Evolutionary Dynamics of Influenza Type B in the Presence of Vaccination:

An Ecological Study

by

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ABSTRACT

Understanding the evolutionary dynamics of influenza type B in human hosts is a public health concern as we strive to minimize the disease burden in seasonal epidemics. Vaccination is considered the best defense against contracting influenza, and everyone over the age of 6 months is advised to get vaccinated before each season. The effect that vaccine-acquired immunity has on the evolution of influenza B remains unclear. In the U.S., vaccine-uptake is irregular across the states, and the differing coverages present an opportunity to study how vaccination influences viral evolution. This thesis analyzes the evolutionary patterns of influenza B in the presence of vaccine-induced selective pressure. Using an ecological study design, estimates on statewide vaccination coverages from the Centers for Disease Control and Prevention were related to influenza B sequence data. The phylogenies and the frequencies of single nucleotide polymorphisms for high and low coverage states across three influenza seasons were compared to evaluate if there was evidence of vaccination influencing evolution. Overall, the results show that vaccination does not significantly impact the evolutionary dynamics of influenza B with both high and low coverage states showing interspersed phylogenetic trees and similar antigenic diversities.

CHAPTER 1:

INTRODUCTION

Influenza, an acute respiratory disease commonly known as *the flu*, is the result of a viral infection. Illness due to influenza can be anywhere from mild to severe, with symptoms appearing one to four days after exposure and successful infection of a host (Heymann, 2014). Figure 1.1 outlines a typical manifestation of influenza from exposure to recovery. Upon infection of a host, influenza virions will begin to replicate within the epithelial cells in the nose, throat, and lungs (Taubenberger & Morens, 2008). The clinical presentation of the illness lasts, on average, from three to seven days, and symptoms include fatigue, coughing, and a sore throat among others (Heymann, 2014).

Ideally, individuals infected with influenza will recover within seven days, and viral shed-

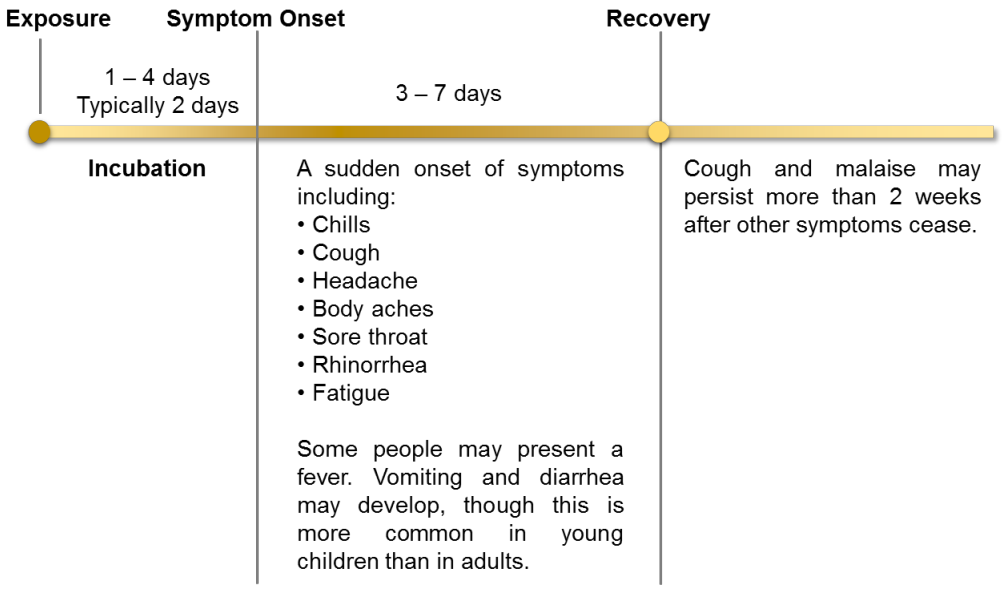

Figure 1.1: Clinical presentation of disease due to influenza infection.

ding will cease. However, even typical presentations can cause significant disease burden. The Centers for Disease Control and Prevention maintains statistics on the estimated number of cases for each influenza season in the United States. Epidemics from the 1979-1980 season up until the 2000-2001 season resulted in an estimated average 226,000 hospitalizations per influenza epidemic (Thompson et al., 2004). The 2016-2017 season presented an estimated 30.9 million reported cases (Centers for Disease Control and Prevention, 2018), and a cumulative incidence for hospitalization of 65 per 100,000 population (Blanton et al., 2017). The following 2017-2018 season was particularly severe. The epidemic, spanning from October 1, 2017, to April 30, 2018, recorded a cumulative incidence of 106.6 influenza-related hospitalizations per 100,000 population and is reported to be the deadliest in over a decade (Garten et al., 2018). Mortality was markedly high in pediatric age groups, and in adults 65 years or older. It is noteworthy that age is among the risk factors for severe influenza. Children younger than 5 and adults 65 years or older are considered high-risk groups for developing complications.

In general, anyone is susceptible to suffering severe complications due to influenza, and these are more likely to present when new strains of the virus, for which immunity has not yet developed, begin circulating in the human population. In the United States, the influenza season spans from October up until May of the following year, and cases spike during the months of February and March. Similar to other diseases, influenza cannot spread as readily in populations with high levels of immunity. Before the beginning of each season, scientists predict the most prevalent influenza strains and develop a vaccine that confers protection. Vaccination is considered the best defense against contracting influenza, and everyone over the age of 6 months is advised to get vaccinated, with an emphasis on individuals at high risk of serious complications.

The immunity conferred from vaccination is known to decline over time, and it is recommended to get a new dose before the start of, or even during, the seasonal epidemic. Aside from waning protection, the virus itself is constantly mutating, making previous vaccina-

tions ineffective. Viral evolution occurs as an attempt to evade the host immune system. Hosts develop natural immunity as a result of infection by creating antibodies to combat the invading virus. In response to this pressure, the influenza virus will alter its structure to avoid detection and continue within-host replication. Analogous to the natural immune system, vaccines stimulate antibody production in the host and, as such, they exert a similar pressure to evolve on the invading virus. However, the overall significance of this pressure in viral evolution remains unclear.

1.1 Statement of the Problem

Understanding the evolutionary dynamics of influenza in human hosts is a concern in public health as we attempt to avoid the next pandemic and minimize disease burden in seasonal epidemics. The role vaccinations play in the process is uncertain as the mechanisms driving mutation are both several and complex. Despite recommendations, vaccine uptake remains irregular in the United States with some states showing lower coverage compared to others. The differing coverage presents an opportunity to study how vaccination influences viral evolution.

Presently, studies regarding the effects of vaccination on evolutionary dynamics have focused on influenza A subtypes (Boni, 2008; Chong & Ikematsu, 2017; Debbink et al., 2017; Dinis et al., 2016), following a long established trend where influenza research centers around influenza A. Previous studies have shown influenza type B to evolve at a slower rate compared to influenza A (Berton, Naeve, & Webster, 1984; Yamashita, Krystal, Fitch, & Palese, 1988; Webster & Berton, 1981), and it is known to cause less disease burden (Glezen, Schmier, Kuehn, Ryan, & Oxford, 2013). However, influenza B still poses a significant public health threat that should not be overlooked.

1.2 Objectives

The purpose of this thesis is to analyze the evolutionary patterns of influenza type B in the presence of vaccine-induced selective pressure. Using an ecological study design, the antigenic diversity for states with high and low vaccine uptake rates will be compared, with the specific objective to answer the question: does influenza type B evolve differently in populations with low vaccination coverage compared to higher coverage populations.

Specific Objectives

To accomplish the objective stated above the following specific objectives are identified:

- Compare the phylogenies of high and low coverage populations to identify evidence of divergence in viral evolution.
- Analyze the antigenic sequences to inspect evidence of clustering by level of vaccination coverage.
- Measure and compare viral diversity by inspecting the frequency of single nucleotide variants in high and low coverage populations.

1.3 Main Contributions

This thesis adds to the body of information available for influenza virus in humans. Specifically, it increases knowledge in two areas that have so far gone understudied. The main contributions are as follows:

1. Increase the body of knowledge available for influenza type B, a generally lesser studied type of influenza.
2. Expand the research regarding the effects of vaccination in viral evolution.

CHAPTER 2:

LITERATURE REVIEW

2.1 Influenza Virus

Influenza is an RNA virus belonging to the *Orthomyxoviridae* family (Couch, 1996). Contrary to DNA viruses, which have deoxyribonucleic acid as their genetic material, RNA viruses use ribonucleic acid to encode genetic information and cannot proofread during replication. The absence of this mechanism results in a higher number of changes in the copied genome called *mutations* (Domingo & Holland, 1997).

An influenza virus can be one of four distinct types: A, B, C, and the most recently discovered type D (Couch, 1996; Hause et al., 2013). Structurally, the virus is characterized by its segmented genome. Types A and B are comprised of eight RNA segments that encode the viral genes, and types C and D are formed by seven segments (Bouvier & Palese, 2008; Su, Fu, Li, Kerlin, & Veit, 2017). The single segment difference between types A and B, and types C and D, is directly related to the cell-binding process that is necessary for viral replication. The proteins responsible for the binding and subsequent release of the virus in influenza A and B are the hemagglutinin (HA) and the neuraminidase (NA), respectively. In types C and D, this dual function is accomplished by the single protein hemagglutinin-esterase-fusion (HEF). All three, HA, NA and HEF, are glycoproteins that reside on the surface of the virus, but, unlike the hemagglutinin-esterase-fusion protein, hemagglutinin and neuraminidase are not antigenically stable. Both the HA and the NA undergo frequent genetic changes in a process known as *antigenic drift* (Couch, 1996).

Human hosts can be afflicted with types A, B and C, though type C has not been

found to cause epidemics. Type D has been found in cattle and is not thought to infect humans. In general, the public health concern for influenza types C and D is much lower due to its antigenic stability (Su et al., 2017). Infection with type C leads to mild symptoms (Jelley et al., 2016), and because the HEF glycoprotein undergoes very little genetic change, it is thought that infection and subsequent recovery from influenza C will result in long-lasting immunity. In fact, studies have shown that humans acquire antibodies to this type of influenza during childhood (Salez et al., 2014; Matsuzaki et al., 2006).

