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Genomic Evolution of Influenza During the 2023–2024 Season, the Johns Hopkins Health System

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Abstract

Influenza, a human disease caused by viruses in the Orthomyxoviridae family, is estimated to infect 5%-10% of adults and 20%-30% of children annually. Influenza A (IAV) and Influenza B (IBV) viruses accumulate amino acid substitutions (AAS) in the hemagglutinin (HA) and neuraminidase (NA) proteins seasonally. These changes, as well as the dominating viral subtypes, vary depending on geographical location, which may impact disease prevalence and the severity of the season. Genomic surveillance is crucial for capturing circulation patterns and characterizing AAS that may affect disease outcomes, vaccine efficacy, or NA antiviral drug activities. In this study, whole-genome sequencing of IAV and IBV was attempted on positive remnant clinical samples collected from 580 patients between June 2023 and February 2024 in the Johns Hopkins Health System (JHHS). Full-length HA segments were obtained from 424 (72.2%) samples. H1N1pdm09 (71.7%) was the predominant IAV subtype, followed by H3N2 (16.7%) and IBV-Victoria clade V1A.3a.2 (11.6%). Within H1N1pdm09 HA sequences, the 6B.1A.5a.2a.1 (60.5%) clade was the most represented. Full-length NA segments were obtained from 421 (71.7%) samples. Within H1N1pdm09 and IBV, AAS previously proposed to change susceptibility to NAIs were infrequently detected. Phylogeny of HA and NA demonstrated heterogeneous HA and NA H1N1pdm09 and IBV subclades. No significant differences were observed in admission rates or use of supplemental oxygen between different subtypes or clades. Influenza virus genomic

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surveillance is essential for understanding the seasonal evolution of influenza viruses and their association with disease prevalence and outcomes.

Keywords

Influenza; IAV; IBV; 2023/2024 season

Introduction

Influenza viruses circulate seasonally across the globe and have the capacity to cause pandemics of severe disease and high mortality (1). Seasonal epidemics of disease are associated with influenza A (IAV) and B (IBV) (2, 3). In the United States, influenza causes an estimated 65% of economic burden of all vaccine-preventable diseases (4, 5). Certain populations including young children, older adults, individuals with chronic health conditions or underlying conditions and pregnant individuals, are at greater risk for severe disease (6). While IBV mainly infects children and causes milder disease, adults and elderly individuals can develop severe illness and require hospitalization, contributing to the global disease burden (1, 7).

The hemagglutinin (HA) and neuraminidase (NA) glycoproteins are important, not only for their function in viral entry and exit, but also because they serve as antigenic sites targeted by host immune responses (2, 8). Positive selective pressures can drive evolution to evade the host immune response (2). Evolution occurs through multiple mechanisms such as antigenic drift and or antigenic shift (5). Antigenic drift refers to a slow accumulation of mutations that result in amino acid substitutions (AAS), potentially altering glycosylation sites, protein functionality, antigenic properties, host adaptability, and virulence (3). In contrast, antigenic shift involves drastic changes in antigenicity caused by the reassortment of genomic segments between different virus strains, leading to the emergence of new subtypes with significant implications for public health. Mutations in HA and NA occur at different rates for IAV and IBV, with IBV evolving at a slower pace (1, 9).

Predominant influenza subtypes differ by geographical location. During the 2022–2023 season, H3N2 dominated the northern hemisphere’s influenza season (10–12); however, H1N1pdm09 predominated in Eastern Europe and much of Africa (12). China, Pakistan, and India reported co-circulation of IBV-Victoria lineage and H1N1pdm09 viruses (11, 13). Most strikingly was the lack of IBV-Yamagata virus detection globally since the beginning of the COVID-19 pandemic (10). This study investigates the genomic evolution of circulating influenza viruses during the 2023–2024 influenza season and describes the associated clinical presentations and outcomes in a large academic center in the eastern United States.

Methods

Ethical considerations and data availability

This work has been approved by the Johns Hopkins Institutional Review Board. The research was performed under protocols IRB00306448 and IRB00331396. Genomes (HA

and NA segments) are attached as supplementary datasets 1– 6. Genomes are available on the Global Initiative on Sharing All Influenza Data (GISAID) database and accession numbers are listed in Table S9.

Study population

Standard-of-care diagnostic influenza testing was conducted for both inpatients and outpatients across the five Johns Hopkins Hospitals. Testing for influenza A/B was performed with Cepheid Xpert Xpress SARS-CoV-2/Flu/respiratory syncytial virus (RSV) test or the ePlex RP/RP2 respiratory panels (14, 15). The Xpert assay targets the matrix (M), PB2, and PA genes of IAV (no IAV subtyping) and the M and NSP of IBV (16). The ePlex panels on the other hand, in addition to targeting the M gene, are able to subtype IAV as H1, H1–2009, and H3, but do not detect or differentiate IAV NA. Study samples were collected between June 2023 and February 2024. Clinical and demographic data were collected as previously described, including bulk extractions of information relating to positive influenza tests (10, 17, 18).

Nucleic acid extraction and whole genome amplification

IAV and IBV samples were differentiated based on clinical laboratory results. Viral nucleic acid was extracted using the Chemagic Viral RNA/DNA Kit following the manufacturer's instructions (Revvity). The whole genome was amplified as described previously (10, 19). IBV amplification utilized a cocktail of 13 primer pairs as described before (20). Library preparation and next-generation sequencing were performed as we previously described, using R9.4.1 flow cells on a GridION (Oxford Nanopore Technologies) (10).

