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Epidemiology and Genetic Evolution of Influenza B Viruses Circulating in the Democratic Republic of Congo from 2015 to 2022: Implication of Vaccination

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Abstract

Introduction: Influenza A (Flu A) and B (Flu B) viruses are responsible for severe acute respiratory infections (SARI) worldwide, with a morbidity of 5 million and mortality of 29,000 - 650,000 deaths per year. Influenza B viruses are an important cause of respiratory infections in humans, but they tend to be underappreciated due to the predominance of Influenza A. No molecular study on Influenza B has been carried out in the DRC. This study was conducted to document the molecular evolution of the hemagglutinin (HA) gene in the circulating Influenza B strains over the eight consecutive epidemic seasons (from 2015 to 2022). **Methods:** Samples were collected from outpatient cases suspected of influenza-like illness (ILI) and in all hospitalized patients with SARI from January 2015 to December 2022. Molecular analysis was done to determine influenza type and subtype, and then segments encoding the HA gene of Influenza B viruses were performed. **Results:** Of 8497 samples collected and tested, 639 (7.5%) were positive for influenza viruses, including 389 (60.8%) for Influenza A viruses and 248 (38,8%) for Influenza B viruses. Of the positive Influenza B samples, 91 were sequenced, including 26 belonging to the B/Yamagata lineage and 65 to the B/Victoria lineage. The HA gene of Influenza B viruses circulating in the DRC showed deletions in the HA1 region. Molecular analysis of Influenza B viruses reflects the genetic diversity of Influenza B/Yam virus clades (Y2, Y3, Y3V1A) alternating with Influenza

B/Victoria virus clades (V1A, V1A.3) depending on the year and influenza seasons. The phylogenetic analysis of these Influenza B strains shows compatibility with the corresponding vaccine strains that the WHO had validated for each influenza season. **Conclusion:** This study underscores the importance of continuous molecular surveillance of Influenza B viruses in the DRC to understand their epidemiology and evolutionary dynamics. Identifying mutations, such as HA deletions, is critical for assessing their impact on transmissibility vaccine efficacy and guiding effective vaccination and control strategies.

Keywords

Epidemiology, Genetic Evolution, Influenza B Viruses, DRC

1. Introduction

Influenza A (Flu A) and B (Flu B) viruses are significant contributors to the global burden of respiratory diseases, including severe acute respiratory infections (SARI) during seasonal epidemics. While Influenza A viruses often receive the most attention due to their role in pandemics, Influenza B viruses represent an important cause of respiratory infections in humans. However, their impact is frequently underestimated because of the predominance of Influenza A [1]. Globally, annual influenza epidemics are estimated to result in 3 - 5 million cases of severe illness and 290,000 - 650,000 deaths [2]. Influenza-like illness (ILI) and SARI caused by Influenza A are more prevalent, but Influenza B still accounts for approximately 30% of all influenza cases during seasonal epidemics [1] [3] [4]. This burden is particularly pronounced in low-income countries, many of which are in tropical regions where influenza activity persists year-round, often with one or more epidemic peaks [4] [5].

Influenza A viruses, known for their zoonotic origins, drive influenza epidemics and pandemics, while Influenza B viruses infect only humans. Despite this, numerous studies highlight the significant impact of Influenza B on respiratory complications in both children and adults [3] [6]. Initially, Influenza B viruses formed a homogenous group, but since the late 1980s, they have evolved into two antigenically and genetically distinct lineages: B/Yamagata/16/88 (Yamagata lineage) and B/Victoria/2/87 (Victoria lineage) [3] [7]. The Yamagata lineage initially dominated globally, while the Victoria lineage remained largely confined to East Asia until its re-emergence in North America and Europe during the 2000/2001 and 2001/2002 seasons, subsequently spreading worldwide [8] [9]. Since then, the two lineages have been co-circulated, with variability in their geographic distribution and genomic evolution [10] [11].

Influenza B viruses evolve primarily through antigenic drift, enabling them to evade host immunity, adapt to new environments, and cause recurrent epidemics [12]. Although they generally exhibit slower genetic and antigenic changes than Influenza A viruses, they can be further classified into specific clades and subclades

[11]-[13]. Differences between the two lineages lie in their transmissibility, genetic dynamics, and antigenic properties [11]. However, the evolutionary dynamics and molecular epidemiology of Influenza B viruses in sub-Saharan Africa remain poorly understood, with limited genomic data available [11] [12].