The concern for influenza type A and B is much greater. Both are known to cause seasonal epidemics and disease burden, and influenza type A specifically has the potential to cause pandemics through a process known as *antigenic shift* (Couch, 1996). Although influenza type A and B contain the same number of RNA segments, important genetic distinctions exist in the segments that encode the hemagglutinin and neuraminidase glycoproteins. For influenza type A, the RNA segments responsible for these two proteins can encode for multiple subtypes of both the hemagglutinin and the neuraminidase. So far, there are a total of 18 known HA subtypes and 11 known NA subtypes (Shao et al., 2017). Influenza A is further identified by the combination of subtypes the virus presents (e.g., H3N2 refers to influenza A with hemagglutinin subtype 3 and neuraminidase subtype 2). The existence of multiple subtypes raises the possibility for pandemic influenza, as combinations that have never existed in human hosts or for which immunity has waned, can potentially arise through zoonosis. Perhaps the most notable occurrence of this is the 1918 influenza pandemic, better known as the *Spanish Flu*. This global epidemic is thought to have infected approximately one-third of the worlds population, and while an exact death toll is not available, accepted estimates range around 50 million deaths (Taubenberger & Morens, 2008).

Unlike influenza type A, multiple subtypes for the HA and NA proteins do not exist for influenza B and as such it does not pose the threat of pandemics. Both types, however, do undergo changes in the nucleotide sequences of the encoding RNA. Antigenic drift is said to occur when the accumulation of changes lead to a different expression of amino acids in either

the HA or NA glycoproteins (Mumford, 2007). Seasonal epidemics are the result of antigenic drift, as this process continually generates new strains of influenza type A and type B capable of evading natural host immunity as well as the immunity conferred by vaccinations.

Mechanisms Behind Viral Evolution

For influenza type A and B, the mechanisms driving evolution are several. As an RNA virus, influenza has short replication times with the first sheddings occurring as quickly as 6 hours post-infection (Couch, 1996). Combined with the absence of a proofreading mechanism to detect errors during transcription, the quick replication times lead to a high volume of imperfect copies known as mutants or quasi-species (Domingo & Holland, 1997). Transmission bottlenecks reduce the level of diversity generated during replication, as not all of the mutations are able to infect host cells, eventually dying out.

Genetic drift occurs as random mutations begin to accumulate, changing amino acid sequences and increasing the genetic distance from the original infecting virus. Antigenic characteristics can change due to drift (i.e., antigenic drift) or as the result of *genetic reassortment* (Mumford, 2007). For both influenza type A and B, reassortment can occur when host cells become infected with more than one influenza virus lineage (or subtype lineage for influenza type A) (Maljkovic Berry et al., 2016; Dudas, Bedford, Lycett, & Rambaut, 2015). Because the virus is segmented, recombination events can lead to the genetic material from both co-infecting viruses being used. For influenza type A, if the co-infecting viruses present different surface glycoproteins HA or NA, the reassortment can result in antigenic shift (Brooke, 2017).

The aforementioned mechanisms result in a genetic diversity for both interhost and intrahost populations. Environmental factors also influence evolutionary dynamics, as the size and structure of the host population can either inhibit or promote the mechanisms driving diversity (Mumford, 2007). For example, closed populations are less likely to become co-infected with more than one virus strain thus inhibiting diversity by reassortment. Likewise,

smaller and sparsely populated communities are unlikely to have sustained transmission.

Internally, the survival and continuous replication of mutations depend on the ability to transmit and on the successful evasion of a host immune response. It has been well established that natural host immunity influences the evolutionary dynamics of viruses by forcing the selection of immune escape variants (Bouvier & Palese, 2008; van de Sandt, Kreijtz, & Rimmelzwaan, 2012). However, the effects of vaccine-acquired immunity on the evolution of influenza type A and B are not fully understood.

2.2 Animal Studies

Several animals can become infected with subtypes of influenza A and develop the disease. Similar to humans, vaccination has been used to curb the spread and eradicate the disease from animal populations. Overall, attempts to understand the role that vaccines play in the viral evolution within animal hosts have reached inconsistent results.

The effects of vaccinating poultry have been previously investigated. Lee, Senne, and Suarez (2004) studied the impacts of long-term vaccination in poultry in Mexico. Since 1995, the country had introduced a large-scale vaccination program that continued for several years to eradicate an endemic strain of low-pathogenic avian influenza H5N2. Results showed significant antigenic drift from the vaccine strain used and higher mutation rates for the Mexican lineage strain where ongoing vaccination occurred compared to strains circulating in the United States. Other studies have reported similar results (Salaheldin et al., 2017; Kandeil et al., 2017).

Cattoli et al. (2011) investigated the relationship between different vaccination policies for poultry on the viral evolution of highly pathogenic avian influenza H5N1. In their study, they analyzed and compared H5N1 virus strains from two countries that adopted vaccination to the strains of the same subtype found in three countries that did not apply vaccination. The results of this study indicated evidence of different evolutionary dynamics, with the two countries that vaccinated their poultry showing higher rates of nucleotide substitution in the

HA proteins.

Not all findings have supported vaccine-induced selective pressure in avian influenza. Contrary to what Cattoli et al. (2011) observed, Long et al. (2011) found no correlation between drift mutations and vaccination status. In their study, 15 HA glycoproteins samples were sequenced, belonging to H5N1 subtype virus strains that were circulating in provinces of southern Vietnam. However, only four of the samples came from farms recently implementing vaccination and the percentage of poultry that was vaccinated with the recommended two doses was unknown.

Other animal hosts for influenza have also exhibited evidence of vaccine-induced pressure. A longitudinal challenge study compared the strains of canine influenza from immunologically naive dogs and vaccinated dogs (Hoelzer et al., 2010). Higher mutation rates were observed in the antigens of the vaccinated group suggesting vaccine-induced escape variants. However, the mutations were mostly transient and were no longer observed after a few days.

A study done by Murcia et al. (2013) investigated the evolutionary dynamics present in equine influenza. Four horses were vaccinated and then exposed to a seeder horse with H3N8 equine influenza and subsequently followed throughout the course of their disease. Viral diversity appeared similar to that of naive horses; however, the authors did note that this may be due to a stronger intrahost bottleneck. They reported similar findings when they studied Eurasian avian-like influenza in pigs (Murcia et al., 2012).

2.3 Human Studies

In human studies, research on the effects of vaccination in evolutionary dynamics is limited and has focused mainly on influenza A subtypes and specifically on the hemagglutinin glycoprotein as this antigen is the main target for protection conferring antibodies (Gomez Lorenzo & Fenton, 2013). Attempts to understand viral evolution have generated both mathematical simulations (Carrat, Lavenu, Cauchemez, & Deleger, 2006; Boni, Gog, Andreasen, & Feldman, 2006) and epidemiological studies. Boni (2008) investigated whether antigenic

drift was observable within single seasons (i.e., 1 to 2 years). In total, samples from 10 influenza seasons from different geographic locations were analyzed to see if genetic distances were larger closer to the end of a season. Although some seasons did display a significant positive correlation between genetic distance and time, it was not a general occurrence, indicating that the time-scale over which antigenic drift occurs is not fixed.

In Japan, Chong and Ikematsu (2017) analyzed the HA gene sequences from the H3N2 isolates collected from vaccinated and immunologically naive individuals over four consecutive influenza seasons, from 2011 to 2015. The sequences for both groups were compared to the vaccine strains used during each season, and it was found that samples from vaccinated individuals presented a greater number of amino acid deviations from the vaccine strain. The rates of amino acid differences within epitope sites were also higher in the vaccinated group, suggesting that vaccination results in more antigenic diversity.

Contrary to the results reported by Chong and Ikematsu (2017), Debbink et al. (2017) did not observe any significant impact from vaccination in the intrahost diversity of H3N2. Using samples gathered from a randomized clinical trial of influenza vaccine efficacy, they examined whether vaccination resulted in observable escape variants using a clustering analysis; however, mutations did not appear to cluster by vaccination status. The study by Dinis et al. (2016) obtained similar results that suggested a purifying selection process occurred limiting intrahost diversity.

Overall, no clear consensus has been reached on how vaccination impacts the evolution of influenza. The focus has been mainly on subtypes of influenza A, and in both animal studies and human studies, results have varied. The public health importance to elucidate the evolutionary dynamics of the influenza virus remain relevant given the significant disease burden faced each season.

CHAPTER 3:

MATERIALS AND METHODS

3.1 Study Design

As part of an effort to reduce the morbidity and mortality caused by influenza in the United States, the Centers for Disease Control and Prevention collaborates with public health partners at the state and local levels to conduct year-round surveillance on influenza infections (Jester et al., 2018). The data collected are used to build weekly statistical reports regarding influenza activity across the United States. Specimens that test positive for influenza are also sequenced and submitted to GenBank along with information about the sample including the virus' classification (e.g., type, subtype or lineage) and the geographical location from which it originated (Clark, Karsch-Mizrachi, Lipman, Ostell, & Sayers, 2016). GenBank provides access to publicly available nucleotide sequences for the HA segment of submitted influenza specimens; however, samples do not come annotated with the vaccination status of the host. The CDC estimates vaccination coverage across the country by using data from several nationally representative surveys. By using an ecological study design, these independent data sources can be leveraged to analyze the evolutionary dynamics of influenza.

Ecological studies aggregate information for entire populations rather than individuals and are ideal for correlating risk factors to geographies and temporal trends. The most notorious limitation of this design is the risk of committing an ecological fallacy (Aschengrau & Seage, 2013). That is, inferring that the findings for the population apply to the individuals. Despite limitations, this design is the most appropriate for answering the research

Table 3.1: Seasonal vaccination coverage and sample availability for Victoria. Coverage data for states is presented as a percentage with standard deviation.

	Low Coverage			High Coverage		
Season	State	% (σ)	N	State	% (σ)	N
2015-2016	Florida	29.7 (1.8)	11	Connecticut	42.6 (2.4)	18
2016-2017	Louisiana	31.8 (2.7)	19	Connecticut	43.6 (2.4)	25
2017-2018	California	29.6 (1.6)	19	Delaware	36.4 (2.7)	14

Table 3.2: Seasonal vaccination coverage and sample availability for Yamagata. Coverage data for states is presented as a percentage with standard deviation.

	Low Coverage			High Coverage		
Season	State	% (σ)	N	State	% (σ)	N
2015-2016	Florida	29.7 (1.8)	12	Virginia	40.7 (2.4)	16
2016-2017	Texas	32.9 (2.9)	18	Washington	40.7 (1.8)	11
2017-2018	Florida	22.8 (2.2)	18	Washington	39.5 (2.2)	18

question. This study uses states belonging to the contiguous United States as the units of analysis and evaluates if an association exists between the level of vaccination coverage and the evolutionary patterns of influenza type B.

3.2 Study Population

Three seasons of influenza across states with high and low vaccination coverage were analyzed. Identification of statewide vaccination coverage percentages was done through estimates from the Centers for Disease Control and Prevention for influenza seasons 2015-2016, 2016-2017 and 2017-2018.

To be included in the study, samples had to belong to human hosts that tested positive for influenza type B. The collection date for the samples must have taken place between October 1st and May 31st of the following year to be considered part of the influenza season. All samples must present a complete HA segment and specify the lineage of influenza type B (i.e., Victoria or Yamagata). To be able to adjust for prior immune experience, the host age had to have been provided and fall between 18 and 64 years.