Virus genome assembly and phylogenetic analysis

Fastq files were analyzed as previously described (10). The alignment of genomes and reference sequences, downloaded from GISAID, was performed using the built-in alignment tool in Nextclade (10). Quality control scores for sequences were assigned using the built-in pipeline available in Nextclade. Sequences with scores of 30 and above were excluded from sequence analysis (21). Sequences were viewed using BioEdit 7.7.1.0. HA and NA sequences with gaps were excluded from the analysis. The phylogenetic trees for the HA and NA were generated using the maximum likelihood method using IQ-Tree version 2.2.6. The visualization was done using FigTree version 1.4.4. Complete reference genomes from GISAID were used in the phylogenetic analysis (Table S7). The ModelFinder in IQ-TREE2, was used to select the best-fitted nucleotide substitution model. The robustness of the tree topology was tested with 1000 nonparametric bootstrap analyses. Bootstrap values >75% were shown on branches of the consensus trees. Nextclade was used to assign subclades based on the hemagglutinin segments. Subclades were confirmed through clustering patterns in the phylogenetic trees. Amino acid substitutions for H1N1pdm09, H3N2, and IBV were assessed using Nextclade compared to their reference genomes (Table S8).

Statistical Analysis

Multivariable logistic regression analyses were performed using STATA/SE 17 to evaluate the odds ratio of admission and the need for supplemental oxygen by subtype and/or clade controlling for patient age, sex, and comorbidities.

Results

Influenza prevalence at JHHS and study cohort

The positivity rate during the 2023/2024 season in JHHS was lower compared to the 2022/2023 season, reaching its peak in late December 2023 (Figure 1). The onset of influenza virus detection coincided with a decline in RSV, a trend observed in both the 2022/2023 and 2023/2024 seasons (Figure 1). Overall, 52,343 tests for influenza and RSV were conducted between June 2023 and February 2024. Among these, 6.5% (3425/52,343) tested positive for either IAV or IBV (Figure 2). IAV accounted for 77.9% of positive tests (2669/3425), while 22.1% were IBV (756/3425) (Figure 2). Out of the 3425 samples that tested positive for influenza, 17.1% (587/3425) were selected for whole genome sequencing (Figure 2). Complete genomes were recovered from 361 (61.5%) plus an additional 63 (10.7%) HA segments which resulted in a total of 424 (72.2%) samples that were used for clade and subclade classifications. The HA sequence analysis showed that 71.7% (304/424) of samples belonged to the H1N1pdm lineage, 16.7% (71/424) to the H3N2 lineage, and 11.3% (48/424) to the B Victoria lineage. Of the total 587 samples, 421 (71.7%) NA complete segments were used for additional analyses (Figure 2).

Metadata of the 2023/2024 cohort was collected for unique patients (580, Table 1). The most represented age group was 18–64 (33.3%), followed by 5–17 years (32.4%) and <5 years (24%) (Table 1). Fever (38.7%, [215/556]), cough (16.2%, [90/556]), and flu-like symptoms (14.2%, [79/556]) were the most commonly reported symptoms (Table 1). Admissions were reported for 23.1% (134/580) of the cohort, with 17.2% (23/134) of admissions requiring ICU level care (Table 1). IBV and H1N1pdm09 infections were found most commonly in children aged 5–17 years (49%, [24/49] and 34.5%, [105/304] respectively) (Table 2). H3N2 infections were most commonly found in adults between 18–64 years old (47.9%, [34/71]) (Table 2). Of the 304 patients infected with H1N1pdm09 and 71 infected with H3N2, 25% (76/304) and 26.7% (19/71) were admitted respectively (Table 2). No statistically significant differences were seen between any of the subclades for the probability of admission or need for supplemental oxygen (Table 3).

Sequence analysis and classification of HA/NA sequences into clades and sub-clades

HA Gene Analysis—Of the 424 samples that were assigned clade and subclade classifications, 71.7% (304/424) were H1N1pdm09 (Table 4). The H1N1pdm09 clades 6B.1A.5a.2a (5a.2a) and 6B.1A.5a.2a.1 (5a.2a.1) were identified from 120 samples (39.5%) and 184 samples (60.5%), respectively. Within the 5a.2a clade, the C.1 and C.1.7 subclades were represented with 83.3% (100/120) and 16.7% (20/120) respectively. Within the 5a.2a.1 clade, 1.1% (2/184) belonged to the C.1.1 subclade, and 98.9% (182/184) belonged to the C.1.1.1 subclade. H3N2 subtype sequences were identified from 16.7% (71/424) of the samples. The majority (97.2%, [69/71]) of sequences were classified

as the 3C.2a1b.2a.2a.3a.1 (2a.3a.1) clade, subclade J.2 (81.2%, [56/69]). IBV sequences represented 11.6% (49/424) of samples and all belonged to the Victoria lineage V1A.3a.2 clade and were primarily C.5.1 subclade (75.5%, [37/49]).

AAS defining the H1N1pdm09 5a.2a and H3N2 clades and lineages (5a.2a.1) were identified in almost all genomes (12, 13). Additionally, our cohort showed T120A and K169Q substitutions in 74% (74/100) and 57% (57/100) of the C.1 genomes, respectively (compared to A/Wisconsin/588/2019) (Table 5). All C.1.7 (20/20) genomes contained the defining AAS D94N and HA2:I206V. T120A was found in 95% (19/20) of genomes but none contained K169Q (Table 5). The C.1.1.1 viruses contained R113K (25.3%, [46/182]) and R45K substitutions (38.5%, [70/182]) (Table 5), which were exclusive to the C.1.1.1 subclade. For H3N2, I223V was found in 92.8% (64/69) of all three subclades (1/1 J, 12/12 J.1, 51/56 J.2) when compared to A/Darwin/6/2021 (Table 5).