Annual influenza vaccination remains the most effective way to prevent influenza and its complications. Due to the constant evolution of influenza viruses, seasonal vaccines are regularly updated through the global monitoring efforts of the World Health Organization's Global Influenza Surveillance and Response System (GISRS). Trivalent or quadrivalent vaccines that include both B lineages are currently available, but their antigenic composition is periodically revised to account for ongoing viral evolution [13].

Local molecular epidemiology of influenza remains the key when selecting influenza strains for inclusion in vaccines for future seasons and understanding observed vaccine effectiveness.

Unfortunately, this information is often unavailable for most low- and middle-income countries which, moreover, do not have a national influenza vaccination policy [13] [14].

In Africa, genomic studies on Influenza B viruses remain limited despite their importance for designing effective control strategies. In the Democratic Republic of Congo (DRC), influenza viruses circulate annually, with Influenza B viruses accounting for approximately 30% of detected cases in patients under influenza surveillance. This highlights the role of Influenza B in the spread of influenza within the DRC. However, no molecular studies on Influenza B viruses have been conducted in the country. To address this gap, this study provides an 8-year overview (2015-2022) of Influenza B virus circulation in the DRC, offering valuable insights into their molecular epidemiology and potential public health implications.

2. Materials and Methods

2.1. Collection of Clinical Samples

Nasopharyngeal and oropharyngeal swabs were collected from outpatients meeting the WHO influenza-like illness (ILI) case definition, or from hospitalized patients with severe acute respiratory infection (SARI).

Swabs were placed in a universal viral transport medium (Becton Dickinson, Italy), stored at 4˚C - 8˚C, and transported to the laboratory within 72 hours of collection for molecular detection of influenza viruses. An aliquot of each sample was stored at −80˚C for biobanking and further studies.

2.2. RNA Extraction and Quantitative Reverse Transcription-PCR (qRT-PCR)

The purification of nucleic acids from respiratory samples was carried out using the QIAGEN kit (QIAamp Viral RNA Mini Kit, Germany), with a matrix volume of 140 µL and a final elution volume of 60 µL, according to the instructions of the manufacturer.

RNA samples were tested Flu A and Flu B using the Human Influenza rRT-PCR Diagnostic Panel and Influenza B Lineage Detection Kit, kindly provided by the Centers for Disease Control and Prevention (US CDC). Amplifications were performed using the AgPath-ID TM One-Step rRT-PCR kit (Termo Fisher Scientific, Austin, Texas, USA), according to the manufacturer's instructions.

Subsequently, Flu A positive samples were tested to identify Influenza A(H3N2) or A(H1N1) pdm09 subtypes using a single-step real-time RT-PCR and Flu B positive samples were tested to identify Influenza B/yamagata or B/Victoria lineage with two pairs of primers and probes for specific detection of Influenza B lineages. HA genes specific to each Influenza B lineage were amplified: B/Yamagata and B/Victoria according to the CDC protocol [15]. After screening for influenza viruses, a selection of Influenza B positive samples was carried out for molecular characterization. Samples selected for sequencing were representative of the entire study period and had a cycle threshold (Ct ≤ 30). They were frozen and shipped in dry ice to the CDC in Atlanta for genetic and antigenic characterization

2.3. Sequencing and Genetic Characterization

The influenza genome was amplified using the SuperScript III One-Step RT-PCR with Platinum Taq High Fidelity (Invitrogen) using the Uni/Inf primer set as previously described [16].

Phylogenetic analyses were done to genetically characterize influenza virus isolates collected at the DRC and to compare them with the vaccine strains recommended by WHO during the 2012-2020 influenza seasons. First, reference tree data sets for Influenza B Yamagata and B Victoria viruses were created by selecting reference viruses from WHO vaccine selection meeting reports since 2012, to assist in visualizing the two genetic lineages, and their HA nucleotide sequences were downloaded from the Global Initiative on Sharing Avian Influenza (GISAID) database [17].

The MEGA11 software was used to build maximum likelihood phylogenetic trees with the General Time Reversible model and annotate branches with amino acid substitutions [18]. Stop codons were removed from the aligned sequences in BioEdit, and the vaccine virus was set as the outgroup and reference at the top of the alignment.

Amino acid sequence alignment was done using MEGA11 to identify mutations in the target genes [19].

2.4. Ethical Considerations

The influenza sentinel surveillance protocol was adapted from World Health Organization (WHO) guidelines with support from the national influenza surveillance program at the DRC Ministry of Health. This protocol was implemented as part of routine public health surveillance by the Ministry of Health and was therefore considered a service and not subject to human subject review. However, some of the authors had access to identifying information of the patients who partici-

pated in the surveillance program.