3.3 Data Sources

Sample data for the hemagglutinin segment of influenza B virus was collected using the EpiFlu Database from the GISAID platform (Shu & McCauley, 2017). The Global Initiative on Sharing All Influenza Data (GISAID) was created in 2008 as an alternative to GenBank although data between the two may overlap. The data is public domain; however, users must agree to certain terms of use before being granted access. Any necessary informed consent authorizations for use of the influenza virus sequences are obtained during primary collection and prior to submission to the EpiFlu database.

A total of 2083 records were reviewed for this study. After applying inclusion criteria, 199 records, belonging to states with high and low vaccination coverage across three influenza seasons, were selected. Tables 3.1 and 3.2 describe the vaccination coverage and sample distribution for each of the three influenza seasons. A total of 29 and 28 samples are available for the Victoria and Yamagata lineages, respectively for the 2015-2016 season; 44 Victoria and 29 Yamagata samples are available for the 2016-2017 season; and 33 Victoria and 36 Yamagata samples are available for the 2017-2018 influenza season.

3.4 Statistical Analysis

Statistical differences between host characteristics for high and low coverage populations were evaluated using a two-tailed t-test with p-values under 0.05 considered significant. Fishers exact test was used to determine any significant differences in gender proportions. For analysis of the antigenic diversity, a two-tailed Welch's t-test for unequal variance was used. Multiple comparisons were addressed using a Bonferroni correction resulting in p-values under 0.0125 being considered significant.

3.5 Bioinformatics Analysis

Traditional epidemiological studies deal with rates of disease cases in the presence of exposures. In these studies, attempting to attribute causation consists in building a mathematical model that explains the relationship between the outcome and the exposure. Investigating viral evolution, unlike traditional studies, does not require analyzing rates of disease cases, but rather the changes in the virus. In these studies, the data used for analysis is biological (e.g., nucleotide or amino acid sequences) and concerns the virus itself instead of the carrier host. As such, when working with data from Biological databases, a bioinformatic analysis must be used.

Sequence Alignment

All sequences were imported into the Influenza Research Database (IRD) workbench (Zhang et al., 2017). For each season, sequences were aligned using the MUSCLE alignment algorithm (Edgar, 2004) provided in the workbench.

Phylogenetic Analysis

Just as a genealogical tree shows the ancestry of families through time, a phylogenetic tree shows the evolution of a species. Through inspection of the branches of the tree diagram, it is possible to elucidate how closely related a set of viruses are to one another by examining the formation of clades. A clade is defined as the entire set of organisms that descend from one common ancestor. Theoretically, viruses that have undergone different evolutionary patterns will diverge to separate branches, whereas those with a similar ancestry will appear to cluster closer together.

For each influenza season, a maximum-likelihood phylogenetic tree for the aligned hemagglutinin sequences was built with PhyML 3.0 (Guindon et al., 2010). Using the *Model Compare* feature from IRD, the HKY85 substitution model was identified as providing the highest

parsimony while still maintaining a high log-likelihood. Statistical confidence in the tree was assessed using bootsrapping with $1,000$ replicates.

Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) occur when there is a genetic variation at a singular position in the sequence. Higher frequencies of SNPs lead to more viral diversity and could potentially indicate the presence of a selective pressure driving viral evolution.

Using SNPGenie (Nelson, Moncla, & Hughes, 2015), estimates of non-synonymous (dN) and synonymous (dS) polymorphisms were calculated separately on the aligned sequences for states with high and low levels of vaccination coverage. Standard errors were calculated using bootstrapping ($n = 1000$ replicates) to assess statistical confidence.

CHAPTER 4:

RESULTS

4.1 Host Characteristics

Tables 4.1 and 4.2 show descriptive statistics for the age and gender of the hosts of the viral samples for each of the three influenza seasons. Overall, host characteristics were not significantly different between populations for either lineage in any season. Individuals infected with the Victoria lineage were on average younger than those infected with Yamagata.

Table 4.1: Host characteristics for Victoria samples.

	Low Coverage	High Coverage	*p-value*
2015-2016			
N	11	18	
Age $\bar{x}(\sigma)$	28.18 (9.49)	21.72 (4.43)	0.05
Gender			0.37
Male	4	3	
Female	7	15	
2016-2017			
N	19	25	
Age $\bar{x}(\sigma)$	25.68 (13.88)	29.48 (8.15)	0.30
Gender			0.22
Male	9	7	
Female	10	18	
2017-2018			
N	19	14	
Age $\bar{x}(\sigma)$	27.53 (9.17)	28.07 (6.78)	0.85
Gender			1.0
Male	6	5	
Female	13	9	

Table 4.2: Host characteristics for Yamagata samples.

	Low Coverage	High Coverage	*p-value*
2015-2016			
N	12	16	
Age $\bar{x}(\sigma)$	46.08 (10.00)	49.38 (13.50)	0.46
Gender∗			0.56
Male	10	10	
Female	2	4	
2016-2017			
N	18	11	
Age $\bar{x}(\sigma)$	46.00 (12.96)	47.64 (11.11)	0.72
Gender			0.69
Male	11	8	
Female	7	3	
2017-2018			
N	18	18	
Age $\bar{x}(\sigma)$	45.56 (11.92)	42.44 (15.46)	0.50
Gender∗			0.31
Male	10	6	
Female	8	11	

∗ Gender was missing for some samples.

4.2 Phylogenetic Analysis

Viral sequences that have undergone divergent evolutionary paths will be shown in a phylogenetic tree to appear on different branches. In the presence of a selective pressure capable of driving viral evolution, the expectation would be to see a separation between the sequences confronted with the pressure and those naive to it.

To examine whether vaccination exerts a selective pressure capable of influencing evolution, the maximum-likelihood phylogenetic tree for each influenza season was built using the sequences from both high and low vaccination coverage populations. Figures 4.1-4.6 display the trees for the Victoria and Yamagata lineages for influenza seasons 2015-2016, 2016-2017 and 2017-2018. All trees were drawn using Dendroscope 3 (Huson et al., 2007). Sequences from low coverage populations are highlighted in bold.

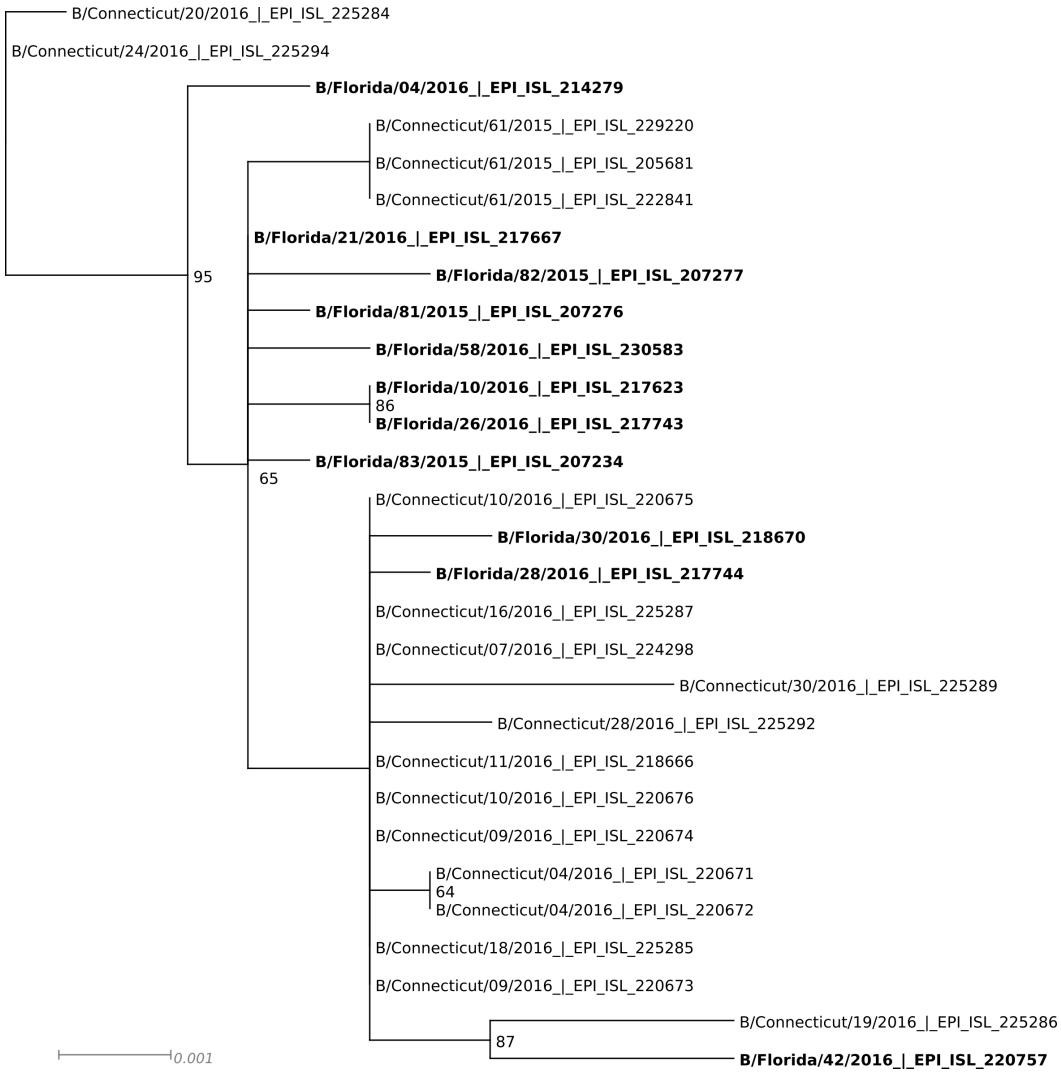

Figure 4.1: Phylogenetic tree for the Victoria 2015-2016 influenza season. The maximum-likelihood bootstrap consensus tree for 1000 replicates is shown with nodes presenting a confidence of <50 collapsed for easier visualization. Sequences from Florida (low coverage) are shown in bold.

