108 (31.8)
	index	3 (60.0)	116 (38.7)	3 (42.9)		124 (38.8)
	secondary	2 (40.0)	80 (26.7)	1 (14.3)		88 (27.5)
Age 5-17y						
n (row %)b		19 (3.1)	520 (84.7)	57 (9.3)		614
Sex (col %)	F	10 (52.6)	260 (50.0)	30 (52.6)	0.921	308 (50.2)
	M	9 (47.4)	260 (50.0)	27 (47.4)		306 (4)
Case Status (col %)	no influenza	8 (42.1)	253 (48.7)	32 (56.1)	0.549	304 (48.2)
	index	8 (42.1)	150 (28.8)	13 (22.8)		176 (28.7)
	secondary	3 (20.0)	117 (22.5)	12 (21.1)		134 (21.8)
Age 18-92y						
n (row %)b		16 (2)	438 (55.7)	325 (41.3)		786
Sex (col %)	F	13 (81.2)	313 (71.5)	255 (78.5)	0.074	586 (74.6)
	M	3 (18.8)	125 (28.5)	70 (21.5)		200 (25.4)
Case Status (col %)	no influenza	12 (75.0)	328 (74.9)	258 (79.4)	0.551	603 (76.7)
	index	0 (0.0)	19 (4.3)	11 (3.4)		30 (3.8)
	secondary	4 (25.0)	91 (20.8)	56 (17.2)		153 (19.5)
Age category (col %)	18-39y	12 (70.6)	307 (68.7)	206 (60.4)	0.028	512 (65.1)
	40-92y	5 (29.4)	140 (31.3)	135 (39.6)		274 (34.9)
Age category (col %)	18-39y		62 (68.1)	39 (69.6)	0.993	101 (68.7)
	40-92y		29 (31.9)	17 (30.4)		46 (31.3)

Table 3.3: Participant Characteristics (continued)

Supplementary Table 1. Participant Characteristics (continued)					
Secondary cases					
		Non-Obeseb	Obese	p	Total
Age 0-4y					
n (row %)b		80 (98.8)	1 (1.2)		81
Sex (col %)	F	41 (51.2)	1 (100.0)	1.000	42 (51.9)
	M	39 (48.8)	0 (0.0)		39 (48.1)
Age 5-17y					
n (row %)b		117 (90.7)	12 (9.3)		129
Sex (col %)	F	70 (59.8)	9 (75.0)	0.474	79 (61.2)
	M	47 (40.2)	3 (25.0)		50 (38.8)
Age 18-92y					
n (row %)b		91 (61.9)	56 (38.1)		147
Sex (col %)	F	63 (69.2)	46 (82.1)	0.123	109 (74.1)
	M	28 (30.8)	10 (17.9)		38 (25.9)
Age category (col %)	18-39y	62 (68.1)	39 (69.6)	0.993	101 (68.7)
	40-92y	29 (31.9)	17 (30.4)		46 (31.3)

Table 3.4: Influenza Type by Age

Supplementary Table 2. Influenza type by age.

All cases with height and weight data

		Age			
	level	0-4y	5-17y	18-92y	Total
	n	197	284	177	658
Influenza Type (%)	H1N1	49 (24.9)	50 (16.7)	42 (23.7)	141 (21.4)
	H3N2	76 (38.6)	127 (44.7)	68 (38.4)	271 (41.2)
	B	72 (36.5)	107 (37.7)	67 (37.92)	246 (37.4)

Secondary cases age 5-92 with height and weight data (used in analyses)

		Age		
		5-17y	18-92y	Total
	n	129	147	276
Influenza Type (%)	H1N1	21 (16.3)	34 (23.1)	55 (19.9)
	H3N2	52 (40.3)	53 (36.1)	105 (38.0)
	B	56 (43.4)	60 (40.8)	116 (42.0)

Table 3.5: Accelerated Failure Time (AFT) Models of Age and Shedding Duration

Supplementary Table 3. Accelerated Failure Time (AFT) Models of Age and Shedding Duration

						Predicted Shedding Duration (days) a		
Age	Age	N	Crude ETRa	LB	UB	Mean	IQR	
All Influenza		596						
	0-4y		1.40	1.22	1.60	7.7	4.9	10.9
	5-17y		1.30	1.15	1.48	7.2	4.6	10.2
	18-92y		ref			5.5	3.5	7.8
Influenza A		377						
	0-4y		1.37	1.17	1.60	7.0	4.5	9.9
	5-17y		1.24	1.07	1.45	6.4	4.1	9.1
	18-92y		ref			5.1	3.3	7.3
Influenza B		219						
	0-4y		1.46	1.16	1.82	9.3	6.1	12.9
	5-17y		1.38	1.12	1.70	8.8	5.8	12.1
	18-92y		ref			6.4	4.2	8.8

a Models use the ARI2 definition of illness onset
all cases (index and secondary) are included

Table 3.6:
Accelerated Failure Time (AFT) Models of Obesity and Shedding Duration Among Older Children by Influenza Type and Strain

Supplementary Table 4. Accelerated Failure Time (AFT) Models of Obesity and Shedding Duration Among Older Children by Influenza Type and Strain

		Crude			Adjusted b			Predicted Shedding Duration (days)		
Children age 5-17a		ETR	LB	UB	ETR	LB	UB	Mean	IQR	
All Influenza (n=97)										
	NO	ref			ref			7.35	4.79	10.31
	Obese	0.82	0.55	1.23	0.89	0.59	1.31	6.46	4.20	9.05
By Influenza Type										
A (n=59)	NO	ref			ref			7.62	5.15	10.39
	Obese	0.77	0.48	1.24	0.89	0.58	1.37	6.80	4.59	9.26
B (n=38)	NO	ref			ref			5.76	3.93	7.77
	Obese	0.89	0.41	1.93	1.32	0.66	2.63	7.60	5.19	10.25
By Strain										
H1N1 (n=19)	NO	ref			ref			10.70	7.79	13.73
	Obese	0.64	0.32	1.26	0.82	0.44	1.55	8.81	6.42	11.31
H3N2 (n=40)	NO	ref			ref			6.37	4.42	8.49
	Obese	0.86	0.49	1.48	0.82	0.48	1.41	5.21	3.62	6.96

NO = non-obeseARI2 = acute respiratory illness, with 2+, of fever, cough, sore throat, runny nose,
a, Models adjusted for age and sex,
b Among secondary cases

Table 3.7: Sensitivity Analyses of Obesity andShedding Duration, Varying Outcome Definitions, in Adults

Supplementary Table 5. Sensitivity Analyses of Obesity and Shedding Duration,Varying Outcome Definitions, in Adults

		Outcome		Crude			Adjusted a		
	N	Definition		ETR	LB	UB	ETR	LB	UB
All Influenza	147	RT-PCR	Obese	1.27	0.97	1.68	1.27	0.97	1.67
	102	ARI2	Obese	1.14	0.86	1.50	1.14	0.87	1.50
	60	ILI	Obese	1.24	0.91	1.71	1.31	0.96	1.78
By Influenza Type									
A	87	RT-PCR	Obese	1.60	1.17	2.20	1.61	1.18	2.21
	62	ARI2	Obese	1.42	1.05	1.92	1.42	1.07	1.89
	42	ILI	Obese	1.68	1.23	2.28	1.65	1.22	2.24
B	60	RT-PCR	Obese	0.84	0.51	1.37	0.83	0.51	1.35
	40	ARI2	Obese	0.80	0.48	1.35	0.82	0.49	1.39
	18	ILI	Obese	0.69	0.36	1.35	0.87	0.44	1.72
By Strain									
H1N1	34	RT-PCR	Obese	1.91	1.21	3.03	1.83	1.16	2.89
	23	ARI2	Obese	1.75	1.12	2.71	1.44	1.02	2.02
	18	ILI	Obese	1.64	1.05	2.57	1.42	0.98	2.06
H3N2	53	RT-PCR	Obese	1.40	0.90	2.16	1.43	0.93	2.22
	39	ARI2	Obese	1.22	0.81	1.84	1.34	0.91	1.98
	24	ILI	Obese	1.70	1.11	2.59	1.68	1.12	2.54