3. Results

3.1. Characteristics of Patients Infected with Influenza Viruses

A total of 8497 samples were collected from the country. Of the samples collected, 639 (7.5%) were positive for influenza, of which 389 (60.8%) were positive for Influenza A, 248 (38.8%) for Influenza B and two (0.3%) mixed infections. Among positive patients, children under 5 years of age were the most affected, followed by those aged 5 - 15 years old. The F/M sex ratio was 0,84 (315/324). We have noted a greater susceptibility to influenza in men than in women. Patients seen in outpatient settings (59%) were more numerous than patients hospitalized for acute and severe respiratory infections (41%). Of the 639 patients who tested positive for influenza, 248 (38.8%) were positive for Influenza B, and women (53%) were more affected (Table 1).

Table 1. Sociodemographic characteristics of ILI and SARI patients received and tested for influenza viruses.

2015-2022	Total samples (N = 8497)	Positives Influenza (N = 639)	Negatives Influenza (N = 7858)	Influenza A (N = 389)	Influenza B (N = 248)	Influenza A&B (2)
Genre						
Female	4363 (51.3%)	315 (49.3%)	4048 (51.5%)	182 (46.7%)	133 (53.6%)	1 (50%)
Male	4134 (48.7%)	324 (50.7%)	3810 (48.5%)	207 (53.2%)	115 (46.3%)	1 (50%)
Age Group						
0 - 5 years	2168 (25.5%)	220 (34.4%)	1948 (24.7%)	143 (36.7%)	77 (31%)	2 (100%)
5 - 15 years	2120 (24.9%)	198 (31%)	1922 (24.4%)	127 (32.6%)	71 (28.6%)	0
15 - 25years	1145 (13.4%)	99 (15.5%)	1046 (13.3%)	48 (12.3%)	51 (20.5%)	0
25 - 50 years	1954 (22.9%)	96 (15%)	1858 (23.6%)	55 (14.1%)	41 (16.5%)	0
>50 Years	1110 (13%)	26 (4%)	1084 (13.7%)	16 (4.1%)	10 (4%)	0
Casa type						
SARI	3483 (41%)	243 (38%)	3240 (41.2%	160 (41.1%)	81 (32.6%)	2 (100%)
ILI	5014 (59%)	396 (62%)	4618 (58.8%)	229 (58.9%)	167 (67.3%)	0

3.2. Prevalence and Molecular Detection of Flu B Types, Subtypes and Lineages

We observed that Influenza A viruses and Influenza B viruses circulated every year, with varying intensities (Figure 1). Overall, Influenza A was the most prevalent virus, except in 2019, where the prevalence of Influenza A and Influenza B was almost similar and then in 2022, the proportion of Influenza B was greater. We have implemented B lineage detection since 2015 and observed that B/Yam was the dominant strain in 2015, 2016 and 2018. However, the Influenza B/Victoria lineage was dominant in 2017, 2019, 2020, 2021and 2022. Of the 248 Influenza B viruses circulating in DRC (2015-2022), molecular detection revealed that 119

samples were of the Influenza B/Yamagata lineage, 114 of the Influenza B/Victoria lineages and 15 non-sub-typeable (Table 2).