The IBV V1A.3a.2 clade genomes had AAS N150K (49/49) and G184E (49/49) identified (Table 5). The subclades C.5, C.5.1 and C.5.6 all contained a N197E substitution (47/47). I117V and A199T AAS were found in all 49 IBV viruses when compared to B/Brisbane/60/2008-egg (Table 5). Further, N129D was found in 81.6% (40/49) of genomes in subclades C.5 (1/2), C.5.1 (37/37) and C.3 (2/2) (Table 5). Three amino acid deletions (K162-, N163- and D164-) were also observed in all genomes (47/47).

NA Gene Analysis—Of the 424 samples with HA subtypes, 421 (99.3%, [421/424]) samples had complete NA gene segments which were used to examine AAS predicted to affect glycosylation patterns or neuraminidase inhibitors (NAI) susceptibility (Figure 2). Within the 304 H1N1pdm09 viruses, six N1 clades were identified (Table 6). The majority of viruses contained the NA C.5.3 (44.4%, [135/304]), followed by C.5 (23.7%, [72/304]) and C.5.2 (22.4%, [68/304]) (Table 6). Within the H3N2 subtype only two N3 clades were identified: B.4 (97.1%, [68/70]) and A.2.2.2 (2.9%, [2/70]) (Table 6). The IBV Victoria lineage had five NA clades: B.7.1 (55.3%, [26/47]), B.7 (21.3%, [10/47]), B.8 (19.1%, [9/47]), B.3 (2.1%, [1/47]) and B (2.1%, [1/47]) (Table 6).

For the H1N1pdm09 sequences, three AAS known to impact NAIs were rarely identified: H275Y (one genome), I223V (one genome) and S247N (three genomes) (22–24). One substitution (K390R (one genome) previously proposed to be in a locus important for N1 antigenicity was identified (Table 7) (8). No AAS suspected of impacting glycosylation or susceptibility to NAIs were detected in H3N2 specimens. For IBV samples, two AAS that may impact NA antigenicity and escape from neutralizing monoclonal antibodies were identified (9). D342N was found in one sample and D342E was identified in another. A395V, which resides in a location known to be important for the function of the NAI peramivir, was identified in 20 genomes (42.55%) (Table 7) (3). Notably, No statistically significant differences were seen between any of the clades and the probability of admission or need for supplemental oxygen (Table 8).

Phylogeny analysis and detection of potential HA/NA subclade reassortments

The Nextclade HA and NA gene analysis were confirmed through a phylogenetic analysis for subtypes H1N1pdm09, H3N2 (Figure 3) and IBV (Figure 4), (also Figures S1–S6). For

H1N1pdm09 viruses, the HA subclades described above (C.1, C.1.7 and C.1.1, C.1.1.1) were found clustered within the respective clades (5a.2a and 5a.2a.10) (Figure 3A). When the phylogeny of the H1N1pdm09 N1 NA segment was examined, five distinct subclades were identified (C.3, C.4, C.5, C.5.1.1, C.5.2 and C.5.3) (Figure 3C). The HA subclade C.1.7 clustered exclusively with the N1 subclade C.4 (Figure 3C). Similarly, the N1 subclade C.5 was identified only in C.1 HA subclades and C.5.1.1 N1 subclade with the HA subclade C.1.1.1 (Figure 3C). However, the C.5.3 N1 subclade was found both within the C.1, C.1.1, and C.1.1.1 HA subclades (Figure 3C). One C.1 HA genome was identified with the C.5.2 N1 subclade (Figure 3C).

For the H3N2 viruses, the HA subclades described above (G.2, J, J.1 and J.2) were found clustered within the respective clades (2b and 2a.3a.1) (Figure 3B). When the phylogeny of the N2 segments were examined, the two distinct subclades were evident (A.2.2.2 and B.4) (Figure 3D). The HA subclade G.2 clustered exclusively with the N2 subclade A.2.2.2 (Figure 3D). The remaining HA subclades (J, J.1 and J.2) were found exclusively with the B.4 N2 subclade (Figure 3D).

When the IBV Victoria lineage HA phylogeny was examined, the subclades described above (C.3, C.5, C.5.1 and C.5.6) clustered within the clade V1A.3a.2 (Figure 4A). When the IBV NA phylogeny was examined, the subclades (B, B.3, B.8, B.7 and B.7.1) were found to distinctly cluster together (Figure 4B). The C.5.1 HA was found with both the B.7 and B.7.1 NA subclades (Figure 4B). The C.5.6 HA viruses were found to have exclusively B.8 NA segments (Figure 4B). One C.5.1 virus (JH24551) was determined to have a B.8 NA subclade (Figure 4B). The C.5 HA viruses were found with different NA subclades, B.3 and B.7, as indicated by the black arrows in Figure 4B.

Discussion

In the years following the emergence of SARS-CoV-2, respiratory virus seasonality was temporarily disrupted (11, 25). Circulation of influenza remained low from March 2020 until May 2021, when H1N1pdm09, H3N2, and IBV resumed at much lower rates, with the striking disappearance of the IBV-Yamagata lineage (10, 11, 25). The 2022–2023 influenza season exhibited activity similar to pre-pandemic seasons, with a national average of 8.9% positivity rates, but peaked earlier than normal in mid-November (24). This early peak was also reported at JHHS, increasing drastically in late 2022 (10). The 2023–2024 activity and seasonality at JHHS was more similar to pre-pandemic seasons, with a 6.5% positivity rate and peak in mid-December (12, 13, 26).

A study from our group reported that in the 2022–2023 influenza season, H3N2, specifically the 2a.2 clade, was dominant (10), consistent with other reports (6, 13, 24). This current season witnessed a shift with the H1N1pdm09, clades 5a.2a and 5a.2a.1 dominating. H1N1pdm09 has been observed to be the dominant subtype circulating in most of the northern hemisphere (13, 26, 27). This shift from H3N2 to H1N1pdm09 was observed in the Russian Federation, with H3N2 dominating the 2021–2022 season and H1N1pdm09, clade 5a.2a, causing a severe 2022–2023 season (28, 29). Peaking at the end of the 2022–2023 season, the V1A.3a.2 clade, which accounted for all IBV detections, circulated similar to

pre-pandemic seasons (6, 10, 24). Similar trends were observed in Canada, Russia, and by the WHO (6, 12, 29).

