

# Phylogenetic Analysis of Seasonal Influenza A H3N2 in Florida from 2017 - 2019

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## Abstract

Influenza A virus subtype H3N2 is widely associated with seasonal influenza and continues to evolve in response to selective pressure of the host immune system in order to avoid antigen recognition and an immune response. Here, we constructed a phylogenetic tree to assess and compare trends of H3N2 strains in Florida during the 2017/2018 and the current 2018/2019 seasons using the HA gene sequences. The analysis showed that the majority of viruses in the 2017/2018 season belong to the 3C.2a clade and that a possible shift in subclade of predominant circulating Influenza A strains between last year and the current year may be occurring.

## Introduction

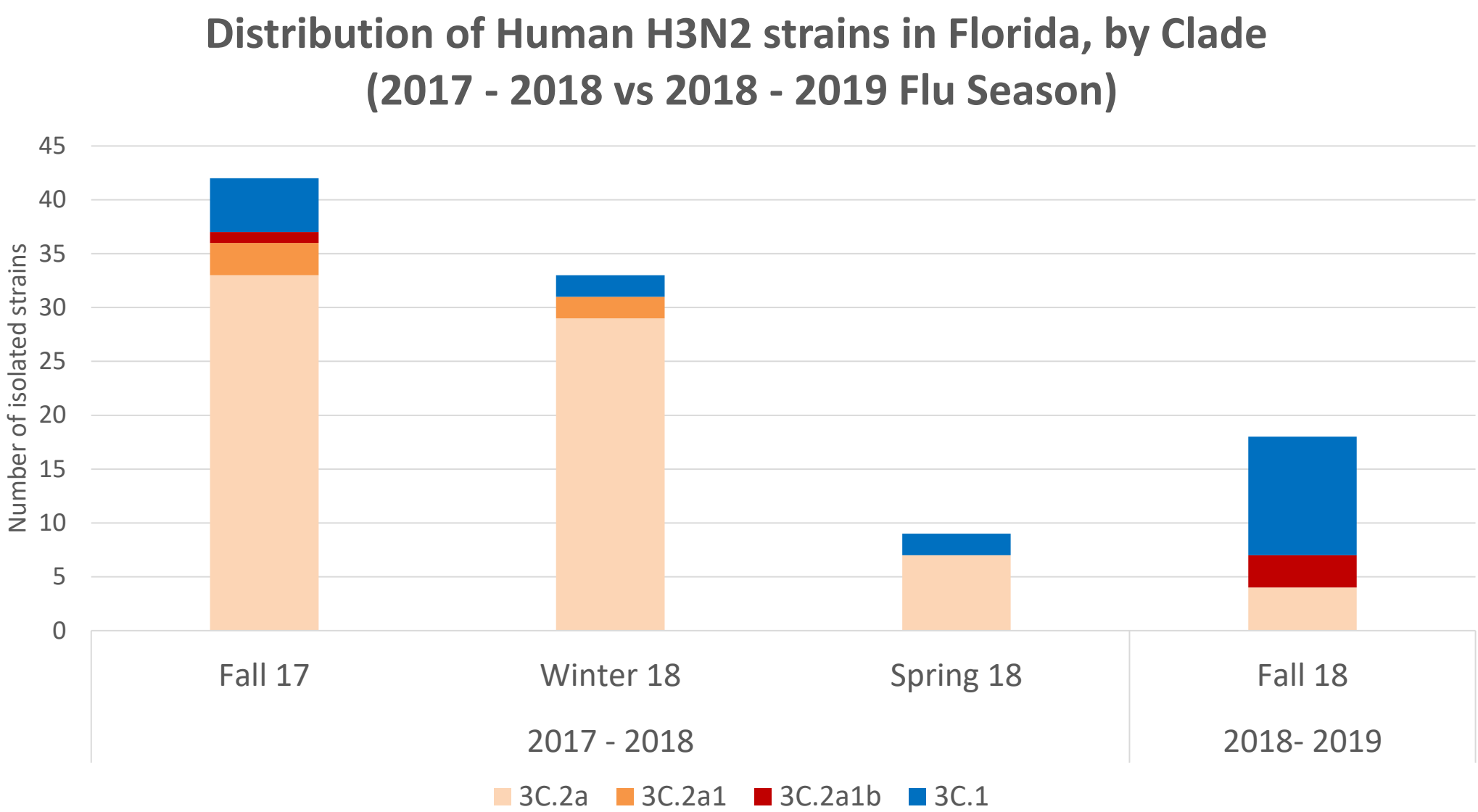
The influenza A virus is a negative-sense, single-stranded RNA with a genome of 8 segments. It is known to be highly infectious and has several subtypes determined by hemagglutinin (HA) and neuraminidase (NA) glycoproteins seen in Figure 3. These are antigens recognized by the immune system and trigger an immune response. Within the United States, the Center for Diseases Control (CDC) estimated that influenza-associated deaths ranged from a low of 12,000 (in 2011-2012) to a high of 56,000 (in 2012-2013) over four years [4].