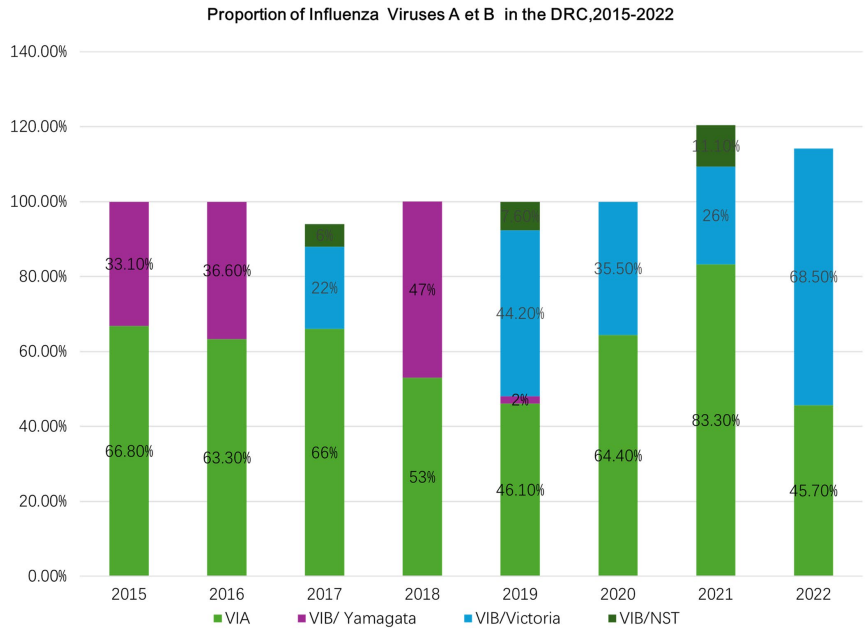

Figure 1. Annual proportion of Influenza A and B viruses circulating in the DRC, 2015-2022.

Table 2. Annual distribution of samples positive for Influenza B in the Yamagata, Victoria and non-subtypeable lines.

Influenza B lineage	2015	2016	2017	2018	2019	2020	2021	2022
Yamagata	60	37	0	16	1	0	0	0
Victoria	0	0	18	0	23	16	14	48
Non-subtypeable	0	0	5	0	4	0	6	0
Total	**60**	**37**	**23**	**16**	**28**	**16**	**20**	**48**

3.3. Matches between Circulating Influenza B Strains and Vaccine Strains

Influenza B lineage suitability was defined as a season in which >60% of the circulating Influenza B lineage virus was like the lineage included in the trivalent or quadrivalent influenza vaccine (TIV) for that season. A partial mismatch was defined if the two lineages co-circulated in equal or near equal proportions (40% - 59%). From 2015 to 2022, the Influenza B /Victoria or B/Yamagata lineages circulated in the DRC with alternating dominance depending on the influenza seasons (Yamagata in 2015, 2016 and 2018 and Victoria in 2017, 2019 until 2022).

However, the trivalent vaccines recommended by the WHO for each influenza season contain only one lineage of Influenza B viruses, unlike the quadrivalent vaccines, which have both B lines. The proportion of circulating Influenza B com-

patible with the vaccine strain was calculated based on the total number of samples successfully typed in the lineage. Thus, during the period 2015-2022, a high degree of concordance of Influenza B with the vaccine strains recommended by the WHO was observed. The dominant sequence lineage during the 2015-2022 seasons corresponded to the lineage of the vaccine strain selected during the same season. No inadequacy was observed with the quadrivalent vaccines containing vaccine strains from two lines Victoria and Yamagata.

3.4. Phylogenetic Analysis of Flu B HA Sequences

The samples were selected based on their Ct value (Ct < 30) for further HA and NA genetic analyses. Only Influenza B positive samples from influenza surveillance sites in the DRC were sent to the CDC in Atlanta for antigenic and genetic characterization, including 26 Yamagata and 65 Victoria.

A total of 91 sequences corresponding to HA genes were successfully generated from the Influenza B-positive samples. The phylogeny of the complete region of the HA gene showed a co-circulation of two Influenza B lineages, with a predominance of the Influenza B/Victoria lineage (71.4%; 65/91) over the B/Yamagata lineage (28.6%; 26/91). Phylogenetic trees showed different genetic diversities of the two lines based on nucleotide differences in the HA protein. We did not observe the co-circulation of the B/Victoria and B/Yamagata lineages in nearly all seasonal epidemics in our study. In 2015, 2016, and 2018, only the Yamagata lineage was detected in the DR Congolese samples, while in 2017 and 2019-2022, only the Victoria lineage was detected.

Rather, we observed genetic diversities in each Influenza B lineage across influenza seasons, with alternating dominances such as the Y2 clades in 2015, the Y3/Y3.V1A clades in 2016 and the Y3 clade in 2018. For Influenza B Victoria, the predominant V1A clades in 2017, the V1A.3 clades in 2019 and 2020, and the V1A.3a.2 clades in 2022. (**Figure 2**)

3.4.1. Genetic Characterization of HA from Flu B/Yamagata

The sequences of the genes which encode the HA of Influenza B/Yamagata detected in the DRC in 2015, 2016, and 2018 were grouped into two distinct genetic clades: clade Y2 and clade Y3. (15). Those from 2015 belonged to clade Y2, closely related to the B/Massachusetts/02/2012 vaccine strains. (Recommended by WHO for the 2015 influenza season); those of 2016 and 2018 belonged to clade 3 (Y3), including the Y3/V1A subclade mainly detected in 2016, closely linked to the vaccine strains B/Phuket/3073/2013). (**Figure 3**)

All Y3 viruses that predominated in 2016 were grouped in the Y3/V1A subclade, standing intralineage between the Y3 clade based on the phylogeny of the HA gene and the 1A B/Victoria clade based on the phylogeny of the NA gene. Since 2020.any Yamagata Influenza B viruses.