The percent hospitalization, ICU admission, and supplemental oxygen usage increased when compared to the previous season at JHHS (10). H1N1pdm09 and H3N2 infections were associated with more admissions than IBV, consistent with reports that show that IBV causes less severe infection than IAV (1), though this was not statistically significant in our cohort. The circulation of H1N1pdm09 dominated and was responsible for the majority of severe acute respiratory infections (SARI) in the European 2022–2023 influenza season (30). The WHO also reported that cumulative hospital admissions are the third highest this season (26).

The CDC interim report on vaccine effectiveness (VE) against IAV for the current season reported 59%-67% efficacy for outpatient visits and 52%-61% efficacy for influenza related hospitalizations in children 6 months-17 years (26). Similarly, in adults 18 and older, protection against outpatient visits was 59%-67% and 52%-61% against hospitalizations (26). The WHO has chosen the following vaccine strains for IAV for the 2024/2025 influenza season: A/Victoria/4897/2022 (H1N1pdm09 egg based) and A/Wisconsin/67/2022 (H1N1pdm09 cell culture). The H1N1pdm09 components remain the same as the 2023/2024 vaccine (12, 13). Some AAS (T120A, K169Q, R113K, and R54K) described in our study, were also reported by a recent genomic surveillance study from Arizona (31), however, are not a part of the 2024/2025 H1N1pdm09 vaccine strain. Even though these substitutions are outside the 130-loop, 150-loop, 190-helix and 220-loop, which are found in the receptor binding domain, these changes may affect glycosylation patterns and epitope masking, affecting antibody binding and vaccine effectiveness, therefore, the potential effects of these AAS should be characterized. However, based on the interim VE studies for the 2023/2024 vaccine, efficacy is expected to remain high for IAV.

In conclusion, this study investigated the genomic evolution and circulation of influenza viruses during the 2023–2024 season. Activity remains similar to national and local observations, with seasonality returning to pre-pandemic norms. The dominant subtype circulating has changed from H3N2 in the 2022–2023 season to predominantly H1N1pmd09 in this current season. IBV circulation has increased this season for the first time since the SARS-CoV-2 pandemic. Clinical outcomes indicated a relative increase in hospitalizations when compared to the previous season, which was most likely associated with the widespread circulation of influenza in general. There appears to be heterogeneous circulation of H1N1pmd09 and IBV HA and NA subclades, and though these were not statistically associated with increased morbidity, they do contain rare AAS known to impact antigenicity or NAI susceptibility, and should be followed into future seasons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- The 2023/ 2024 respiratory viral season was predominated by influenza A H1N1pdm09 with the co-circulation of influenza A H2N2 and influenza B Victoria lineage.
- No significant differences were observed in admission rates or use of supplemental oxygen between different subtypes or clades.
- Amino acid substitutions that might impact neuraminidase inhibitors activity were rarely identified.

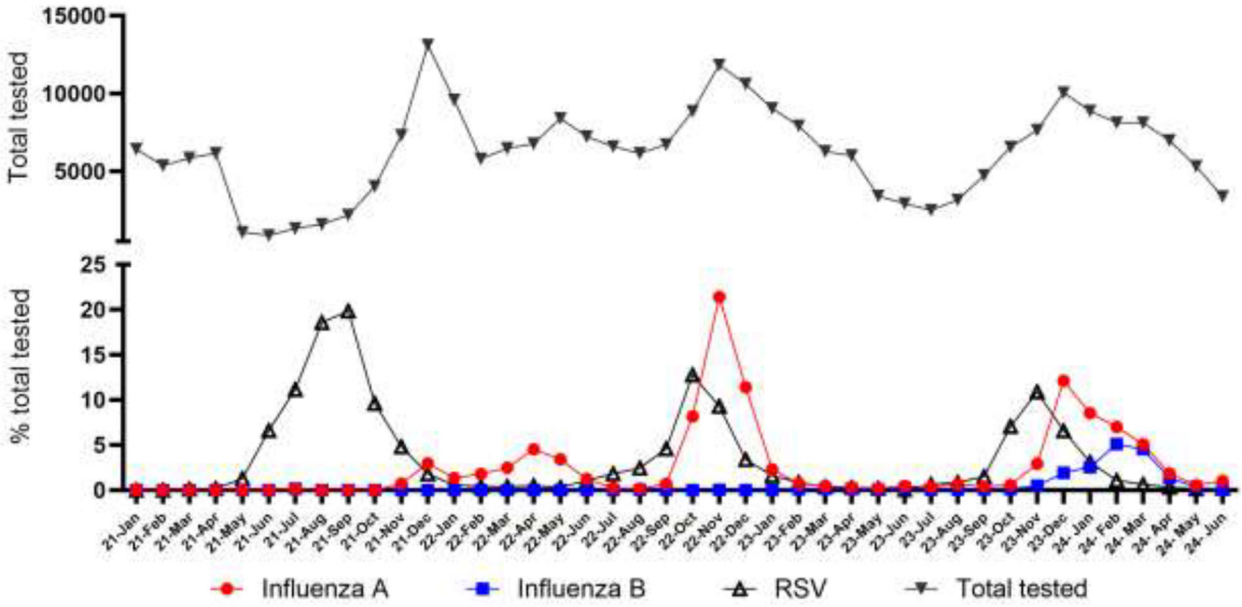

Figure 1:
Percent positive IAV, IBV, and RSV tests diagnosed at JHHS between January 2021 and June 2024.