ARI2 = Acute Respiratory Illness with 2+ of fever, sore throat, cough, or runny nose,
ILI = Influenza-Like Illness: fever with cough or sore throat,
a Models adjusted for age and sex

Table 3.8: Illness Severity by Obesity Status

Supplementary Table 6. Illness Severity by Obesity Status				
	Non-Obesea	Obese	p	Total
Age 5-17y				
N	117	12		129
ILI (%)	60 (51.3)	7 (58.3)	0.871	62 (48.1)
ARI2 (%)	86 (73.5)	11 (91.7)	0.300	97 (75.2)
Paucisymptomatic (%)	9 (7.7)	0 (0.0)	0.688	9 (7.0)
Asymptomatic (%)	19 (16.2)	1 (8.3)	0.763	20 (15.5)
Age 18-92y				
n	91	56		147
ILI (%)	33 (36.3)	27 (48.2)	0.283	60 (40.8)
ARI2 (%)	57 (62.6)	45 (80.4)	0.068	102 (69.4)
Paucisymptomatic (%)	15 (16.5)	2 (3.6)	0.035	17 (11.6)
Runny Nose = 1 (%)	6 (40.0)	0 (0.0)		
Sore Throat = 1 (%)	3 (20.0)	0 (0.0)		
Cough = 1 (%)	7 (46.7)	2 (100.0)		
Asymptomatic (%)	17 (18.7)	9 (16.1)	0.857	26 (17.7)
a Non-obese does not include underweight				

CHAPTER IV

Obesity and influenza A susceptibility in households in Managua, Nicaragua

4.1 Abstract

Background: Obesity has been shown to increase the risk of severe outcomes and death for influenza virus infections. Recently we also found that obesity increased the duration of influenza A viral shedding among adults, suggesting that obesity may play an important role in influenza virus transmission. Little is known yet of whether obesity is associated with *susceptibility* to influenza and other less severe outcomes.

Methods: Briefly, we performed a case-ascertained, community-based study of influenza transmission within households. Index cases were identified at the study health clinic and their families were invited to enroll. Heights and weights were collected at enrollment, and obesity was defined as a BMI $\geq$30 kg/m^2 for adults or a BMI Z score $>$ 2 SD above the WHO reference populations for children under 18 years. Households were then intensively monitored for 10 to 15 days. We use logistic regression models to investigate whether obesity increases the likelihood of influenza A infection within households.

Results: Between 2015 and 2017 we enrolled a total of 217 index cases with influenza A and 867 of their household contacts. Of the household contacts, 242

(28%) developed RT-PCR-confirmed influenza A infections. Prevalence of obesity was 41% in adults aged 18 and older, and 7% in children aged 0-17. We found that obesity was associated with increased susceptibility to symptomatic A(H1N1)pdm infection among adults, but not children, and that this association increased with age (older adults aged 35-85y OR: 6.3, 95%CI: 1.5-27.1). There was also an increase in susceptibility to A(H1N1)pdm infection regardless of symptoms among older adults (OR: 1.99, 95%CI: 0.68-5.86). A one-unit increase in BMI was also associated with increased susceptibility to symptomatic A(H1N1)pdm infection, although primarily among older adult females (OR: 1.2, 95%CI: 1.04-1.41). Obesity was not associated with increased H3N2 susceptibility.

Conclusions: We found that obesity is associated with susceptibility to symptomatic influenza A(H1N1)pdm infection, and not to A(H3N2) infection, in adults. This association was stronger in females. These findings will help target prevention and therapeutics to this high-risk population.

4.2 Introduction

Obesity has been recognized as a risk factor for increased influenza severity since the 2009 influenza A(H1N1) swine flu pandemic (A(H1N1)pdm) [14, 92]. Since then, several studies have additionally linked obesity to less severe influenza outcomes: a study of college age participants with mild to moderate influenza A and B infections found that BMI was positively associated with increased viral load in fine and coarse aerosols, representing infection in the lung, while BMI was not significantly associated with viral load on nasopharyngeal swabs [105]. More recently we showed that obesity is also associated with increased influenza A viral shedding duration among adults, even among adults with no or mild symptoms, suggesting that obesity may also influence influenza transmission [52].

The precise mechanism linking obesity to increased disease severity is not known,

but many potential pathways exist. Molecular studies have shown that obesity increases proinflammatory and decreases anti-inflammatory cytokine levels as adipose tissue expands, leading to defective innate and adaptive immune function and chronic inflammation which is associated impairs host defenses [53]. Chronic inflammation increases with age and is associated with chronic diseases [33]. Obesity can also impair wound healing and lead to mechanical difficulties in breathing and increased oxygen requirements [53, 56]. The prevalence of obesity has been rising rapidly in all parts of the world, in children and adults [94, 30], and the contribution of obesity to the burden of influenza is not known but could potentially be large.

Here we investigate the association of obesity on influenza susceptibility in households, an important setting to study risk factors for influenza transmission because a large portion of influenza transmission occurs in households [15]. The case-ascertained household-based study design we use here allowed us to efficiently identify individuals that have just been or are about to be naturally exposed to influenza virus infection. We used logistic regression models to assess the association of obesity and BMI on susceptibility of household contacts to influenza A infection.

4.3 Methods

4.3.1 Ethics Statement

This study was approved by the institutional review boards at the Nicaraguan Ministry of Health and the University of Michigan. Informed consent or parental permission for minors was obtained from all participants. Assent was obtained for children aged 6 years and older.

4.3.2 Study Design

This work uses data from two household influenza transmission studies, a case-ascertained study and a household cohort study, conducted in the same area of Managua, Nicaragua. The Household Influenza Transmission Study (HITS) is a case-ascertained study of households in the catchment area of the Health Center Sócrates Flores Vivas (HCSFV), the study health clinic, that ran from 2012-2017 [37]. The Household Influenza Cohort Study (HICS) is a cohort of 330 households that are influenza-free at baseline; it has a transmission study embedded within the cohort with the same design features as HITS, allowing data from the two studies to be combined.

Briefly, in both studies household influenza index cases were identified, and household members were then intensively monitored for influenza virus infection for 10-15 days. In HITS, index influenza cases were recruited at the study health clinic and through the ongoing Nicaraguan Pediatric Influenza Cohort Study (NPICS) [34] and their households were invited to enroll. Inclusion criteria for index cases in HITS were 1) a positive influenza QuickVue A+B rapid test, 2) experienced onset of Acute Respiratory Illness (ARI) within the previous 48 hours, and 3) live with at least one other household member, and 4) no household members had Influenza-like Illness (ILI) symptoms in the last two weeks. In HICS, families with at least one child were enrolled in the cohort between May and July 2017. All HICS participants are provided with primary medical care through study physicians. Once a household member enrolled in HICS becomes ill with rapid test or RT-PCR-confirmed influenza, the household enters the intensive monitoring period.

Three influenza seasons are included in this analysis, starting when heights and weights were collected: late 2015, 2016/2017, and mid/late 2017.

4.3.3 Data Collection

Height and weight, blood samples, and household questionnaires were collected at enrollment, which for HITS was when the index case was identified, and for HICS was at the beginning of the influenza season. Height and weight measurements were taken twice and if there was a difference $> 5\%$ between the 2 measurements, a third was taken; measurements were averaged.

For both studies, after index cases were identified, study staff would visit houses every 2-3 days for up to 10 to 15 days to collect nasal/oropharyngeal swabs and reported daily symptom diaries. Up to 6 swabs were collected for each household member.