Among the 26 Yamagata Influenza B viruses tested by HI were susceptible to oseltamivir and zanamivir. Within the Y3 genetic group, there was a reassortment event leading to viruses with the HA of the B/Yamagata Y3 genetic group and the

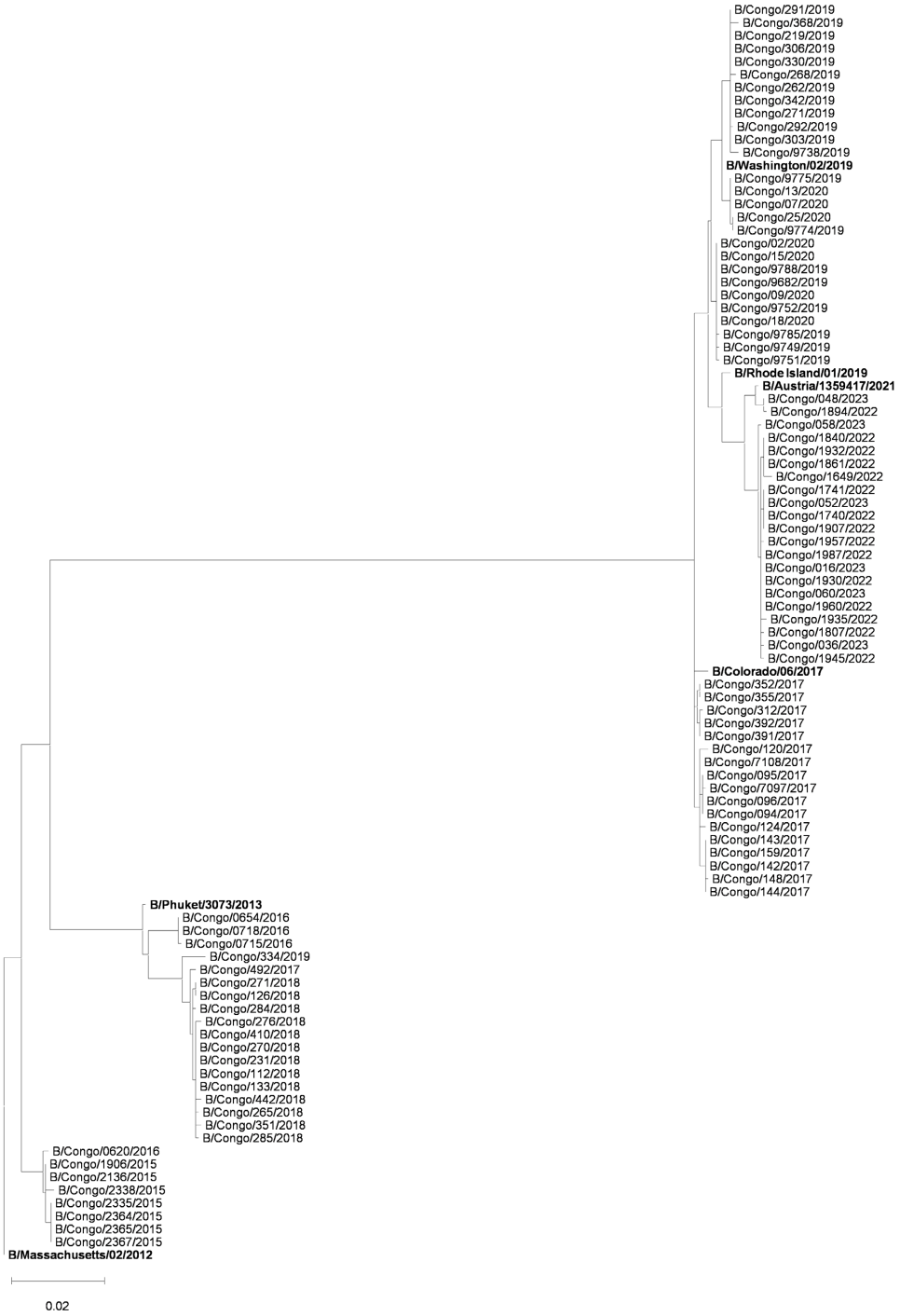

Figure 2. Phylogenetic tree of Influenza B hemagglutinin genes collected from Democratic Republic of Congo between 2015 and 2022. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model [18]. The tree with the highest log likelihood (−4449.43) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (6 categories (+*G*, parameter = 0.3075)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 97 nucleotide sequences. Codon positions included were 1^{st} + 2^{nd} + 3^{rd} + Noncoding. There were a total of 1710 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [19].