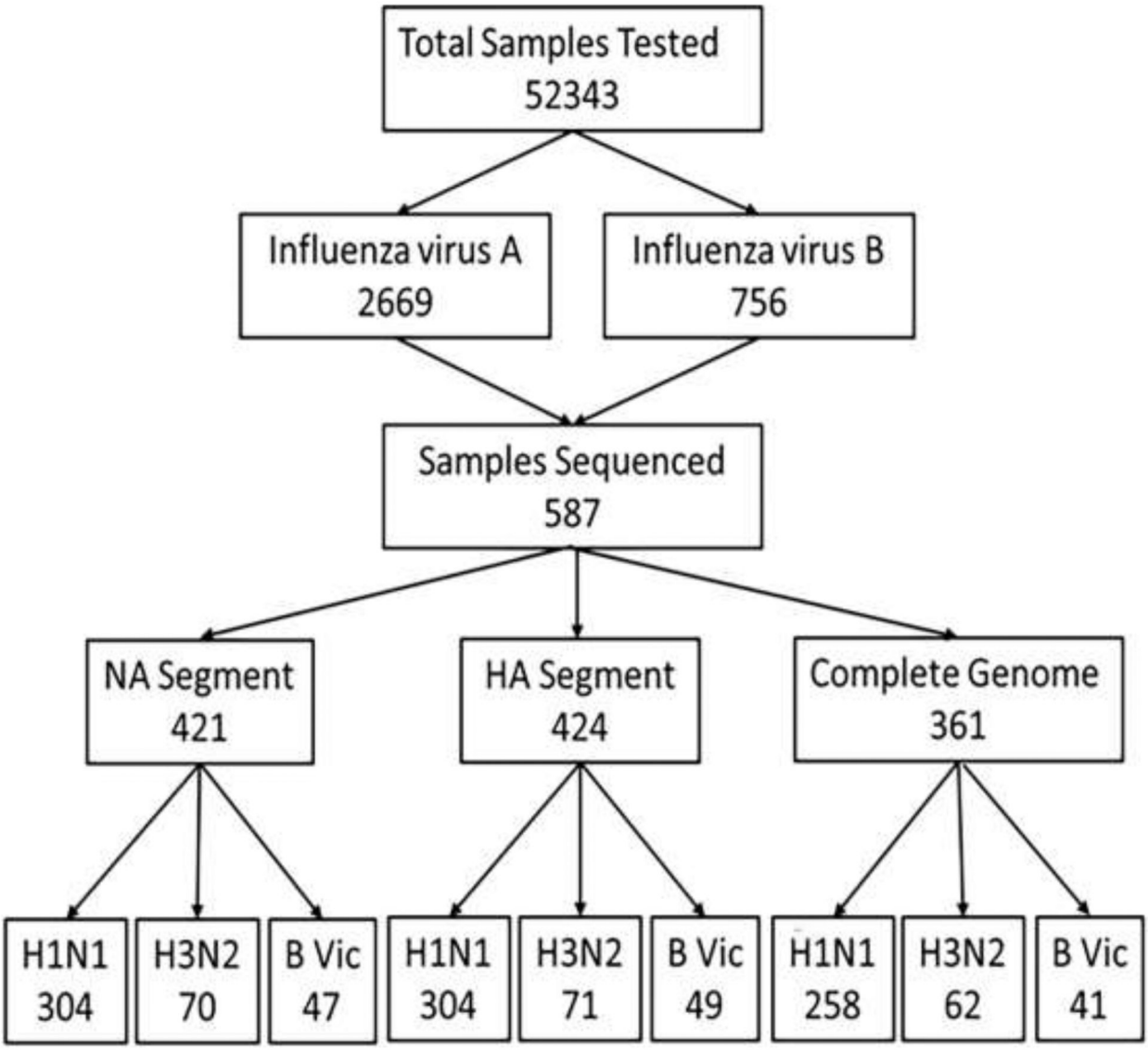

Figure 2.

Flow chart illustrating the number of samples selected for whole genome sequencing. HA and NA segments illustrates specimens where full-length HA segments were recovered..