4.3.4 Obesity Definition

Obesity was defined as a Body Mass Index (BMI) ≥ 30 kg/m^2 for adults. BMI Z scores were calculated for children < 5 years based on the World Health Organization (WHO) growth reference standard, and for children aged 5-17 years using a separate WHO growth reference [98, 25]. Obesity in children was defined as a BMI Z score $>$ 2 SD above the WHO reference population median.

4.3.5 Laboratory Testing and Influenza Definitions

Pooled nasal and oropharyngeal swabs maintained in viral transport media at 4°C and blood samples were sent within 48 hours to the Nicaraguan National Virology Laboratory at the Nicaraguan Ministry of Health. Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed on RNA extracted from the swab samples per validated CDC protocols for influenza A(H1N1)pdm detection [68, 96].

Influenza infection was defined as a participant with RT-PCR-confirmed influenza regardless of symptoms. *Symptomatic* infection was defined as additionally having

Influenza-like Illness (ILI), reported fever with a cough or sore throat.

4.3.6 Statistical Methods

We used logistic regression models to investigate the association of obesity and susceptibility to influenza A infection. For separate models of each influenza A subtype, A(H1N1)pdm and A(H3N2), participants in households where the index case had this type of infection were included. We present results separately for each subtype. Models of obesity and influenza A susceptibility were adjusted for age as a linear predictor, age squared, sex, and an obesity by age squared interaction. Akaike Information Criterion (AIC) was used to compare models including combinations of the terms just mentioned.

To present interpretable associations for obesity and influenza A susceptibility, we stratified this model by age groups: ages 0-4, 5-17, 18-34, and 35-85 years. Age groups for children were chosen based on the two WHO references used to define obesity. Age stratified models of obesity and influenza A susceptibility were run as crude models and as models adjusted for sex and age as a linear predictor.

Among adults, we also looked at the association of BMI as a linear predictor, in addition to categorical obesity. A model adjusting for BMI squared, age group, sex, and an interaction term of BMI squared and sex fit best, and the BMI by sex interaction term was borderline significant, so we stratified the models by sex and age group (younger and older adults, 18-34 and 35-85 years). The age and sex-stratified models only included BMI as a linear predictor.

Analyses were performed in R version 3.5.2 (`http://www.R-project.org/`) with the following packages: tidyverse v1.2.1 (for data wrangling), broom v0.5.1 (to present tidy versions of model output), modelr v0.1.2 (to augment data values with model predicted probabilities for plots), and grid and gridExtra v2.3 for arranging plots [78, 99, 82, 100, 1]. The "glm" function was used to run logistic regressions.

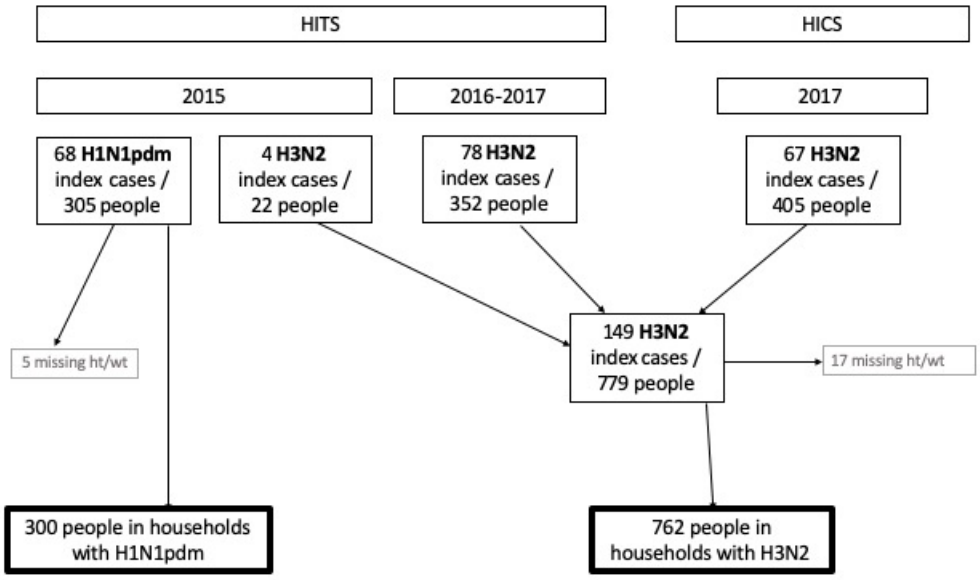

Figure 4.1: Flowchart of Participants Included (2015-2017)

4.4 Results

4.4.1 Study Population

Between 2015 and 2017 we enrolled a total of 217 index cases with influenza A and 867 of their household contacts (Table 4.2, Figure 4.1). Fifty percent of the participants lived in households with 5 or more people and 21% living in households with 7 or more people (Table 4.2). There was an approximately equal representation of all age groups in this study. Sex was balanced in children, but adult male participation was low (22% in young adults aged 18-34, 30% in older adults aged 35-85, Table 4.1). Height and weight data were available for 1,067 (98.4%) participants. Prevalence of obesity was 41% in adults aged 18 and older, and 7% in children aged 0-17, with obesity prevalence increasing dramatically with age, up to 47% in adults aged 35-85 (Table 4.1). Throughout the intensive monitoring periods, 4,603 swabs were collected (average: 4.2 per person), and 10,482 total person days of symptoms were recorded (average: 9.7 days per person). Index cases presented to the study

health clinic average within 1.0 days of symptom onset. Of the household contacts, 242 (28%) developed RT-PCR-confirmed influenza A infections matching the strain of their index case; 6 influenza A cases did not match the strain of the index case.

4.4.2 Logistic Regression Model

Figure 4.2 shows the predicted probability of H1N1pdm and H3N2 infection and symptomatic infection, in logistic regression models by age and sex for obese and non-obese individuals. Overall, young children were at the highest risk for influenza A infection and symptomatic infection. In adults, a dramatic increase in probability of A(H1N1)pdm infection began in middle age for both obese and non-obese individuals, however, probability of *symptomatic* A(H1N1)pdm infection rose sharply only for obese individuals. In addition, across age groups and obesity status males generally had a slightly higher risk of infection, most notably for symptomatic A(H1N1)pdm (Figure 4.2, Table 4.3).

4.4.2.1 Obesity and Influenza A Susceptibility

In univariate models, obesity was protective against A(H1N1)pdm and A(H3N2) infection (Table 4.3), because adults were at lower risk and obese individuals were mostly adults (Table 4.1).

In the adjusted model with symptomatic H1N1pdm infection as the outcome (Figure 4.2B and Table 4.3), there was a significant interaction of obesity and age with no association among children, but steeply increasing risk among obese adults with increasing age. For H1N1pdm infection regardless of symptoms, there also appeared to be some increased risk among obese, but this was not significant. Interestingly, obesity was not associated with increased risk of H3N2 infection, and even appeared slightly (not significantly) protective (Figure 4.2C and D).