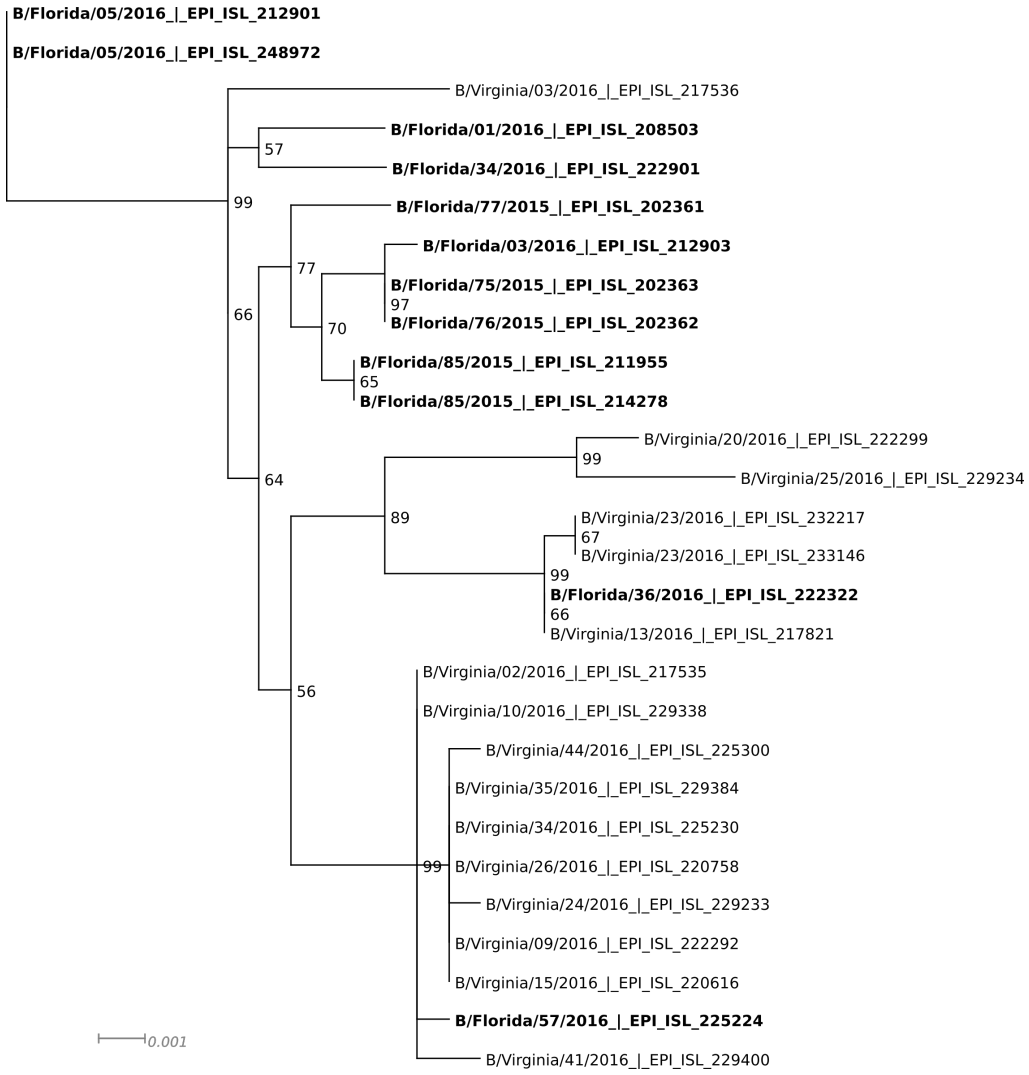

Figure 4.2: Phylogenetic tree for the Yamagata 2015-2016 influenza season. The maximum-likelihood bootstrap consensus tree for 1000 replicates is shown with nodes presenting a confidence of <50 collapsed for easier visualization. Sequences from Florida (low coverage) are shown in bold.

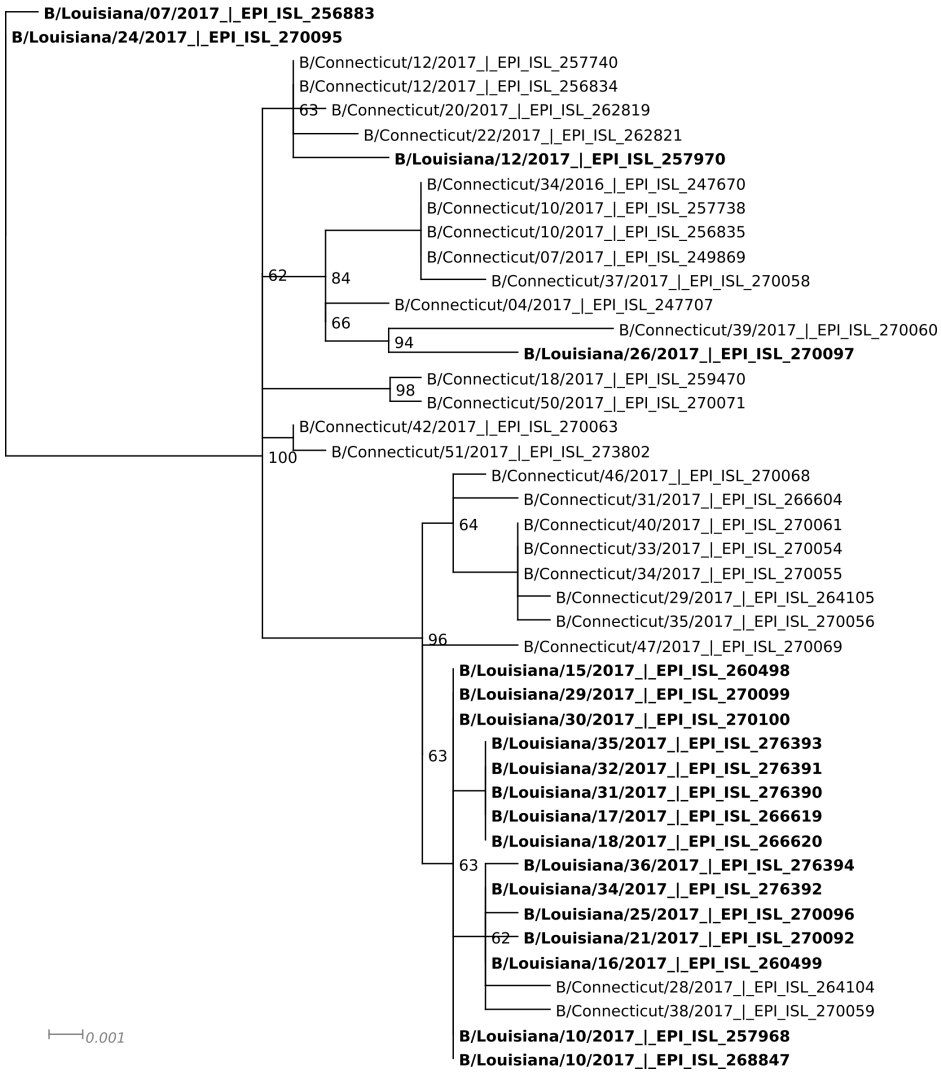

Figure 4.3: Phylogenetic tree for the Victoria 2016-2017 influenza season. The maximum-likelihood bootstrap consensus tree for 1000 replicates is shown with nodes presenting a confidence of <50 collapsed for easier visualization. Sequences from Louisiana (low coverage) are shown in bold.

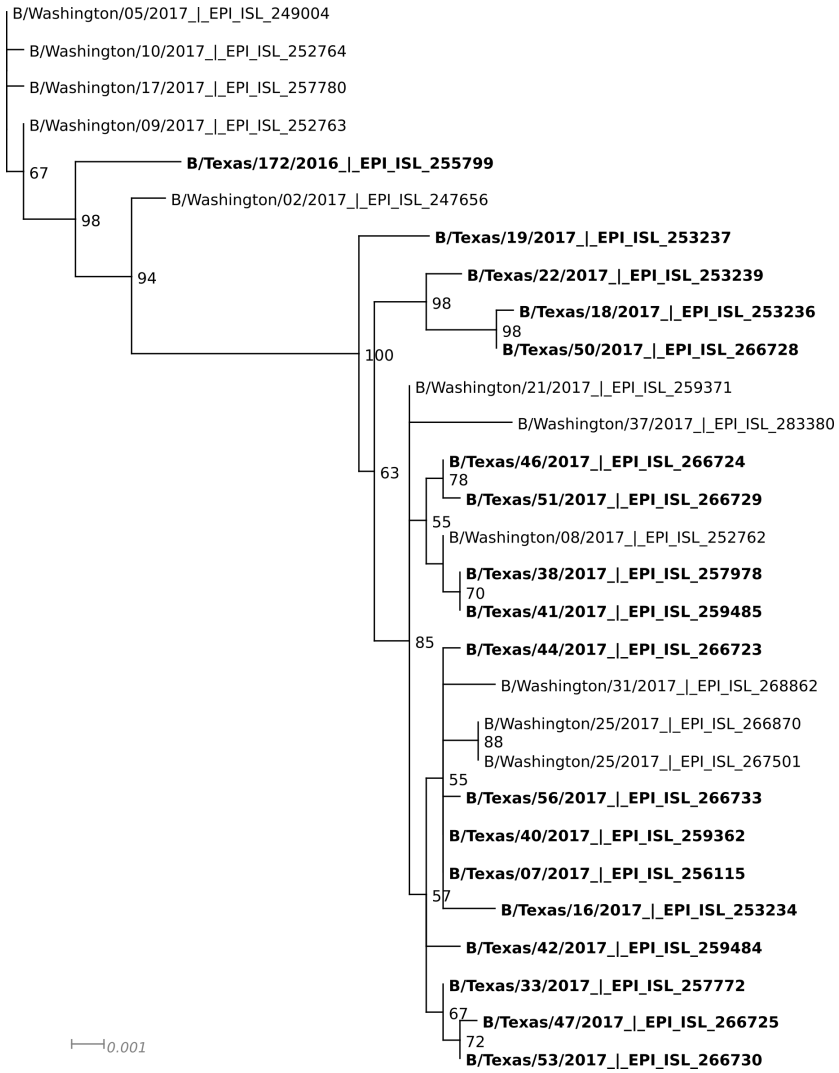

Figure 4.4: Phylogenetic tree for the Yamagata 2016-2017 influenza season. The bootstrap consensus tree for 1000 replicates is shown with nodes presenting a confidence of <50 collapsed for easier visualization. Sequences from Texas (low coverage) are shown in bold.