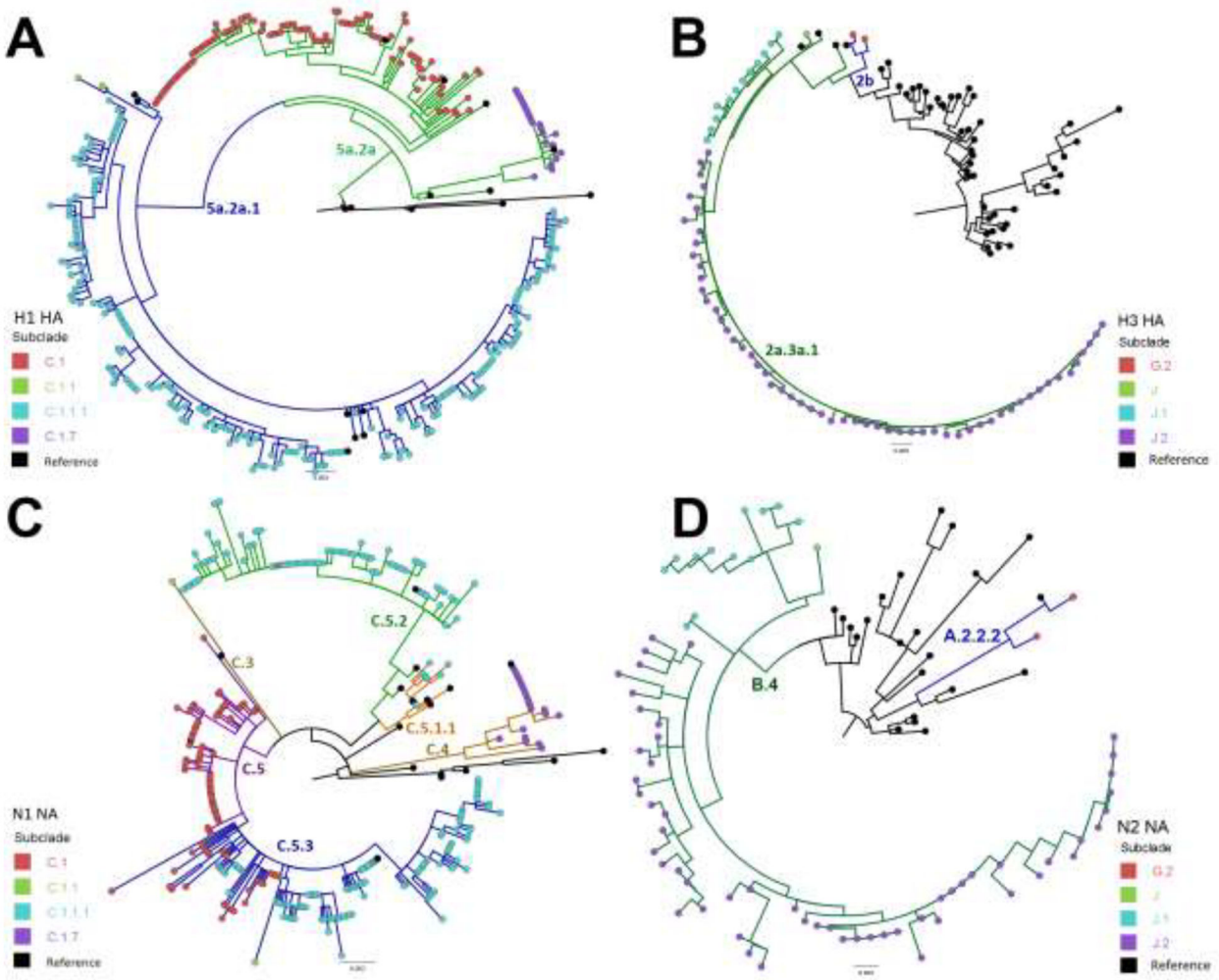

Figure 3.
Phylogenetic trees of the HA and NA segments annotated by subclades.

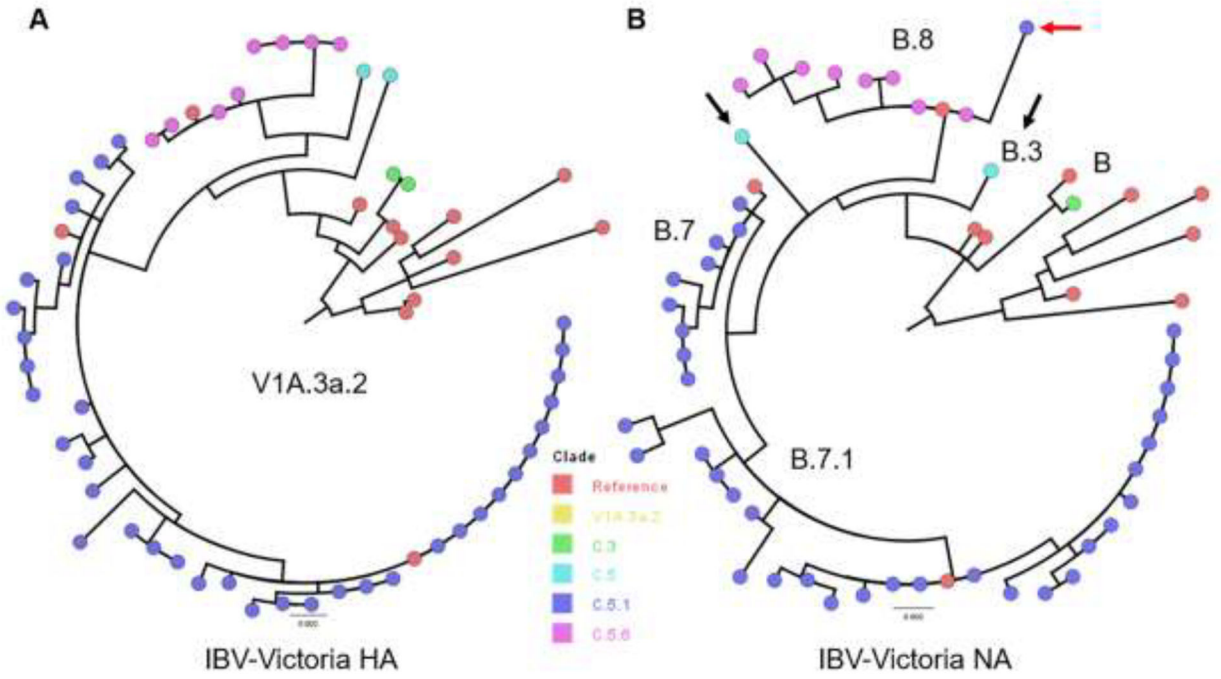

Figure 4.

Phylogenetic trees of the HA and NA segments annotated by subclades. Sample with a red arrow indicates the only C.5.1 IBV HA subclade identified with NA segment belonging to subclade B.8. The black arrows indicate samples belonging to the C.5 HA subclade with differing NA subclade segments.

Table 1:

Clinical and metadata of the 2023/2024 study cohort. Breathing problems include wheezing and shortness of breath. Seizures include febrile seizures.