Results of the same association of obesity and influenza A susceptibility stratified

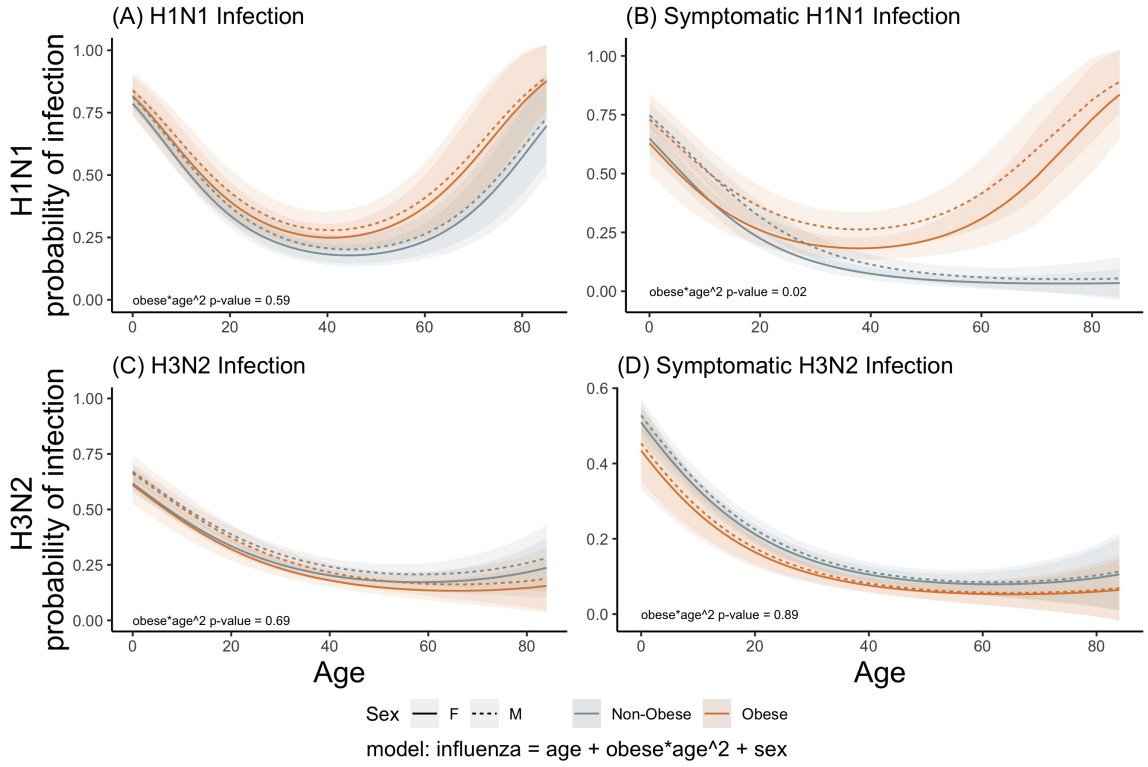

Figure 4.2: Obesity and H1N1pdm and H3N2 Susceptibility. Curves show probability of infection (A and C) and *symptomatic* infection (B and D) by age (x-axis), sex (solid line=female), and obesity (obese = orange). H1N1pdm is shown in panels A and B, H3N2 in C and D. The logistic regression models include predictors obese, sex, age, age squared, and an age squared by obese interaction term.

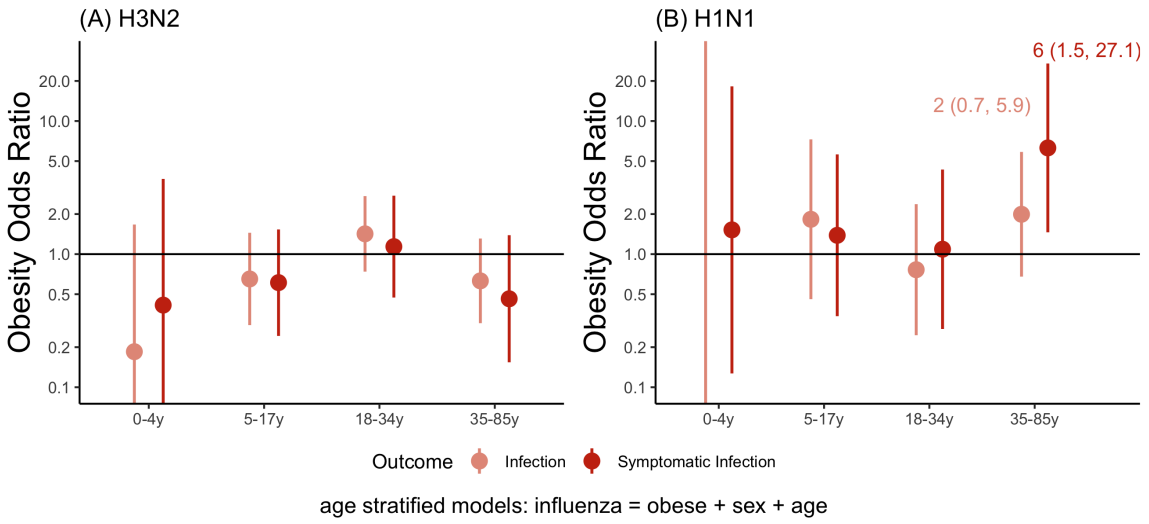

Figure 4.3: Obesity and A(H1N1)pdm and A(H3N2) Susceptibility Stratified by Age. Odds Ratios (ORs) for obesity from age-stratified models (0-4, 5-17, 18-34, and 35-85 years) adjusted for age and sex are presented for H3N2 (A) and H1N1pdm (B). Obesity Odd Ratios for symptomatic infection are in red and ORs for infection regardless of symptoms are in pink. These models correspond to those in Figure 4.2 but are stratified by age.

by age are presented in Figure 4.3 (odds ratios for obesity) and Tables 4.4 and 4.5 (odds ratios for all variables in the model. Among the older adults (age 35-85y), obese individuals had 6.3 times higher odds of symptomatic H1N1pdm infection compared to non-obese older adults (95%CI: 1.5-27.1), and 2 times higher odds of H1N1pdm infection (95%CI: 0.7, 5.9). Obese children also had slightly increased odds of H1N1pdm infections, but the results were not significant. Only 3 children under 5 years were obese and all 3 had H1N1pdm infection (Fisher's exact test: p=1). There appeared to be no association of obesity and H1N1pdm susceptibility for younger adults (age 18-34y, Table 4.4). In contrast, obesity was not associated with increased susceptibility to H3N2 (Figure 4.3 and Table 4.5).

4.4.2.2 BMI and sex

In adults, we also looked at BMI as a linear predictor of symptomatic influenza A infection (Figure 4.4). The average adult BMI was higher in females than males (30.6

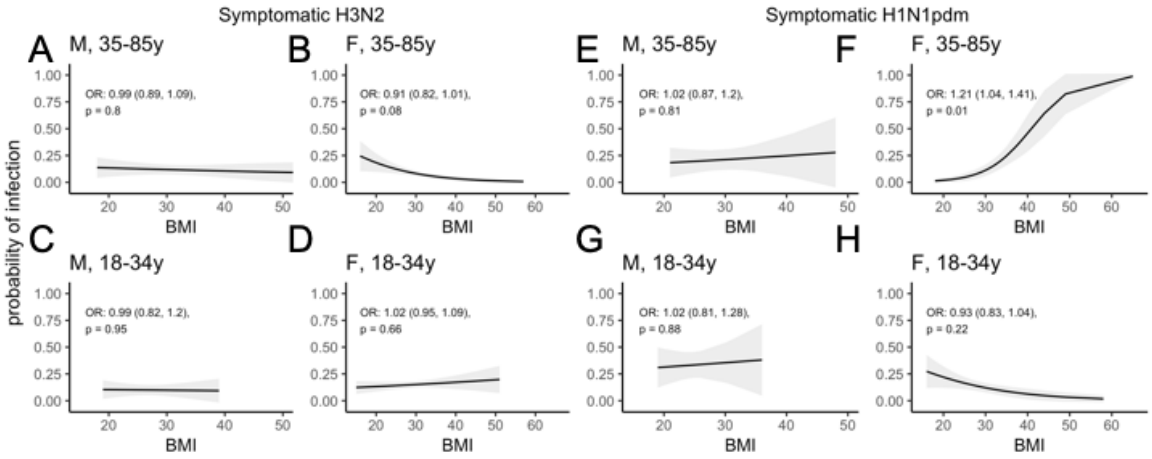

Figure 4.4: BMI and Susceptibility to Symptomatic Influenza A Infection, by Strain, in Adults, Stratified by Age and Sex. Probability of infection with BMI as a linear predictor is shown for H3N2 (panels A-D) and H1N1pdm (E-H), with older adults on the top row (A,B,E,F) and younger adults on the bottom (C,D,G,H), and also stratified by sex (males: A, C, E, G, females: B,D,F,H).

vs 28.9). Among younger adults (bottom row of Figure 4.4), BMI was not associated with symptomatic A(H3N2) or A(H1N1)pdm infection for either sex. And among older adults, BMI was also not associated with symptomatic H3N2 infection (Figure 4.4A and B).