Of the many distinct Influenza A subtypes, H3N2 is recognized as one of the most dominant, widespread strains in the human population particularly during flu season. In the Northern Hemisphere, the season runs from October through May, with January and February typically considered as the peak. In cooperation with other partners in the World Health Organization (WHO), the Center for Disease Control (CDC) routinely tests and recommends strains as components for the seasonal flu vaccine. Selection is based on several factors including the expected dominant circulating subtype and clade for the season.[5] In H3N2, 3C.2a has been the predominant clade in recent seasons starting from 2011-2012 [3].

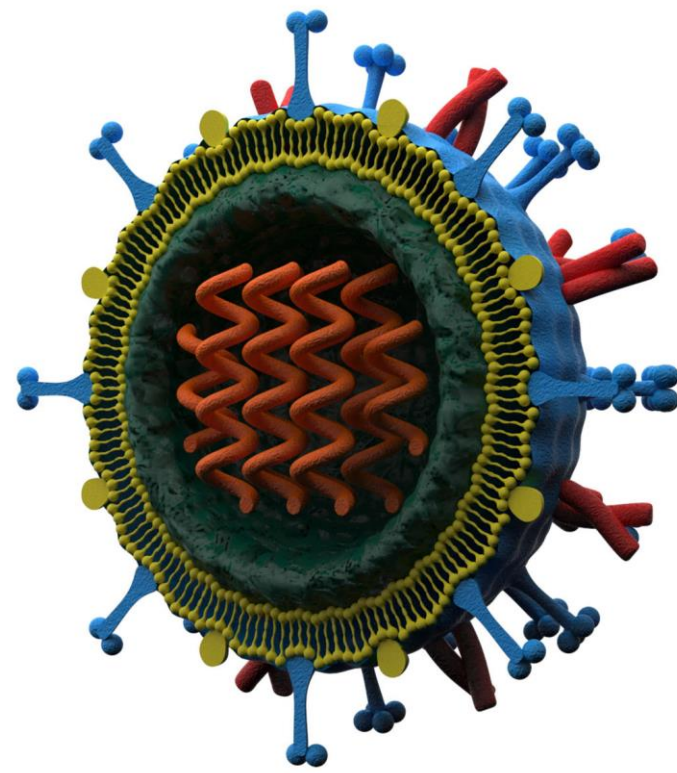
This study looks at strains isolated in Florida from the 2017/2018 and 2018/2019 influenza seasons as of March 1<sup>st</sup>, 2019. Its goal is to examine whether or not 3C.2a continues to be the dominant clade between the previous and current seasons.