Characteristic	Number of Patients (% cohort)
No. of Patients	580 *
Sex, N (%)	
Female	301 (51.9)
Male	279 (48.1)
Age Group in years, N (%)	
<5	139 (24)
5–17	188 (32.4)
18–64	193 (33.3)
≥65	60 (10.3)
Clinical Signs, N (%)	
Patients with Symptoms in Charts	556 (95.9)
Fever	215 (38.7)
Cough	90 (16.2)
Headache	14 (2.5)
Breathing Problems	40 (7.2)
Chest Pain	19 (3.4)
Sore Throat	16 (2.9)
Upper Respiratory Infection	11 (2)
Abdominal Pain	20 (3.6)
Emesis	30 (5.4)
Flu-like Symptoms	79 (14.2)
Generalized Weakness or Body Ache	8 (1.4)
Seizures	10 (1.8)
Comorbidity, N (% whole cohort)	
≥1 underlying medical condition	306 (52.8)
Hypertension	128 (22.1)
Pregnancy	7 (1.2)
Lung Disease	169 (29.1)
Kidney Disease	67 (11.6)
Immunosuppression	101 (17.4)
Diabetes	63 (10.9)
Heart Failure	48 (8.3)
Cerebrovascular Disease	43 (7.4)
Cancer	152 (26.2)
Outcome, N (%)	

Characteristic	Number of Patients (% cohort)
Admitted	134 (23.1)
ICU	23 (17.2% admitted) (4% whole cohort)
Supplemental Oxygen	89 (15.3)

* Unique patients used for clinical analysis. Seven samples (Figure 2) were from same patients.

Table 2:

Clinical and metadata of the 2023/2024 cohort stratified by subtype: IBV-Victoria, H1N1pdm09 and H3N2. Breathing problems include wheezing and shortness of breath. Seizures include febrile seizures.

	IBV-Victoria	H1N1pdm09	H3N2
No Samples	49	304	71
Characteristic	**Number of Patients (% subtype)**		
Age Group in years, N (%)			
<5	14 (28.6)	77 (25.3)	13 (18.3)
5–17	24 (49)	105 (34.5)	12 (18.3)
18–64	11 (22.4)	91 (29.9)	34 (47.9)
≥65	0	31 (10.2)	11 (15.5)
Clinical Signs, N (%)			
Patients with Symptoms Reported in Charts	46 (93.9)	288 (94.7)	71 (100)
Fever	27 (58.7)	125 (43.4)	17 (23.9)
Cough	6 (13)	49 (17)	10 (14.1)
Headache	4 (8.7)	7 (2.4)	1 (1.4)
Breathing Problems	2 (4.3)	19 (6.6)	5 (7)
Chest Pain	0	7 (2.4)	6 (8.4)
Sore Throat	3 (6.5)	8 (2.7)	3 (4.2)
URI	2 (4.3)	3 (1)	2 (2.8)
Abdominal Pain	3 (6.5)	7 (2.4)	3 (4.2)
Emesis	0	19 (6.6)	4 (5.6)
Flu-like Symptoms	7 (15.2)	34 (11.8)	19 (26.8)
Generalized Weakness or Body Ache	0	5 (1.7)	1 (1.4)
Seizures	0	6 (2.1)	1 (1.4)
Outcome, N (%)			
Admitted	2 (4.1)	76 (25)	19 (26.7)
ICU	2 (100% admitted)	14 (18.4% admitted)	1 (5.3% admitted)
	(4% IBV)	(4.6% H1N1pdm09)	(1.41% H3N2)
Supplemental Oxygen	4 (8.2)	53 (17.4)	11 (15.5)

Table 3:

Multivariate Logistic Regression by Influenza subtype

	Admitted	Supplemental Oxygen
Female	0.99 (0.57–1.71)	1.23 (0.67–2.26)
Patient Age		
<2	Reference	Reference
2–4	0.74 (0.15–3.58)	0.39 (0.08–1.88)
5–17	1.35 (0.36–4.99)	0.66 (0.19–2.24)
18–44	2.79 (0.75–10.39)	0.64 (0.18–2.36)
44–64	6.87 (1.69–27.91)	3.24 (0.86–12.18)
65–79	10.18 (2.18–47.51)	3.56 (0.84–15.06)
80+	14.72 (1.57–138.11)	3.10 (0.49–19.71)
Comorbidities		
Atrial Fibrillation	0.46 (0.15–1.45)	0.55 (0.20–1.51)
Cancer	1.01 (0.51–1.98)	0.81 (0.39–1.70)
Cerebrovascular Disease	1.88 (0.62–5.71)	2.21 (0.86–5.67)
Coronary Artery Disease	4.72 (2.00–11.14)	3.75 (1.57–8.95)
Diabetes	0.97 (0.40–2.35)	1.29 (0.55–3.00)
Heart Failure	1.11 (0.40–3.08)	1.35 (0.55–3.27)
Hypertension	0.83 (0.36–1.91)	0.52 (0.20–1.38)
Immunosuppression	5.41 (2.64–11.10)	3.06 (1.46–6.38)
Kidney Disease	1.81 (0.72–4.59)	1.43 (0.59–3.47)
Lung Disease	1.58 (0.87–2.87)	2.04 (1.08–3.85)
Smoker	0.60 (0.27–1.36)	1.06 (0.47–2.39)
ED Visit	2.41 (0.28–20.68)	2.71 (0.25–29.90)
coinfection	0.82 (0.17–3.94)	1.47 (0.28–7.71)
Strain		
H1N1	reference	reference
H3N2	0.79 (0.34–1.80)	0.73 (0.29–1.83)
IVB	0.30 (0.07–1.39)	1.19 (0.36–3.85)
unknown	0.98 (0.53–1.82)	0.68 (0.33–1.36)
N	580	580

Table 4:

Clades and subclades of IAV and IBV identified from the 2023/2024 cohort.