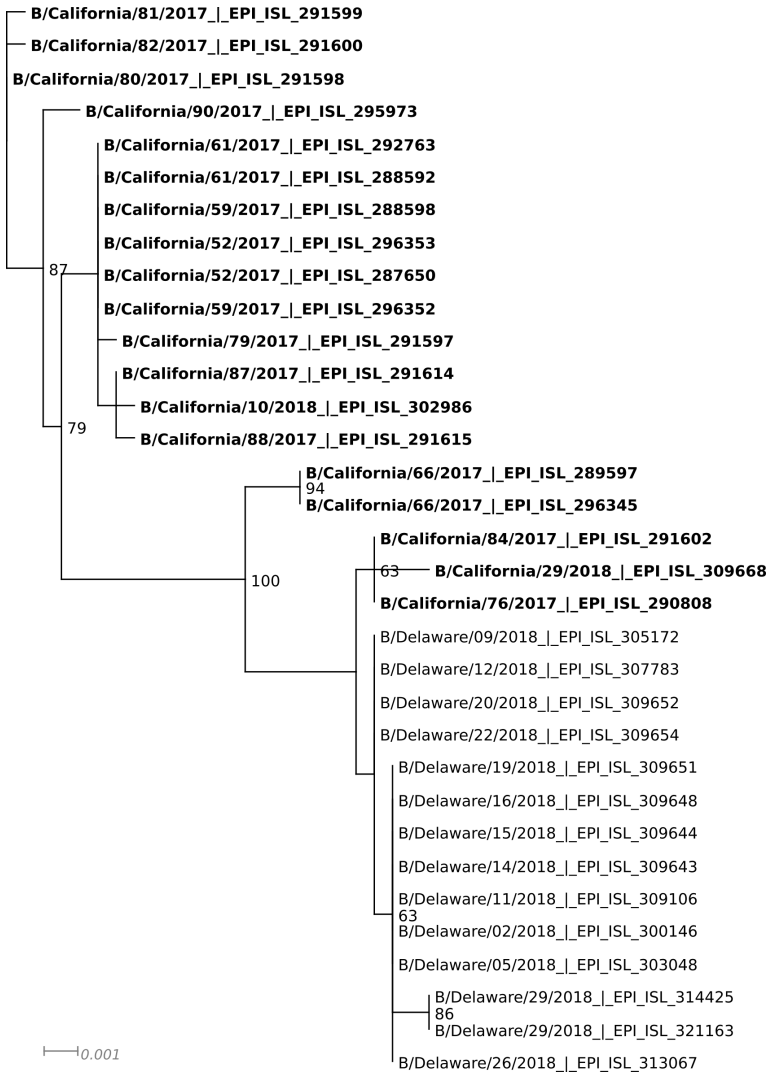

Figure 4.5: Phylogenetic tree for the Victoria 2017-2018 influenza season. The bootstrap consensus tree for 1000 replicates is shown with nodes presenting a confidence of <50 collapsed for easier visualization. Sequences from California (low coverage) are shown in bold.

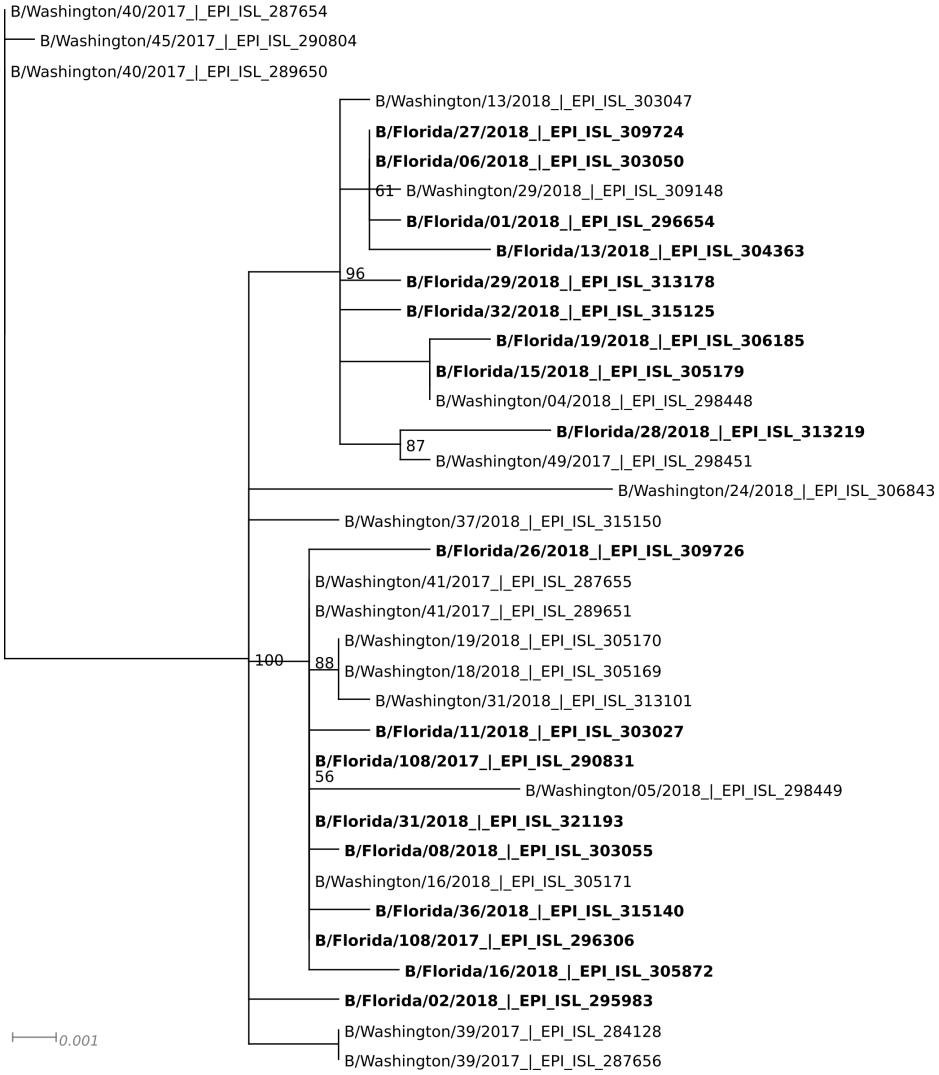

Figure 4.6: Phylogenetic tree for the Yamagata 2017-2018 influenza season. The bootstrap consensus tree for 1000 replicates is shown with nodes presenting a confidence of <50 collapsed for easier visualization. Sequences from Florida (low coverage) are shown in bold.

Overall, the HA viral sequences from populations with high and low vaccination coverage are interspersed throughout the trees and do not appear to cluster based on coverage. One notable exception is the phylogenetic tree for the Victoria lineage of the 2017-2018 influenza season. In this case, the viral sequences from Delaware (high coverage) achieve perfect separation from the samples belonging to California (low coverage), clustering into a single clade. Outside of this instance, the largest clade seen for a high or low coverage population has a total of 9 sequences and belongs to the Victoria lineage seen in Connecticut (high coverage) during the 2015-2016 season.

4.3 Antigenic Diversity

In most instances, even without vaccination, hosts will respond to viral infection by creating antibodies that combat the invading organism. The pressure to evade host antibodies combined with the absence of a proofreading mechanism during replication result in mutations; most notably in the antigens. As such, single nucleotide polymorphisms in the hemagglutinin segment are expected to occur naturally, even without any external pressure. A polymorphism that results in a change of amino acid is called non-synonymous, and alternatively, one that continues to encode the same amino acid is said to be synonymous. The dN and dS statistics calculate the frequency of non-synonymous and synonymous substitutions, respectively. An elevated frequency in these values may be an indication of an outside force influencing evolution.

To evaluate if populations with higher vaccination coverage presented any discernible difference in frequency of SNPs, the dN and the dS statistics were estimated for both high and low coverage states. As done by Dinis et al. (2016), the mean dN and mean dS values were then compared between populations.

Tables 4.3 and 4.4 show the comparisons of the dN and dS statistics, respectively for both the Victoria and Yamagata lineages in each of the three influenza seasons. A statistically significant difference was found in the Victoria lineage for the 2017-2018 season. The

Table 4.3: Comparison of mean dN values for high and low coverage populations.

	Low Coverage		High Coverage		
	dN	SE	*dN*	SE	*p-value*
Victoria					
2015-2016	0.52	0.31	0.87	0.34	0.45
2016-2017	1.45	0.56	2.02	0.69	0.52
2017-2018	1.46	0.64	*NA*		0.03∗
Yamagata					
2015-2016	1.30	0.51	1.32	0.51	0.97
2016-2017	0.66	0.26	2.10	0.84	0.10
2017-2018	0.50	0.21	0.33	0.16	0.53

Mean dN values and standard errors are shown in scientific notation ($\times 10^{-3}$).
All p-values were calculated using a two-tailed Welch's t-test.
$*$ p-value for test evaluating deviance from zero due to zero mean and SE.

Table 4.4: Comparison of mean dS values for high and low coverage populations.

	Low Coverage		High Coverage		
	dS	SE	*dS*	SE	*p-value*
Victoria					
2015-2016	8.31	2.07	5.58	1.75	0.32
2016-2017	8.58	2.15	11.52	2.54	0.38
2017-2018	16.60	3.46	2.19	1.31	<0.001
Yamagata					
2015-2016	18.70	3.67	16.61	3.67	0.69
2016-2017	18.12	3.21	25.91	5.50	0.22
2017-2018	17.32	3.68	20.60	3.69	0.53

Mean dS values and standard errors are shown in scientific notation ($\times 10^{-3}$).
All p-values were calculated using a two-tailed Welch's t-test.

difference in mean dS values was highly significant ($p < 0.001$) with California presenting a higher mean dS compared to Delaware (16.60×10^{-3} vs. 2.19×10^{-3}). In the same year and lineage, Delaware did not exhibit any non-synonymous polymorphisms and presented a standard error equal to zero. Because a zero variance in one sample reduces the formula for Welch's t-test to a one-sample t-test, the p-value obtained for this instance is not a comparison between populations, but rather an evaluation on whether the mean dN value for California deviates significantly from zero. This value was not found to be significant, and no other comparisons between populations achieved statistical significance.

Table 4.5: *dN/dS* ratios for high and low coverage populations.

	Low Coverage		High Coverage	
	dN/dS	*p-value*	*dN/dS*	*p-value*
Victoria				
2015-2016	0.06	<0.001	0.15	<0.01
2016-2017	0.17	<0.01	0.18	<0.001
2017-2018	0.09	<0.001	0	0.09
Yamagata				
2015-2016	0.07	<0.001	0.08	<0.001
2016-2017	0.04	<0.001	0.08	<0.001
2017-2018	0.03	<0.001	0.02	<0.001

Table 4.5 shows the results of calculating the *dN/dS* ratio for high and low coverage states. The *dN/dS* statistic indicates the type of natural selection taking place. A *dN/dS* ratio > 1 shows that positive selection is occurring as more non-synonymous polymorphisms are present, leading to increased viral diversity. Negative selection is said to be taking place when the *dN/dS* ratio is <1, indicating that non-synonymous SNPs are present only at a lower frequency, theoretically as the result of a purification process that removes deleterious mutations. A $dN/dS = 1$ signifies neutrality. All *dN/dS* ratios for both high and low coverage populations were <1 indicating purifying selection. *p*-values were calculated to determine if there was a significant difference from neutrality, with all except one falling under 0.01. The high coverage Delaware population for the Victoria lineage of the 2017-2018 influenza season did not present any non-synonymous polymorphisms and as such had a $dN/dS = 0$ but did not achieve statistical significance ($p = 0.09$).