Although increased BMI was associated with increased susceptibility to symptomatic H1N1pdm infection in older adults (Figure 4.4E and F), this association was driven nearly entirely by the association among women (Figure 4.4F). Older adult women (age 35-85y) had a 21% increase in odds of symptomatic H1N1pdm infection for every unit increase in BMI (95%CI: 1.0-1.4) (Figure 4.4F).

4.5 Discussion

In this study we found that obesity was associated with increased susceptibility to symptomatic influenza A(H1N1)pdm infection in older adults and this association increased with age (Figures 4.2B and 4.3B). In addition, obesity was associated with

A(H1N1)pdm infection regardless of symptoms, however it was not significant (Figures 4.2A and 4.3B). Older adults aged 35-85 years who were obese had 6 times the odds of symptomatic H1N1pdm infection (OR: 6, 95%CI: 1.5-27.1, Figure 4.3B) and twice the odds of H1N1pdm infection (OR: 1.99, 95%CI: 0.68-5.86, Figure 4.3B) compared to non-obese older adults. Interestingly, we found no association, for obesity and H3N2 infections (Figures 4.2C and D and 4.3A).

There are many mechanisms through which obesity may act to increase susceptibility to and severity of influenza infections. Two reviews ([44, 53]) summarize some of the pathways; many are identified based on mice studies. Briefly, obesity can affect lung function, reducing lung volume and increasing the respiratory rate [56], it can impair wound healing in the lungs [69], and it leads to production of more proinflammatory and less anti-inflammatory cytokines, leading to systemic inflammation which can ultimately impair innate and adaptive immune responses [44, 53]. In the innate immune system, cytokines, natural killer cells, macrophages, and dendritic cells have been shown to be affected by obesity [84, 85, 44, 53]. T cells and the adaptive immune system have also been shown to be reduced and less effective under obese conditions [45, 67, 71, 43, 81].

Our findings here, coupled with the existing literature on severe disease, demonstrate that obesity impacts the risk of influenza disease across the disease spectrum. Previous studies reporting associations of obesity and influenza outcomes in humans have primarily focused on severe outcomes such as death, hospitalization, and secondary pneumonia [14, 92, 32, 18, 55, 106]. This work demonstrates that obesity is also associated with susceptibility to symptomatic influenza A(H1N1)pdm in older adults, and with H1N1pdm infection regardless of symptoms. This finding is supported by another study that found vaccinated adults who are obese had higher risk of ILI, despite having HAI titers [64]. Reported associations of obesity and influenza-associated death, the most severe outcome, have been larger than associations for

hospitalization or other less severe outcomes [92], and we also found a larger association for A(H1N1)pdm infection with symptoms vs all A(H1N1)pdm infections. One possible explanation for this is that there might be more pathways obesity can act through to increase severity, such as impaired wound healing in the lungs [53, 69], rather than increase *susceptibility* to infection in the first place. Further, that the association was specific to older adults and increased with age fits with our understanding that obesity can affect influenza outcomes through chronic inflammation, the effects of which increase over time [53].

Importantly, in our study the effect of obesity on influenza disease observed was specific to H1N1pdm. The difference in association of obesity and H1N1pdm compared to H3N2 susceptibility is interesting and while the literature generally cites obesity as a risk factor for severe influenza, existing studies have primarily reported on specific effects of H1N1pdm [14, 92, 32] or combined effects of all influenza types [106, 50, 108]. These studies of combined influenza types found some evidence for increased hospitalization and mortality for seasonal influenza [106, 50, 108]. One study of a season in which H3N2 predominated found no increased severity among hospitalized adults [3]. It is possible that there could be biologic differences between the virus strains or that there is an important difference in immune history between obese adults exposed to H1N1pdm, a newer virus, compared to obese adults exposed to H3N2, which adults likely have some immunity to.

Our results support that the effect of obesity (as measured by continuous BMI) varies by sex. Older adult females (aged 35-85 y) had increased risk of symptomatic H1N1pdm with increasing BMI (OR: 1.21, 95% CI: 1.04-1.41, Figure 4.4), but there was no association for males. This finding of a sex-specific association with increasing BMI was surprising and we could not find any other studies that looked at the association of obesity and influenza susceptibility by sex, although pregnancy was noted as a risk factor for hospitalization and complications during the 2009 pandemic [44].

There is however some knowledge of sex differences for influenza outcomes: women mount stronger antibody and humoral immune responses to vaccination than men and pandemic influenza outcomes are generally worse for women [47, 62]. Worse outcomes in females are shown in mice studies to correspond to increased cytokine and chemokine production in lungs along with greater immunopathology [47]. Sex hormones also affect levels of inflammatory cytokines and chemokines, and influenza infection in mice has been shown to interrupt the estrous cycle, keeping estradiol levels low and consequently leading to excessive inflammation, which can lead to higher pathogenesis [47]. Additionally, studies have also shown that sex and sex hormones play a role in regulating obesity-induced inflammation [59]. Future studies, including molecular and immunological studies, should consider the effect of sex on the relationship between obesity and influenza infection and disease.

A strength of this study is that the efficient study design allowed us to identify influenza virus infections across individuals of all ages and those with comorbid conditions. This allowed us to observe influenza infections that were mild enough to not require treatment or hospitalization and are less frequently studied, but are critical to inform us about susceptibility to influenza infection and disease. One limitation of this study is that we had relatively low adult male participation which limited our ability to examine sex differences by BMI. It is therefore possible that there is an association with increased BMI in males that we could not observe. Another limitation is that we only collected swabs to test for virus every 2-3 days, so we may not have detected infections that only shed virus briefly. Because of this, we may be limited in our ability to discern whether obese adults are more susceptible to H1N1pdm infection which trended towards significance in our study.

These findings extend our understanding of the connection of obesity to infectious diseases, indicating that the extent of obesity's influence is larger than previously understood. Obesity is not only associated with severe outcomes and death, but also

with susceptibility to symptomatic infection. This combined with our previous finding that obesity increases the duration of viral shedding [52] demonstrate that obesity may effect transmission and the overall burden of influenza. With the increasing worldwide obesity epidemic, obesity has the potential to increase the burden and transmission of influenza and other infectious diseases. Physicians should be informed of the importance of obesity as a risk factor for influenza outcomes and vaccination, antiviral therapy, and other preventive and therapeutic measures should consider targeting obesity as an important upstream risk factor for influenza. Future efforts should focus on identifying the pathways through which obesity increases the risk of influenza outcomes, and how this varies by sex, to better inform the design of interventions and therapeutics.