H1N1pdm09		
	Number of Samples (% Cohort)	**% of Type or Clade**
Total	304 (71.7)	
6B.1A.5a.2a	120	39.5
C.1	100	83.3
C.1.7	20	16.7
6B.1A.5a.2a.1	184	60.5
C.1.1	2	1.1
C.1.1.1	182	98.9
H3N2		
Total	71 (16.7)	
3C.2a1b.2a.2a.3a.1	69	97.2
J	1	1.5
J.1	12	17.4
J.2	56	81.2
3C.2a1b.2a.2b	2	2.8
G.2	2	100
IBV-Victoria		
Total	49 (11.6)	
V1A.3a.2	49	100
C.5.1	37	75.5
C.5.6	8	16.3
C.3	2	4.1
C.5	2	4.1

Table 5:

Additional AAS identified in our cohort within the HA proteins of H1N1pdm09 (Reference: A/Wisconsin/588/2019), H3N2 (Reference: A/Darwin/6/2021), and IBV (Reference: B/Brisbane/60/2008-egg).

H1N1pdm09						
Clade	**Subclade**	**AAS (number of samples / %)**				
6B.1A.5a.2a	C.1	T120A (74/74%)	K169Q (57/57%)			
	C.1.7	T120A (19/95%)				
6B.1A.5a.2a.1	C.1.1.1	R113K (46/25.3%)	R45K (70/38.5%)			
H3N2						
Clade	**Subclade**	**AAS (number of samples / %)**				
3C.2a1b.2a.2a.3a.1	J	I223V (1/100%)				
	J.1	I223V (12/100%)				
	J.2	I223V (51/91.1%)				
3C.2a1b.2a.2b	G.2	G53D (2/100%)				
IBV-Victoria						
Clade	**Subclade**	**AAS (number of samples / %)**				
V1A.3a.2	**C.5**	N150K (49/100%)	G184E (49/100%)	I117V (49/100%)	A199T (49/100%)	N129D (1/50%)
	C.5.1	N150K (49/100%)	G184E (49/100%)	I117V (49/100%)	A199T (49/100%)	N129D (37/100%)
	C.5.6	N150K (49/100%)	G184E (49/100%)	I117V (49/100%)	A199T (49/100%)	
	C.3	N150K (49/100%)	G184E (49/100%)	I117V (49/100%)	A199T (49/100%)	N129D (2/100%)

Table 6:

Subclades identified based on NA segment analysis.

H1N1pdm09	
NA subclade	**Number of Samples (% of total)**
Total	304
C.3	1 (0.3)
C.4	20 (6.6)
C.5	72 (23.7)
C.5.1.1	8 (2.6)
C.5.2	68 (22.4)
C.5.3	135 (44.4)
H3N2	
Total	70
A.2.2.2	2 (2.9)
B.4	68 (97.1)
IBV-Victoria	
Total	47
B	1 (2.1)
B.3	1 (2.1)
B.7	10 (21.3)
B.7.1	26 (55.3)
B.8	9 (19.1)

Table 7:

previously reported AAS within the N1and IBV NA that could be associated with NAI resistance or impact antigenicity.

H1N1pdm09			
Mutation	**Potential Impact**	**NA Subclade**	**Number of Samples (%)**
H275Y	NAI resistance	C.4	1 (0.33)
I223V	NAI resistance	C.5.3	1 (0.33)
S247N	NAI resistance	C.5.3	3 (0.99)
K390R	Antigenicity	C.5.3	1 (0.33)
IBV-Victoria			
D342N	Antigenicity	B.3	1 (2.13)
D342E	Antigenicity	B.7	1 (2.13)
A395V	NAI resistance	B.8 and B.7	20 (42.55)

Table 8:

Multivariate Logistic Regression by H1N1 Clade

	Admitted	Supplemental Oxygen
Female	0.93 (0.42–2.03)	1.76 (0.73–4.23)
Patient Age		
<2	Reference	Reference
2–4	0.77 (0.11–5.24)	0.52 (0.07–4.15)
5–17	1.44 (0.29–7.13)	0.64 (0.12–3.57)
18–44	1.55 (0.27–8.88)	0.35 (0.05–2.53)
44–64	4.11 (0.65–25.82)	3.26 (0.52–20.52)
65–79	15.75 (1.91–130.04)	2.79 (0.37–21.10)
80+	8.69 (0.65–116.27)	4.87 (0.37–63.79)
Comorbidities		
Atrial Fibrillation	0.44 (0.09–2.06)	0.85 (0.21–3.44)
Cancer	0.95 (0.36–2.53)	1.11 (0.41–3.06)
Cerebrovascular Disease	1.90 (0.46–7.78)	4.02 (1.15–14.09)
Coronary Artery Disease	6.92 (1.72–27.81)	2.10 (0.59–7.49)
Diabetes	1.17 (0.31–4.37)	0.94 (0.30–2.96)
Heart Failure	1.06 (0.25–4.55)	1.45 (0.41–5.08)
Hypertension	0.72 (0.21–2.41)	0.58 (0.15–2.22)
Immunosuppression	5.27 (1.81–15.36)	1.89 (0.62–5.82)
Kidney Disease	1.95 (0.51–7.43)	2.70 (0.80–9.14)
Lung Disease	1.21 (0.52–2.84)	2.75 (1.11–6.82)
Smoker	0.72 (0.21–2.49)	1.01 (0.32–3.19)
ED Visit	1.28 (0.07–24.86)	0.75 (0.03–15.96)
coinfection	0.43 (0.03–5.85)	0.16 (0.01–4.67)
Clade		
5a.2a	reference	reference
5a.2a.1	0.72 (0.13–3.82)	0.95 (0.10–8.77)
6B.1A.5a.2a	1.06 (0.23–4.84)	2.21 (0.31–15.71)
6B.1A.5a.2a.1	0.87 (0.20–3.76)	1.66 (0.24–11.39)
N	304	304

*There were not enough H3N2 clades to include in multivariate regression