## Methods and Results

103 HA sequences from H3N2 strains isolated in Florida from the 2017/2018 and 2018/2019 flu seasons were edited and aligned using the Basic Local Alignment Search Tool (BLAST) provided by the NCBI. Molecular Evolutionary Genetics Analysis software (MEGA) was used to build the phylogenetic tree (Fig 1), subclades are distinguished by amino acid substitutions, noted in blue at their respective nodes. The model was constructed using a maximum-likelihood tree where the tree is determined by the highest probability score. Percentage Identity (seen in Table 1) between reference strains and isolated sample strains for both the nucleotide and amino acid sequences were also calculated in BLAST. The seasonal samples, lineage reference, and vaccine strains were queried from the NCBI Influenza Virus Database. Lineage reference and vaccine strains were selected according to the World Health Organization recommendations for seasonal vaccine compositions and lineage strains [3,5].



**Fig 2. The distribution of sampled strains between flu seasons, defined by clades.** The above chart shows the change in distribution of Florida H3N2 strains from Fall 2017 through Fall 2018. In a year-over-year comparison, 78.6% of Florida strains were within the 3C.2a clade during Fall 2017. In Fall 2018, however, 61% of strains were found to be within another 3C clade.



**Fig 3. Structure of Influenza A Virus**  
A rendering of Influenza A virus (right). Eight, segmented RNA genes (orange) make up the virus genome. The matrix of the virus surrounds the genome (in yellow) and surface proteins that characterize the virus. Neuraminidase is in blue and Hemagglutinin, the gene of interest in this study, is in red.

Year	Clade	Strains	Vaccine	Percentage identity (%) of HA	
				Nucleotide	Amino Acid
2017- 2018	3C.2a	75	A/Hong Kong/4801/2014	99	99
	3C.2a1	1		98.5	98.4
	3C.2a1b	3		98.5	98.4
	3C.1	5		98	97
2018-2019	3C.2a1b	4	A/Singapore/INFIMH-16-0019/2016-like	97.4	96.4
	3C.1	15		98.1	97.1

**Table 1. H3N2 HA gene comparison between vaccine, and the isolated Florida strains, by season.** This is a comparison of the HA genes between the circulating Florida strains and the selected H3N2 component for seasonal influenza for their respective years. Overall, there is a > 97% similarity in the nucleotide sequences from year to year and while the amino acid similarity drops from about 98% similarity to around 96%

## Results and Discussion

- Results**
- Overall, phylogenetic clustering along the tree appears to be chronological.
  - 89% of strains circulating in the 2017 – 2018 season belong to the 3C.2a subclade (similar to A/Hong Kong/1408/2012 strain), seen in orange and green in Figure 1.
  - Of the Florida strains within 3C.2a, 8% belong to further subclades, 3C.2a1 or 3C.2a1b. (see Figure 2)
  - However, 78% the available isolated strains from the current 2018/2019 season (in pink in Figure 1) appear to belong to a 3C.1 subclade (in blue, Figure 2), reflecting the known tendency for multiple, antigenically distinct groups to circulate with higher proportions in some areas – this is a factor that can delay recommendations for upcoming influenza seasons.

**Discussion**  
Although the currently suggested vaccine shares over 95% identity with the circulating strains in the season (see Table 1), these preliminary results suggest that the currently recommended H3N2 component may change in order to maintain effectiveness. This is dependent on continued study using a wider distribution of isolated strains. As of March 21<sup>st</sup>, 2019 – the WHO did change the recommended Vaccine strain. One of the stated reasons for a late recommendation included the different distributions of predominant subclades in different parts of the northern hemisphere.

It should be noted that the 19 available samples in the 2018 – 2019 season are only from October to December and thus, too small to be considered a representative sample. Phylogenetic analysis could be improved with a wider selection (both geographically and chronologically) of H3N2 reference strains. Additionally, a wider selection of samples and reference strains than what is currently available in GenBank could improve the confidence in results, comparable studies also use the GISAID database. Larger data sets will impact model generation times and should be considered when deciding on which method to build trees.

## References

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