Table 4.1: Participant Characteristics (2015-2017)

Age		Non-obese	Obese	p	Missing*	Overall
All	n (row %)	824 (76.0)	243 (22.4)		17 (1.6)	1,084
	Male (%)	341 (41.4)	64 (26.3)	<0.01		414 (38.2)
0-4y	n (row %)	192 (93.0)	6 (3.0)		8 (3.9)	206
	Male (%)	98 (51.0)	3 (50.0)	1		105 (51.0)
	Index (%)	79 (41.1)	3 (50.0)	0.99		85 (41.3)
5-17y	n	340 (89.0)	34 (8.9)		8 (2.1)	382
	Male (%)	159 (46.8)	18 (52.9)	0.61		181 (47.4)
	Index (%)	101 (29.7)	9 (26.5)	0.84		112 (29.3)
18-34y	n	168 (63.6)	95 (36.0)		1 (0.4)	264
	Male (%)	43 (25.6)	14 (14.7)	0.06		58 (22.0)
	Index (%)	9 (5.4)	4 (4.2)	0.91		13 (4.9)
35-85y	n	124 (53.4)	108 (46.6)		0 (0)	232
	Male (%)	41 (33.1)	29 (26.9)	0.38		70 (30.2)
	Index (%)	5 (4.0)	2 (1.9)	0.56		7 (3.0)

*no height/weight data available

Table 4.2: HITS and HICS Index and Household Characteristics (2015-2017)

Characteristic	Non-obese	Obese	p	Overall
Index Cases				
n	194	18		217
Male (%)	108 (55.7)	10 (55.6)	1	121 (55.8)
Age (%)			<0.01	
0-4y	79 (40.7)	3 (16.7)		85 (39.2)
5-17y	101 (52.1)	9 (50.0)		112 (51.6)
18-34y	9 (4.6)	4 (22.2)		13 (6.0)
35-85y	5 (2.6)	2 (11.1)		7 (3.2)
H1N1 (%)	60 (30.9)	6 (33.3)	1	68 (31.3)
H3N2 (%)	134 (69.1)	12 (66.7)	1	149 (68.7)
# in hh (%)			0.28	
2-4	99 (51.0)	6 (33.3)		108 (49.8)
5-6	56 (28.9)	6 (33.3)		63 (29.0)
7-16	39 (20.1)	6 (33.3)		46 (21.2)
# fluA in hh (%)			0.32	
1	89 (45.9)	9 (50.0)		101 (46.5)
2	51 (26.3)	2 (11.1)		54 (24.9)
3-9	54 (27.8)	7 (38.9)		62 (28.6)
Contacts				
n	630	225		867
Male (%)	233 (37.0)	54 (24.0)	<0.01	293 (33.8)
Age, y (%)			<0.01	
0-4y	113 (17.9)	3 (1.3)		121 (14.0)
5-17y	239 (37.9)	25 (11.1)		270 (31.1)
18-34y	159 (25.2)	91 (40.4)		251 (29.0)
35-85y	119 (18.9)	106 (47.1)		225 (26.0)
FluA (%)	179 (28.4)	52 (23.1)	0.15	236 (27.2)
H1N1 (%)	57 (9.0)	20 (8.9)	1	79 (9.1)
H3N2 (%)	122 (19.4)	32 (14.2)	0.1	157 (18.1)

Table 4.3: Obesity and H1N1pdm and H3N2 Susceptibility. Results correspond to Figure 4.2

		Infection			Symptomatic Infection		
Subtype	Variable	OR	CI	p	OR	CI	p
H1N1	Obese (crude)	0.63	(0.36, 1.09)	0.1	0.68	(0.38, 1.21)	0.19
	Age (linear)	0.88	(0.84, 0.92)	<0.001	0.9	(0.86, 0.94)	<0.001
	Obese	1.21	(0.53, 2.78)	0.65	0.92	(0.38, 2.22)	0.84
	Age^2	1	(1, 1)	<0.001	1	(1, 1)	0.12
	SexM	1.17	(0.69, 1.98)	0.57	1.6	(0.93, 2.74)	0.09
	Obese*Age	1	(1, 1)	0.59	1	(1, 1)	0.02
H3N2	Obese (crude)	0.43	(0.3, 0.63)	<0.001	0.32	(0.19, 0.51)	<0.001
	Age (linear)	0.93	(0.91, 0.96)	<0.001	0.92	(0.89, 0.95)	<0.001
	Obese	0.97	(0.54, 1.73)	0.91	0.74	(0.37, 1.49)	0.4
	Age^2	1	(1, 1)	0.01	1	(1, 1)	0.03
	SexM	1.26	(0.92, 1.73)	0.16	1.08	(0.76, 1.54)	0.67
	Obese*Age	1	(1, 1)	0.69	1	(1, 1)	0.89

Table 4.4: Obesity and H1N1pdm Susceptibility Stratified by Age. Model corresponding to Figure 4.3B.

H1N1		Infection			Symptomatic Infection		
Age	term	OR	CI	p	OR	CI	p
0-4y	Obese (c*)	**	-	-	1.33	(0.11, 15.54)	0.818
	Obese	**	-	-	1.52	(0.13, 18.22)	0.74
	Male	1.22	(0.36, 4.17)	0.755	1.45	(0.52, 4.04)	0.481
	Age	1.03	(0.65, 1.63)	0.9	1.23	(0.84, 1.8)	0.297
5-17y	Obese (c*)	1.15	(0.32, 4.04)	0.833	0.87	(0.25, 3.08)	0.833
	Obese	1.83	(0.46, 7.28)	0.393	1.38	(0.34, 5.62)	0.648
	Male	0.95	(0.39, 2.28)	0.906	1.05	(0.43, 2.55)	0.919
	Age	0.78	(0.69, 0.89)	<0.001	0.76	(0.67, 0.87)	<0.001
18-34y	Obese (c*)	0.65	(0.22, 1.93)	0.435	1.8	(0.63, 5.13)	0.271
	Obese	0.76	(0.25, 2.37)	0.642	1.09	(0.27, 4.32)	0.904
	Male	1.92	(0.56, 6.51)	0.297	3.53	(0.88, 14.05)	0.074
	Age	0.96	(0.86, 1.07)	0.454	0.9	(0.78, 1.03)	0.12
35-85y	Obese (c*)	0.77	(0.21, 2.79)	0.694	6.18	(1.48, 25.79)	0.013
	Obese	1.99	(0.68, 5.86)	0.21	6.28	(1.46, 27.05)	0.014
	Male	1.08	(0.34, 3.46)	0.898	1.42	(0.34, 6.02)	0.634
	Age	1.02	(0.98, 1.06)	0.332	1	(0.95, 1.05)	0.958

c* = crude model, ** only 3 children were obese and all had H1N1pdm

Table 4.5: Obesity and H3N2 Susceptibility Stratified by Age. Model results corresponding to Figure 4.3A.

H3N2		Infection			Symptomatic Infection		
Age	term	OR	CI	p	OR	CI	p
0-4y	Obese (c*)	0.25	(0.03, 2.2)	0.212	0.44	(0.05, 3.85)	0.458
	Obese	0.18	(0.02, 1.67)	0.133	0.41	(0.05, 3.67)	0.429
	Male	1.35	(0.76, 2.41)	0.303	1.09	(0.6, 2.01)	0.769
	Age	0.76	(0.61, 0.95)	0.015	0.94	(0.75, 1.18)	0.603
5-17y	Obese (c*)	0.64	(0.29, 1.4)	0.262	0.57	(0.23, 1.41)	0.223
	Obese	0.65	(0.29, 1.45)	0.292	0.61	(0.24, 1.53)	0.294
	Male	1.18	(0.77, 1.81)	0.443	1.05	(0.66, 1.67)	0.831
	Age	0.97	(0.91, 1.02)	0.259	0.93	(0.87, 0.99)	0.032
18-34y	Obese (c*)	1.36	(0.72, 2.58)	0.341	1.08	(0.46, 2.56)	0.859
	Obese	1.42	(0.74, 2.73)	0.292	1.14	(0.47, 2.75)	0.772
	Male	1.01	(0.48, 2.16)	0.972	0.87	(0.31, 2.46)	0.798
	Age	0.98	(0.92, 1.04)	0.482	0.96	(0.88, 1.05)	0.371
35-85y	Obese (c*)	0.65	(0.32, 1.34)	0.241	0.48	(0.16, 1.41)	0.18
	Obese	0.63	(0.3, 1.31)	0.216	0.46	(0.15, 1.39)	0.169
	Male	1.51	(0.71, 3.22)	0.286	1.56	(0.53, 4.58)	0.417
	Age	0.98	(0.95, 1.01)	0.279	0.98	(0.94, 1.03)	0.431

c* = crude model

CHAPTER V

Conclusions

5.1 Summary of Findings

This dissertation addresses factors affecting susceptibility to influenza infection and disease, and influenza transmission. Influenza viral shedding duration, the outcome used in chapters II and III, is directly related to transmission as the longer a person sheds virus the longer the duration that they can infect other people (the higher their *infectivity*). Transmission is a function of both infectivity and *susceptibility*, which is the outcome in chapter IV.