CHAPTER 5:

DISCUSSION

This thesis set out to determine if there was any evidence indicating a difference in the evolutionary patterns for influenza type B based on vaccine uptake. Using an ecological study design, statewide vaccination coverage estimates were related to biological data for influenza B viruses. Although an ecological study cannot by itself establish a causal link, it can identify associations that merit further consideration.

Influenza vaccine uptake estimates were obtained through data reported to the Centers for Disease Control and Prevention, and states with high and low coverage were identified for three influenza seasons. Nucleotide sequences for the hemagglutinin segment of influenza B viruses were collected for these states using the EpiFlu database. Using bioinformatic tools, the phylogenetic trees and frequencies of single nucleotide polymorphisms were estimated for populations reporting high and low influenza vaccine uptake. Overall, there is minimal evidence suggesting that vaccination induces a significant selective pressure on the viral evolution of influenza type B.

Phylogenetic analyses for the 2015-2016- 2016-2017 and 2017-2018 HA segments were conducted with the expectation that if vaccination was inducing a significant selective pressure, then this would be evidenced by the formation of distinct clades based on vaccination coverage. With one notable exception, the HA segments from both low and high coverage populations were mostly interspersed throughout the trees. These results are consistent with what has been observed for the phylogenies for different strains of the influenza A virus (Chong & Ikematsu, 2017; Debbink et al., 2017; Dinis et al., 2016), suggesting that like with influenza A, vaccination status does not appear to impact the evolution of the influenza B

lineages.

The antigenic diversity present in each of the three influenza seasons was estimated by calculating the number of single nucleotide polymorphisms, both as synonymous and non-synonymous mutations. If vaccination produces a selective pressure on influenza B, then more diversity would be expected in populations with higher coverages. Furthermore, the increased diversity would be reflected in the number of non-synonymous changes present as these are escape mutations that allow the virus to evade antibody recognition. Neither population consistently presented more synonymous mutations compared to the other. However, higher coverage states generally showed a larger amount of non-synonymous substitutions. The exceptions to this trend occurred during the 2017-2018 influenza season in both the Victoria and Yamagata lineages but were not found to be significantly different after applying a Bonferroni correction to adjust for multiple comparisons. Thus, the larger amount of non-synonymous mutations seen for high coverage states provides only weak evidence of vaccination influencing viral evolution.

Natural selection for high and low vaccination coverage populations as measured by dN/dS ratios showed to be, in all instances, purifying. All except one measure deviated significantly from neutrality ($dN/dS = 1$), with the single non-significant result occurring in the 2017-2018 season for the Victoria lineage, where no non-synonymous mutations were identified. Altogether, these results do not support a conclusion of vaccine-induced selective pressure and are in line with what has been observed in other studies. Both Debbink et al. (2017) and Dinis et al. (2016) found antigenic diversity of influenza A strains to be similar between vaccinated and naive individuals and concluded that seasonal vaccination had limited impact on diversity.

The results for the Victoria lineage from the 2017-2018 influenza season appear to stand in contrast to the previously stated findings. During this season, the HA segments from California, the low coverage population, achieved perfect separation in the phylogenetic tree from the samples belonging to Delaware. Moreover, mean dS estimates were found to be

significantly different between the two states. Although a visual inspection of the phylogenetic tree suggests that vaccination may be associated with the diverging evolutionary paths, further analysis of the mutations present in both populations refutes this conclusion. Both mean dS and dN estimates were smaller for Delaware, the high coverage state, indicating less diversity overall. These findings do not align with the expectation that a selective pressure will produce more mutational variants. Furthermore, there were no non-synonymous mutations found in the samples belonging to Delaware indicating that the changes that led to branching events in the phylogenetic tree were likely driven by synonymous mutations. Synonymous mutations generally do not result in immune escape. Lastly, the dN/dS ratio does not deviate significantly from neutrality and as such does not support a finding of positive selection.

Despite findings being mostly consistent with what has been observed in other studies for influenza A, these results cannot be considered thoroughly conclusive, and the limitations of the study should be noted. The ecological study design has a well-known disadvantage that impedes projecting inferences made from a group onto individuals. Samples were collected based on the statewide vaccination coverage percentages the CDC reported; however, the actual vaccination status for the hosts of the viral samples is unknown. As such, it is plausible that the results observed in this study are due to sampling. Further exacerbating this possibility is a small sample size. The median number of samples available for high and low coverage populations infected with the Victoria and Yamagata lineages was 18.5 and 17, respectively. With so few data points, the likelihood of the samples correctly representing the reported coverages is greatly diminished. It should be noted that under an ecological study design, the objective is not to accurately represent the distribution of the variable measured in aggregate, but rather to maximize the probability that the sample holds the actual exposure or outcome of interest. In this thesis, vaccination status is measured in aggregate as a coverage percentage where, ideally, samples from low coverage populations will belong to unvaccinated hosts and vice versa. As is usually the case, larger sample sizes

would benefit the analyses by increasing the likelihood of correctly obtaining the desired exposure. Statistical testing would also be improved as a post hoc analysis revealed the power for the tests shown in tables 4.3 and 4.4 to be consistently under 5%.

Differences in host immunological characteristics may also be confounding analyses and results. As suggested by Dinis et al. (2016)., when possible, analyses should adjust for differences between hosts by grouping participant data by their biological characteristics and analyzing each stratum separately. Such in-depth information about host genetics is rarely if ever collected outside of a specialized study. Instead, variables that code for external traits that may be associated with differences in host genetics are used. The age of the host was used as a proxy to adjust for prior immune experience to influenza B by restricting samples to belong to hosts between 18 and 64 years. Although the age distributions for hosts were not significantly different between low and high coverage populations (tables 4.1 and 4.2), controlling for age may not be sufficient as exposure histories can vary given other factors like socioeconomic status, type of employment, etc. Unlike with age, it was not possible to control for the different racial and ethnic makeup of the states used for the study. Due to disparities in health coverage, vaccine uptake will likely vary among races, and, depending on their ethnic background, hosts may present different biological and immunological characteristics making this a potential source for residual confounding.

This study uses viral samples taken from hosts at a single point in time. As such, it is only a snapshot of the viral evolution taking place during infection. Because no follow-up data were available for analyses, it is uncertain if samples taken at a separate point in time would have produced different results. Although the findings of this study concur with what has been observed for the H3N2 and pandemic H1N1 strains of influenza A virus (Debbink et al., 2017; Dinis et al., 2016), influenza B has been shown to undergo a slower evolution (Berton et al., 1984; Yamashita et al., 1988; Webster & Berton, 1981), and it is possible that any selective pressure produced by vaccination will not be observable within a single season.

Human challenge studies, where volunteers are intentionally infected with a virus to fur-

ther research could answer some of the limitations of this thesis provided they are conducted under ethical guidelines. In the past, challenge trials have been used to evaluate vaccine efficacy for influenza (Balasingam & Wilder-Smith, 2016) as well as for studying the disease's natural history (Carrat et al., 2008). Under a controlled experiment, questions regarding confounding and study power would be more easily addressed. There is also the potential to follow individuals for more than a single influenza season, thus allowing to asses the impact vaccination has over an extended period of time.

Although not thoroughly conclusive, the results obtained in this thesis suggest that vaccination does not significantly impact the evolution of either lineage of influenza B. The higher frequencies of non-synonymous substitutions found for higher coverage populations were the only evidence for differing evolution, and more research is needed to evaluate if the trend consistently persists under study designs that produce stronger evidence. Further analysis comparing the divergence between high and low coverage populations to the vaccine strain should also be undertaken. Using the yearly vaccine strain as a reference, Chong and Ikematsu (2017) identified a significantly greater rate of amino acid differences at epitope sites in vaccinated individuals. For this thesis, dN and dS values were calculated using pairwise comparisons between populations and did not show any significant differences. However, whether either population evolves away from the vaccine strain at a different rate is not answered with this analysis.

The purpose of this thesis was to analyze the evolutionary patterns of influenza B in the presence of vaccine-induced selective pressure. The results of this study should not serve as definitive answers, but rather as a basis for further research into viral evolution. This study adds to the body of information available for influenza B virus in humans. Under the persisting threat of another pandemic, research has mostly focused on influenza A subtypes, and influenza B lineages have gone understudied. The main contribution of this work is to increase knowledge about the effects of vaccination in the viral evolution of influenza B.

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APPENDICES

Appendix A: Submitting Laboratories

Table A-1: Authors, originating and submitting laboratories of the sequences.