In chapter II we found that pre-existing neuraminidase antibodies were associated with shorter influenza A(H1N1)pdm viral shedding duration and that this relationship was much stronger in adults. Importantly, we found that adults had a dose-response relationship of shorter shedding with increasing levels of neuraminidase antibodies (Figures 2.1 and 2.4). In adults, higher neuraminidase antibodies were also associated with shortened *symptom* duration among those with influenza A(H1N1)pdm infections (Figure 2.5. Further, we found that neuraminidase antibodies were *independently* associated with shortened shedding duration (Figure 2.3 after adjusting for hemagglutination inhibiting and hemagglutinin stalk antibodies. Taken together with another finding from our group that antibodies to hemagglutinin and hemagglutinin stalk, but not neuaraminidase, were independently associated with protection from

infection [66], our findings fit with the understanding of the mechanisms of action of these antibodies [49]—that HA antibodies play an important role in preventing influenza virus from entering cells, whereas NA antibodies are more important in preventing virus from leaving cells and spreading. These findings suggest that increased immune response to NA could shorten illness duration and thus also limit transmission.

In chapter III we found that obesity increases the duration of influenza A viral shedding in adults, suggesting that obesity may play a role in influenza transmission (Figure 3.1). Importantly, we found that even paucisymptomatic/asymptomatic obese adults shed virus longer (Table 3.1). In chapter IV we found that obesity was associated with increased susceptibility to symptomatic influenza A(H1N1)pdm infection, and that this association was larger among older adults (Figure 4.2). We also found, surprisingly, that increased BMI (looking at the continuous predictor) was associated with increased susceptibility to symptomatic H1N1pdm infection in older adult females but not males (Figure 4.4. We also found no associations with obesity and increased susceptibility to influenza A(H3N2) infection. While obesity was first noticed as a risk factor for severe influenza A(H1N1)pdm outcomes, it has been assumed that obesity is a risk factor for severe influenza for both influenza A subtypes. Our findings linking just the influenza A H1N1pdm subtype to severity in obese individuals will help to elucidate the mechanism of this interaction.

5.2 Significance

Our findings from chapter II that NA antibodies are associated with shorter viral shedding duration informs the design of next generation influenza vaccines. Our findings support that formulating influenza vaccines to generate an immune response to NA could lead to reduced illness duration and influenza transmission. Given these findings and understanding of how NA antibodies work—by preventing virus from

leaving cells and spreading [49]—consideration should be given to find better/more endpoints, such as reduction in viral shedding or illness duration, for vaccine effectiveness clinical trials, rather than focusing only on HAI antibody response and prevention of infection [27].

Both chapters III and IV identify obesity as a risk factor for influenza A-related outcomes: increased viral shedding duration and increased susceptibility to symptomatic H1N1pdm infection, respectively. Chapter IV also found a sex difference in the association of increased BMI with susceptibility to symptomatic H1N1pdm, with the association only present for females, not males. Recognizing obesity as a new risk factor for susceptibility and transmission can inform the development of efficient policies to help target vaccines and antivirals.

5.3 Future Work

This dissertation has made important contributions in identifying neuaraminidase as a target for future vaccines and identifying obesity as a risk factor for susceptibility to influenza A(H1N1)pdm infection, but there are numerous future directions in which to take this research. To expand on our findings from Chapter II, which only included influenza A(H1N1)pdm, future work will examine the association of antibodies to NA and shedding duration to other virus types: influenza A(H3N2) and influenza B. In addition, it will be important to use household transmission models to examine the potential effects of neuraminidase antibodies in index cases on subsequent transmission of influenza.

Given our findings on obesity from Chapters III and IV, future work is needed to examine sex differences in the association of obesity and influenza susceptibility and transmission. Additionally, a more detailed investigation of the effects of obesity on influenza outcomes in children is needed, as the definition of obesity used in this work may not reflect levels of BMI in children that correspond to increased health risks

for children and there may be other important confounding factors for children. We also need to understand the specifics of the mechanisms by which obesity increases susceptibility and severity in humans, to understand the how well interventions will work in an obese population. To this aim, future work should study whether obesity affects antibody levels and immune response to infection and vaccination and whether similar levels of antibodies are protective in obese as in normal weight individuals.

Future work should also follow up on chapter III more specifically to confirm whether increased viral shedding among obese adults actually corresponds to increased transmission. We set up a model of household influenza transmission to answer this question (details in Appendix A), but were not able to investigate the effect of obesity on *transmissibility* at this time due to low numbers of obese adult index cases but plan to do so when new data becomes available. We were however able to model the effects of obesity on susceptibility and verify our results from chapter IV with another method (results presented in Appendix A).

5.4 Conclusion

In summary, this work has identified neuaraminidase as a target for next generation influenza vaccines to reduce influenza burden by shortening viral shedding and illness duration, which in addition to reducing burden in infected vaccinated individuals may reduce influenza transmission. We also identified obesity as a risk factor for influenza susceptibility and transmission, and found that obesity and sex may interact, with females experiencing increased susceptibility with increased BMI. Understanding obesity as a risk factor can guide prevention and treatment strategies. Taken together, our findings for the interaction of obesity and influenza infection will help target interventions, our finding for neuraminidase will help create better next generation influenza vaccines, and both findings will help prevent influenza infections at the base of the influenza burden pyramid (Figure 1.1), thus lowering the overall

burden of influenza and controlling epidemics.

APPENDIX

APPENDIX A

Household Transmission Model

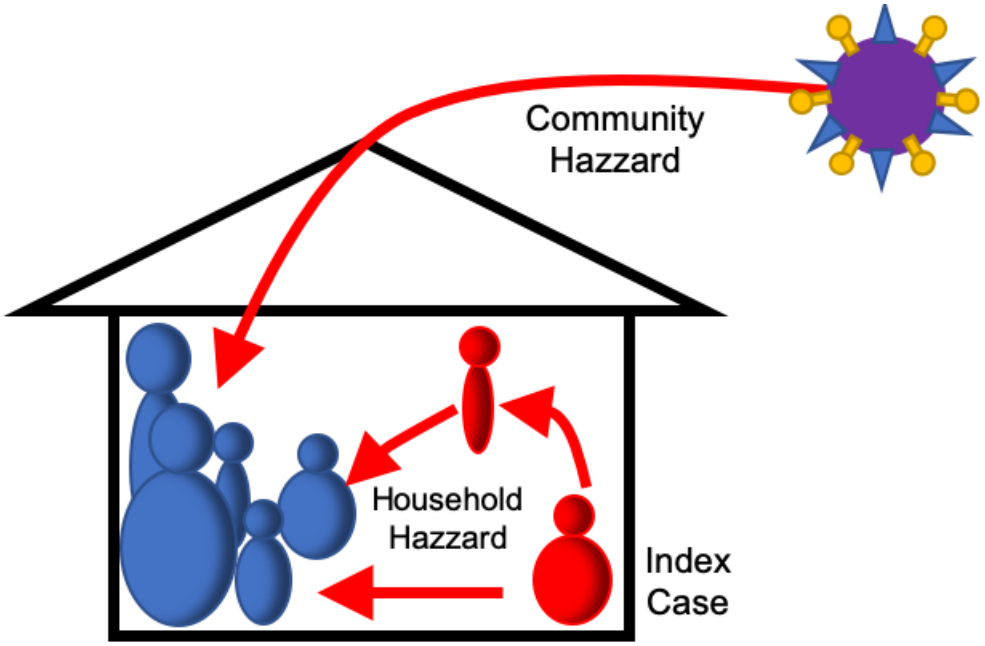

Figure A.1: Household Transmission Model Diagram.