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI816151	05/03/16	B/Florida/58/2016	Florida Department of Health-Tampa	CDC
EPI745091	02/29/16	B/Florida/30/2016	Florida Department of Health-Tampa	CDC
EPI738441	01/19/16	B/Florida/10/2016	Florida Department of Health-Tampa	CDC
EPI721278	01/14/16	B/Florida/04/2016	Florida Department of Health-Tampa	CDC
EPI694914	12/06/15	B/Florida/83/2015	Florida Department of Health-Tampa	CDC
EPI806671	12/01/15	B/Connecticut/61/2015	Connecticut Department. of Public Health	CDC
EPI777524	04/08/16	B/Connecticut/24/2016	Connecticut Department. of Public Health	CDC
EPI777508	04/19/16	B/Connecticut/28/2016	Connecticut Department. of Public Health	CDC
EPI777484	04/21/16	B/Connecticut/30/2016	Connecticut Department. of Public Health	CDC
EPI777468	03/18/16	B/Connecticut/16/2016	Connecticut Department. of Public Health	CDC
EPI777460	03/24/16	B/Connecticut/19/2016	Connecticut Department. of Public Health	CDC
EPI777453	03/24/16	B/Connecticut/18/2016	Connecticut Department. of Public Health	CDC
EPI777446	03/29/16	B/Connecticut/20/2016	Connecticut Department. of Public Health	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI773016	02/29/16	B/Connecticut/07/2016	Connecticut Department. of Public Health	CDC
EPI765301	12/01/15	B/Connecticut/61/2015	Connecticut Department. of Public Health	CDC
EPI754469	02/09/16	B/Connecticut/10/2016	Connecticut Department. of Public Health	CDC
EPI754465	02/09/16	B/Connecticut/10/2016	Connecticut Department. of Public Health	CDC
EPI754457	02/08/16	B/Connecticut/09/2016	Connecticut Department. of Public Health	CDC
EPI754450	02/08/16	B/Connecticut/09/2016	Connecticut Department. of Public Health	CDC
EPI754442	02/18/16	B/Connecticut/04/2016	Connecticut Department. of Public Health	CDC
EPI754434	02/18/16	B/Connecticut/04/2016	Connecticut Department. of Public Health	CDC
EPI745059	03/02/16	B/Connecticut/11/2016	Connecticut Department. of Public Health	CDC
EPI686370	12/01/15	B/Connecticut/61/2015	Connecticut Department. of Public Health	CDC
EPI755090	03/09/16	B/Florida/42/2016	Florida Department of Health-Jacksonville	CDC
EPI739358	02/17/16	B/Florida/28/2016	Florida Department of Health-Jacksonville	CDC
EPI739350	02/17/16	B/Florida/26/2016	Florida Department of Health-Jacksonville	CDC
EPI738770	02/06/16	B/Florida/21/2016	Florida Department of Health-Jacksonville	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI695253	12/11/15	B/Florida/82/2015	Florida Department of Health-Jacksonville	CDC
EPI695245	12/07/15	B/Florida/81/2015	Florida Department of Health-Jacksonville	CDC
EPI917763	01/10/16	B/Florida/05/2016	Florida Department of Health-Tampa	CDC
EPI776977	04/25/16	B/Florida/57/2016	Florida Department of Health-Tampa	CDC
EPI763438	03/04/16	B/Florida/36/2016	Florida Department of Health-Tampa	CDC
EPI721270	12/21/15	B/Florida/85/2015	Florida Department of Health-Tampa	CDC
EPI714717	01/17/16	B/Florida/03/2016	Florida Department of Health-Tampa	CDC
EPI714701	01/10/16	B/Florida/05/2016	Florida Department of Health-Tampa	CDC
EPI710601	12/21/15	B/Florida/85/2015	Florida Department of Health-Tampa	CDC
EPI700918	01/02/16	B/Florida/01/2016	Florida Department of Health-Tampa	CDC
EPI674905	10/21/15	B/Florida/75/2015	Florida Department of Health-Tampa	CDC
EPI674889	10/14/15	B/Florida/77/2015	Florida Department of Health-Tampa	CDC
EPI765772	02/20/16	B/Florida/34/2016	Florida Department of Health-Jacksonville	CDC
EPI674897	10/14/15	B/Florida/76/2015	Florida Department of Health-Jacksonville	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI808062	04/18/16	B/Virginia/41/2016	Virginia Division of Consolidated Laboratories	CDC
EPI807941	04/02/16	B/Virginia/35/2016	Virginia Division of Consolidated Laboratories	CDC
EPI807598	02/15/16	B/Virginia/10/2016	Virginia Division of Consolidated Laboratories	CDC
EPI777572	04/26/16	B/Virginia/44/2016	Virginia Division of Consolidated Laboratories	CDC
EPI777020	03/16/16	B/Virginia/34/2016	Virginia Division of Consolidated Laboratories	CDC
EPI755098	03/13/16	B/Virginia/26/2016	Virginia Division of Consolidated Laboratories	CDC
EPI739957	02/23/16	B/Virginia/13/2016	Virginia Division of Consolidated Laboratories	CDC
EPI830996	04/21/16	B/Virginia/23/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI825619	04/21/16	B/Virginia/23/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI806775	04/21/16	B/Virginia/25/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI806768	04/21/16	B/Virginia/24/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI763264	04/06/16	B/Virginia/20/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI763208	03/10/16	B/Virginia/09/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI753996	03/26/16	B/Virginia/15/2016	University of Virginia, Medical Labs/Microbiology	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI737780	02/25/16	B/Virginia/03/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI737772	02/27/16	B/Virginia/02/2016	University of Virginia, Medical Labs/Microbiology	CDC
EPI977594	03/15/17	B/Texas/40/2017	Texas Department of State Health Services-Laboratory Services	CDC
EPI969665	03/06/17	B/Texas/33/2017	Texas Department of State Health Services-Laboratory Services	CDC
EPI971059	03/20/17	B/Texas/38/2017	San Antonio Metropolitan Health	CDC
EPI943818	02/15/17	B/Texas/22/2017	San Antonio Metropolitan Health	CDC
EPI943780	01/26/17	B/Texas/16/2017	San Antonio Metropolitan Health	CDC
EPI941873	02/09/17	B/Washington/10/2017	Seattle and King County Public Health Lab	CDC
EPI941865	02/07/17	B/Washington/09/2017	Seattle and King County Public Health Lab	CDC
EPI908918	01/04/17	B/Washington/02/2017	Seattle and King County Public Health Lab	CDC
EPI1089749	02/10/17	B/Washington/37/2017	Washington State Public Health Laboratory	CDC
EPI1020592	04/26/17	B/Washington/31/2017	Washington State Public Health Laboratory	CDC
EPI1011767	04/09/17	B/Washington/25/2017	Washington State Public Health Laboratory	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1009757	04/09/17	B/Washington/25/2017	Washington State Public Health Laboratory	CDC
EPI977663	03/19/17	B/Washington/21/2017	Washington State Public Health Laboratory	CDC
EPI969729	03/04/17	B/Washington/17/2017	Washington State Public Health Laboratory	CDC
EPI941857	01/29/17	B/Washington/08/2017	Washington State Public Health Laboratory	CDC
EPI918011	01/15/17	B/Washington/05/2017	Washington State Public Health Laboratory	CDC
EPI978520	03/20/17	B/Texas/41/2017	Houston Department of Health and Human Services	CDC
EPI978512	03/21/17	B/Texas/42/2017	Houston Department of Health and Human Services	CDC
EPI960416	01/28/17	B/Texas/07/2017	Houston Department of Health and Human Services	CDC
EPI943802	02/13/17	B/Texas/19/2017	Houston Department of Health and Human Services	CDC
EPI943795	02/13/17	B/Texas/18/2017	Houston Department of Health and Human Services	CDC
EPI1008824	03/27/17	B/Texas/56/2017	Baylor Scott and White Health	CDC
EPI1008800	03/18/17	B/Texas/53/2017	Baylor Scott and White Health	CDC
EPI1008792	03/13/17	B/Texas/51/2017	Baylor Scott and White Health	CDC
EPI1008784	03/03/17	B/Texas/50/2017	Baylor Scott and White Health	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1039897	04/19/17	B/Connecticut/51/2017	Connecticut Department. of Public Health	CDC
EPI1026043	04/19/17	B/Connecticut/50/2017	Connecticut Department. of Public Health	CDC
EPI1026027	04/10/17	B/Connecticut/47/2017	Connecticut Department. of Public Health	CDC
EPI1026019	04/09/17	B/Connecticut/46/2017	Connecticut Department. of Public Health	CDC
EPI1025987	03/26/17	B/Connecticut/42/2017	Connecticut Department. of Public Health	CDC
EPI1025971	03/16/17	B/Connecticut/40/2017	Connecticut Department. of Public Health	CDC
EPI1025963	03/14/17	B/Connecticut/39/2017	Connecticut Department. of Public Health	CDC
EPI1025955	03/13/17	B/Connecticut/38/2017	Connecticut Department. of Public Health	CDC
EPI1025947	03/13/17	B/Connecticut/37/2017	Connecticut Department. of Public Health	CDC
EPI1025931	03/10/17	B/Connecticut/35/2017	Connecticut Department. of Public Health	CDC
EPI1025923	03/07/17	B/Connecticut/34/2017	Connecticut Department. of Public Health	CDC
EPI1025915	03/03/17	B/Connecticut/33/2017	Connecticut Department. of Public Health	CDC
EPI1025931	03/10/17	B/Connecticut/35/2017	Connecticut Department. of Public Health	CDC
EPI1025923	03/07/17	B/Connecticut/34/2017	Connecticut Department. of Public Health	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1025931	03/10/17	B/Connecticut/35/2017	Connecticut Department. of Public Health	CDC
EPI1025923	03/07/17	B/Connecticut/34/2017	Connecticut Department. of Public Health	CDC
EPI1025915	03/03/17	B/Connecticut/33/2017	Connecticut Department. of Public Health	CDC
EPI1007822	04/28/17	B/Connecticut/31/2017	Connecticut Department. of Public Health	CDC
EPI1001974	04/20/17	B/Connecticut/29/2017	Connecticut Department. of Public Health	CDC
EPI1001966	04/18/17	B/Connecticut/28/2017	Connecticut Department. of Public Health	CDC
EPI995079	03/22/17	B/Connecticut/22/2017	Connecticut Department. of Public Health	CDC
EPI995063	03/18/17	B/Connecticut/20/2017	Connecticut Department. of Public Health	CDC
EPI978401	03/03/17	B/Connecticut/18/2017	Connecticut Department. of Public Health	CDC
EPI969420	01/26/17	B/Connecticut/12/2017	Connecticut Department. of Public Health	CDC
EPI969404	01/24/17	B/Connecticut/10/2017	Connecticut Department. of Public Health	CDC
EPI963635	01/24/17	B/Connecticut/10/2017	Connecticut Department. of Public Health	CDC
EPI963627	01/26/17	B/Connecticut/12/2017	Connecticut Department. of Public Health	CDC
EPI924150	01/16/17	B/Connecticut/07/2017	Connecticut Department. of Public Health	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI909320	01/09/17	B/Connecticut/04/2017	Connecticut Department. of Public Health	CDC
EPI909030	12/28/16	B/Connecticut/34/2016	Connecticut Department. of Public Health	CDC
EPI1051533	04/11/17	B/Louisiana/36/2017	Louisiana Department of Health and Hospitals	CDC
EPI1051525	04/04/17	B/Louisiana/35/2017	Louisiana Department of Health and Hospitals	CDC
EPI1051520	04/03/17	B/Louisiana/34/2017	Louisiana Department of Health and Hospitals	CDC
EPI1051512	04/03/17	B/Louisiana/32/2017	Louisiana Department of Health and Hospitals	CDC
EPI1051504	04/03/17	B/Louisiana/31/2017	Louisiana Department of Health and Hospitals	CDC
EPI1026272	03/28/17	B/Louisiana/30/2017	Louisiana Department of Health and Hospitals	CDC
EPI1026264	03/27/17	B/Louisiana/29/2017	Louisiana Department of Health and Hospitals	CDC
EPI1026248	03/21/17	B/Louisiana/26/2017	Louisiana Department of Health and Hospitals	CDC
EPI1026240	03/14/17	B/Louisiana/25/2017	Louisiana Department of Health and Hospitals	CDC
EPI1026232	03/13/17	B/Louisiana/24/2017	Louisiana Department of Health and Hospitals	CDC
EPI1026208	03/06/17	B/Louisiana/21/2017	Louisiana Department of Health and Hospitals	CDC
EPI1020474	03/06/17	B/Louisiana/10/2017	Louisiana Department of Health and Hospitals	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1007945	04/19/17	B/Louisiana/18/2017	Louisiana Department of Health and Hospitals	CDC
EPI1007937	04/18/17	B/Louisiana/17/2017	Louisiana Department of Health and Hospitals	CDC
EPI984850	03/27/17	B/Louisiana/16/2017	Louisiana Department of Health and Hospitals	CDC
EPI984842	03/27/17	B/Louisiana/15/2017	Louisiana Department of Health and Hospitals	CDC
EPI971002	03/07/17	B/Louisiana/12/2017	Louisiana Department of Health and Hospitals	CDC
EPI970988	03/06/17	B/Louisiana/10/2017	Louisiana Department of Health and Hospitals	CDC
EPI964011	02/22/17	B/Louisiana/07/2017	Louisiana Department of Health and Hospitals	CDC
EPI1228854	03/01/18	B/California/29/2018	California Department of Health Services	CDC
EPI1196436	01/30/18	B/California/10/2018	California Department of Health Services	CDC
EPI1165288	10/27/17	B/California/52/2017	California Department of Health Services	CDC
EPI1165280	11/24/17	B/California/59/2017	California Department of Health Services	CDC
EPI1165224	10/22/17	B/California/66/2017	California Department of Health Services	CDC
EPI1162634	12/28/17	B/California/90/2017	California Department of Health Services	CDC
EPI1147819	11/25/17	B/California/61/2017	California Department of Health Services	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1165288	10/27/17	B/California/52/2017	California Department of Health Services	CDC
EPI1165280	11/24/17	B/California/59/2017	California Department of Health Services	CDC
EPI1165224	10/22/17	B/California/66/2017	California Department of Health Services	CDC
EPI1162634	12/28/17	B/California/90/2017	California Department of Health Services	CDC
EPI1147819	11/25/17	B/California/61/2017	California Department of Health Services	CDC
EPI1141681	12/24/17	B/California/88/2017	California Department of Health Services	CDC
EPI1141673	12/22/17	B/California/87/2017	California Department of Health Services	CDC
EPI1141578	12/12/17	B/California/84/2017	California Department of Health Services	CDC
EPI1141562	12/12/17	B/California/82/2017	California Department of Health Services	CDC
EPI1141554	12/12/17	B/California/81/2017	California Department of Health Services	CDC
EPI1141546	12/11/17	B/California/80/2017	California Department of Health Services	CDC
EPI1141538	12/11/17	B/California/79/2017	California Department of Health Services	CDC
EPI1137617	12/22/17	B/California/76/2017	California Department of Health Services	CDC
EPI1130539	10/22/17	B/California/66/2017	California Department of Health Services	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1124626	11/24/17	B/California/59/2017	California Department of Health Services	CDC
EPI1137617	12/22/17	B/California/76/2017	California Department of Health Services	CDC
EPI1130539	10/22/17	B/California/66/2017	California Department of Health Services	CDC
EPI1124626	11/24/17	B/California/59/2017	California Department of Health Services	CDC
EPI1124578	11/25/17	B/California/61/2017	California Department of Health Services	CDC
EPI1117868	10/27/17	B/California/52/2017	California Department of Health Services	CDC
EPI1273402	05/07/18	B/Delaware/29/2018	Delaware Public Health Lab	CDC
EPI1253844	05/07/18	B/Delaware/29/2018	Delaware Public Health Lab	CDC
EPI1246626	04/17/18	B/Delaware/26/2018	Delaware Public Health Lab	CDC
EPI1228743	02/08/18	B/Delaware/22/2018	Delaware Public Health Lab	CDC
EPI1228727	02/07/18	B/Delaware/20/2018	Delaware Public Health Lab	CDC
EPI1228719	02/07/18	B/Delaware/19/2018	Delaware Public Health Lab	CDC
EPI1228695	02/06/18	B/Delaware/16/2018	Delaware Public Health Lab	CDC
EPI1228664	02/04/18	B/Delaware/15/2018	Delaware Public Health Lab	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1228656	02/01/18	B/Delaware/14/2018	Delaware Public Health Lab	CDC
EPI1225766	03/09/18	B/Delaware/11/2018	Delaware Public Health Lab	CDC
EPI1220788	03/20/18	B/Delaware/12/2018	Delaware Public Health Lab	CDC
EPI1206765	02/26/18	B/Delaware/09/2018	Delaware Public Health Lab	CDC
EPI1196880	01/27/18	B/Delaware/05/2018	Delaware Public Health Lab	CDC
EPI1182443	01/19/18	B/Delaware/02/2018	Delaware Public Health Lab	CDC
EPI1273639	05/07/18	B/Florida/31/2018	Florida Department of Health-Tampa	CDC
EPI1257422	05/26/18	B/Florida/36/2018	Florida Department of Health-Tampa	CDC
EPI1257306	05/09/18	B/Florida/32/2018	Florida Department of Health-Tampa	CDC
EPI1247801	04/19/18	B/Florida/28/2018	Florida Department of Health-Tampa	CDC
EPI1247460	04/24/18	B/Florida/29/2018	Florida Department of Health-Tampa	CDC
EPI1229301	04/07/18	B/Florida/26/2018	Florida Department of Health-Tampa	CDC
EPI1212154	03/12/18	B/Florida/19/2018	Florida Department of Health-Tampa	CDC
EPI1206820	02/26/18	B/Florida/15/2018	Florida Department of Health-Tampa	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1196896	01/24/18	B/Florida/06/2018	Florida Department of Health-Tampa	CDC
EPI1196736	02/06/18	B/Florida/11/2018	Florida Department of Health-Tampa	CDC
EPI1167320	01/02/18	B/Florida/01/2018	Florida Department of Health-Tampa	CDC
EPI1162714	01/08/18	B/Florida/02/2018	Florida Department of Health-Tampa	CDC
EPI1229285	04/07/18	B/Florida/27/2018	Florida Department of Health-Jacksonville	CDC
EPI1210465	03/05/18	B/Florida/16/2018	Florida Department of Health-Jacksonville	CDC
EPI1202639	02/20/18	B/Florida/13/2018	Florida Department of Health-Jacksonville	CDC
EPI1196929	01/28/18	B/Florida/08/2018	Florida Department of Health-Jacksonville	CDC
EPI1164971	12/05/17	B/Florida/108/2017	Florida Department of Health-Jacksonville	CDC
EPI1137799	12/05/17	B/Florida/108/2017	Florida Department of Health-Jacksonville	CDC
EPI1206749	03/02/18	B/Washington/19/2018	Seattle and King County Public Health Lab	CDC
EPI1206741	03/02/18	B/Washington/18/2018	Seattle and King County Public Health Lab	CDC
EPI1196872	02/02/18	B/Washington/13/2018	Seattle and King County Public Health Lab	CDC
EPI1175148	01/12/18	B/Washington/05/2018	Seattle and King County Public Health Lab	CDC