In chapter III we found that obese adults shed influenza A virus longer than non-obese adults. In the work presented in this appendix, we followed up on that finding, by building a model to answer the question of whether obesity is also associated with

increased transmissibility. This model is more appropriate to study factors affecting transmission because it doesnâĂŹt assume that all cases arise from the index case, which logistic regression does, and it allows us to easily account for characteristics of the index case and susceptible contacts.

We set up a an individual hazard-based transmission model as previously developed [6, 7, 88] and broadly used [89, 91, 90, 74, 37], including in this study population [37]. This model estimates factors affecting person-to-person risk of transmission by allowing for transmission from the community as well as chains of transmission within the household (tertiary cases, Figure A.1). We used the same study population as described in chapter IV.

We modeled the hazard of infection of an individual in the household as the sum of the hazards from the community and from the household over the approximately two week intensive monitoring period. The influenza A subtype-specific hazard of transmission from the *community* was modelled, using data from our local pediatric cohort (NPICS), as being proportional to the weekly incidence of influenza A. The hazard (λ) of *household* transmission from infected person i to susceptible contact j was modelled as the sum of the hazards for each day (t) since $i's$ onset to the end of $j's$ susceptible period (either when they became infected or the end of follow-up), $\lambda_{i \to j}(t) = \lambda_h * S_j$, where λ_h is the baseline household transmission hazard and S_j is the factors affecting transmission. The baseline household transmission hazard was a function of the serial interval, which we modeled as having a discretized Weibull distribution (with shape and scale parameters, $W1$ and $W2$), as the Weibull distribution has previously been shown to fit the distribution of influenza serial intervals best [37, 26].

We fit this model in a Bayesian framework, using priors informed by previous work (Table A.1) and sampling new values and updating them using a Markov chain Monte Carlo (MCMC) algorithm as done previously [7, 88]. Based on previous studies'

Table A.1: Household Transmission Model Parameter Prior Values

Parameter	Description	Prior (mean, sd)
W1*	Weibull shape	4, 1
W2	Weibull scale	2, 1
hh_base	baseline household transmission	0.01, 0.3
c_base	baseline community transmission*	0.04, 0.2
j_age	age (<35)	0.5, 1
j_obese	obesity of susceptible contact	0.75, 5
j_male	male sex	0.75, 1
j_obese_age	obses age interaction term	0, 4

*this parameter is then scaled by multiplying by the relative size of the epidemic, peak = 1

estimates of the serial interval (2-4 days generally [7, 91, 26], and 3.1 days in this setting [37]), we constrained the model so that that 50% of secondary cases should occur within 4 days of the onset in the first case. We also constrained the baseline household and community hazard parameters to be non-negative.

We looked at models of RT-PCR-confirmed infection depending on obesity of either the household contact or the infected person, to examine the effect of obesity on influenza virus susceptibility and transmissibility. Models were adjusted for age category of the household contact. Convergence was assessed visually. Analyses were performed in R version 3.5.2 (http://www.R-project.org/).

Results from this transmission model (Figure A.2) do confirm our findings from chapter IV for A(H1N1)pdm: obese older adults (35-85) had 2.89 times higher risk of symptomatic H1N1pdm infection (Hazard Ratio (HR) 2.89 95% Credible Interval: 1.01, 8.00), and a non-significant increase in risk of H1N1pdm infection (HR: 1.51, 95% CI: 0.66-3.42), compared to non-obese older adults, in models adjusted for age and sex. These associations are smaller than what we found with logistic regression (Figure 4.3), and are likely closer to the true value because the OR can overestimate when the outcome is common.

Once enough data is available, we will use this model to examine the effects of

obesity on transmissibility of influenza in the household setting.

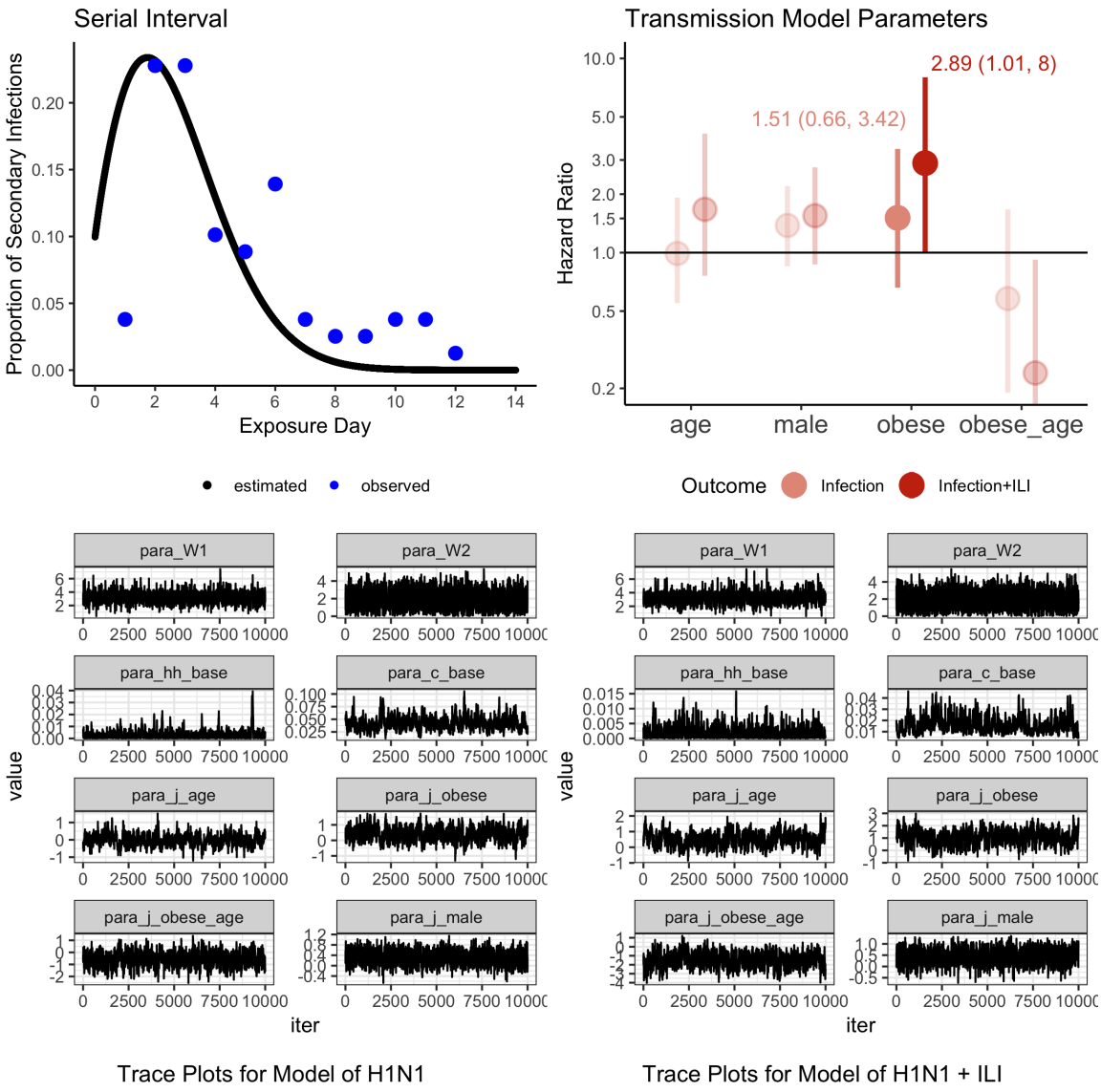

Figure A.2: Household Transmission Models of Susceptibility to H1N1pdm Infection and Symptomatic Infection. Models adjust for age (<35y), male, obesity, and an age (<35y) by obesity interaction term. (A): estimated and observed Serial Interval. The serial interval has a Weibull distribution, with shape and scale parameters W1 and W2, estimates shown in C and D. (B): Hazard Ratios (HR) for model parameters. HRs for obesity are printed. (C) and (D): MCMC trace plots of model parameters for H1N1pdm (C) and symptomatic H1N1pdm (D).

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