Table A-1 (Continued)

Segment ID	Collection date	Isolate name	Originating Lab	Submitting Lab
EPI1175140	01/08/18	B/Washington/04/2018	Seattle and King County Public Health Lab	CDC
EPI1257502	04/10/18	B/Washington/37/2018	Washington State Public Health Laboratory	CDC
EPI1246875	04/05/18	B/Washington/31/2018	Washington State Public Health Laboratory	CDC
EPI1226102	03/28/18	B/Washington/29/2018	Washington State Public Health Laboratory	CDC
EPI1214776	03/18/18	B/Washington/24/2018	Washington State Public Health Laboratory	CDC
EPI1206757	02/13/18	B/Washington/16/2018	Washington State Public Health Laboratory	CDC
EPI1175164	12/15/17	B/Washington/49/2017	Washington State Public Health Laboratory	CDC
EPI1137585	11/30/17	B/Washington/45/2017	Washington State Public Health Laboratory	CDC
EPI1130965	10/25/17	B/Washington/41/2017	Washington State Public Health Laboratory	CDC
EPI1130957	10/12/17	B/Washington/40/2017	Washington State Public Health Laboratory	CDC
EPI1117914	10/15/17	B/Washington/39/2017	Washington State Public Health Laboratory	CDC
EPI1117906	10/25/17	B/Washington/41/2017	Washington State Public Health Laboratory	CDC
EPI1117898	10/12/17	B/Washington/40/2017	Washington State Public Health Laboratory	CDC
EPI1094295	10/15/17	B/Washington/39/2017	Washington State Public Health Laboratory	CDC

Appendix B: IRB Study Approval

USF
UNIVERSITY OF
SOUTH FLORIDA

RESEARCH INTEGRITY AND COMPLIANCE
Institutional Review Boards, FWA No. 00001669
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799
(813) 974-5638 • FAX (813) 974-7091

4/4/2019

Lindsey Fiedler
College of Public Health
Tampa, FL 33612

RE: **Expedited Approval for Initial Review**
IRB#: Pro00040038
Title: Evolutionary Dynamics of Influenza Type B in the Presence of Vaccination: An Ecological Study

Study Approval Period: 4/4/2019

Dear Dr. Fiedler:

On 4/4/2019, the Institutional Review Board (IRB) reviewed and **APPROVED** the above application and all documents contained within, including those outlined below. **Please note this study is approved under the 2018 version of 45 CFR 46 and you will be asked to confirm ongoing research annually in place of a full Continuing Review. Amendments and Reportable Events must still be submitted per USF HRPP policy.**

Approved Item(s):

Protocol Document(s):

Proposal

It was the determination of the IRB that your study qualified for expedited review which includes activities that: (1) present no more than minimal risk to human subjects, and (2) involve only procedures listed in one or more of the categories outlined below. The IRB may review research through the expedited review procedure authorized by 45 CFR 46.110 and 21 CFR 56.110. The research proposed in this study is categorized under the following expedited review category:

(5) Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).

Your study qualifies for a waiver of the requirements for the informed consent process for this retrospective chart review as outlined in the federal regulations at 45 CFR 46.116 (f), which states that an IRB may approve a consent procedure which does not include, or which alters, some or all of the elements of informed consent, or waive the requirements to obtain informed consent provided the IRB finds and documents that: (1) the research involves no more than minimal risk to the subjects; (2) the research could not practicably be carried out without the requested waiver or alteration; (3) if the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format; (4) the waiver or alteration will not adversely affect the rights and welfare of the subjects; and (5) whenever appropriate, the subjects or legally authorized representatives will be provided with additional pertinent information after participation.

As a reminder, please contact USF IT at help@usf.edu to set up your Box.com study folder before storing data on the cloud. You will need to include the name of the Principal Investigator (folder owner), study title, data to be stored, and a list of IRB-approved study team members in your email to USF IT. For additional information, please see section 12.2 of USF HRPP Policy.

As the principal investigator of this study, it is your responsibility to conduct this study in accordance with IRB policies and procedures and as approved by the IRB. Any changes to the approved research must be submitted to the IRB via an Amendment for review and approval. Additionally, all unanticipated problems must be reported to the USF IRB within five (5) business days.

We appreciate your dedication to the ethical conduct of human subjects research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

V Jorgensen MD

E. Verena Jorgensen, M.D., Chairperson
USF Institutional Review Board