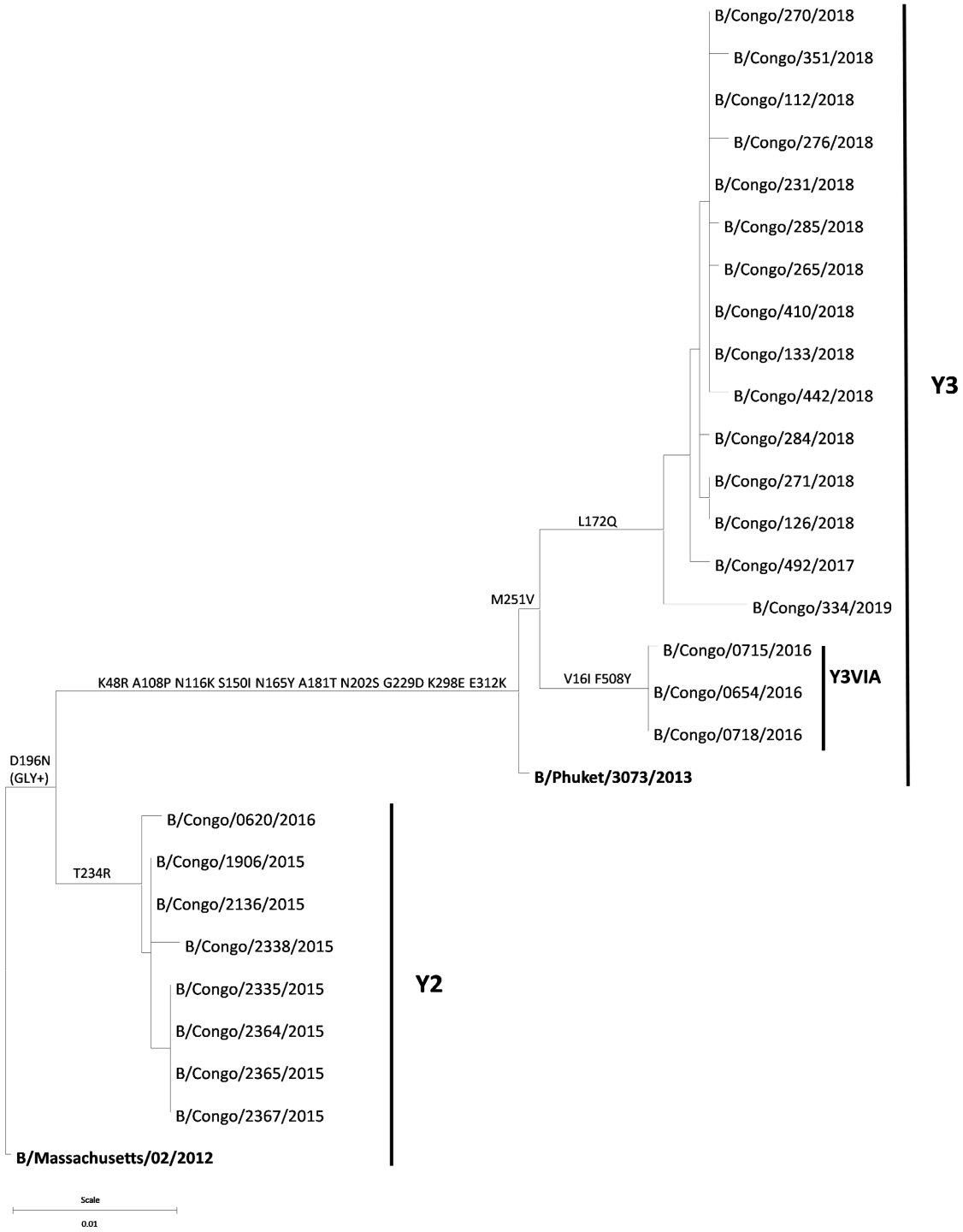

Figure 3. Phylogenetic tree of Influenza B (Yamagata-lineage) hemagglutinin genes collected from the Democratic Republic of Congo between 2015 and 2019. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model [18]. The tree with the highest log likelihood (−3067.13) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (6 categories (+*G*, parameter = 0.0689)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 28 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 1710 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [19].

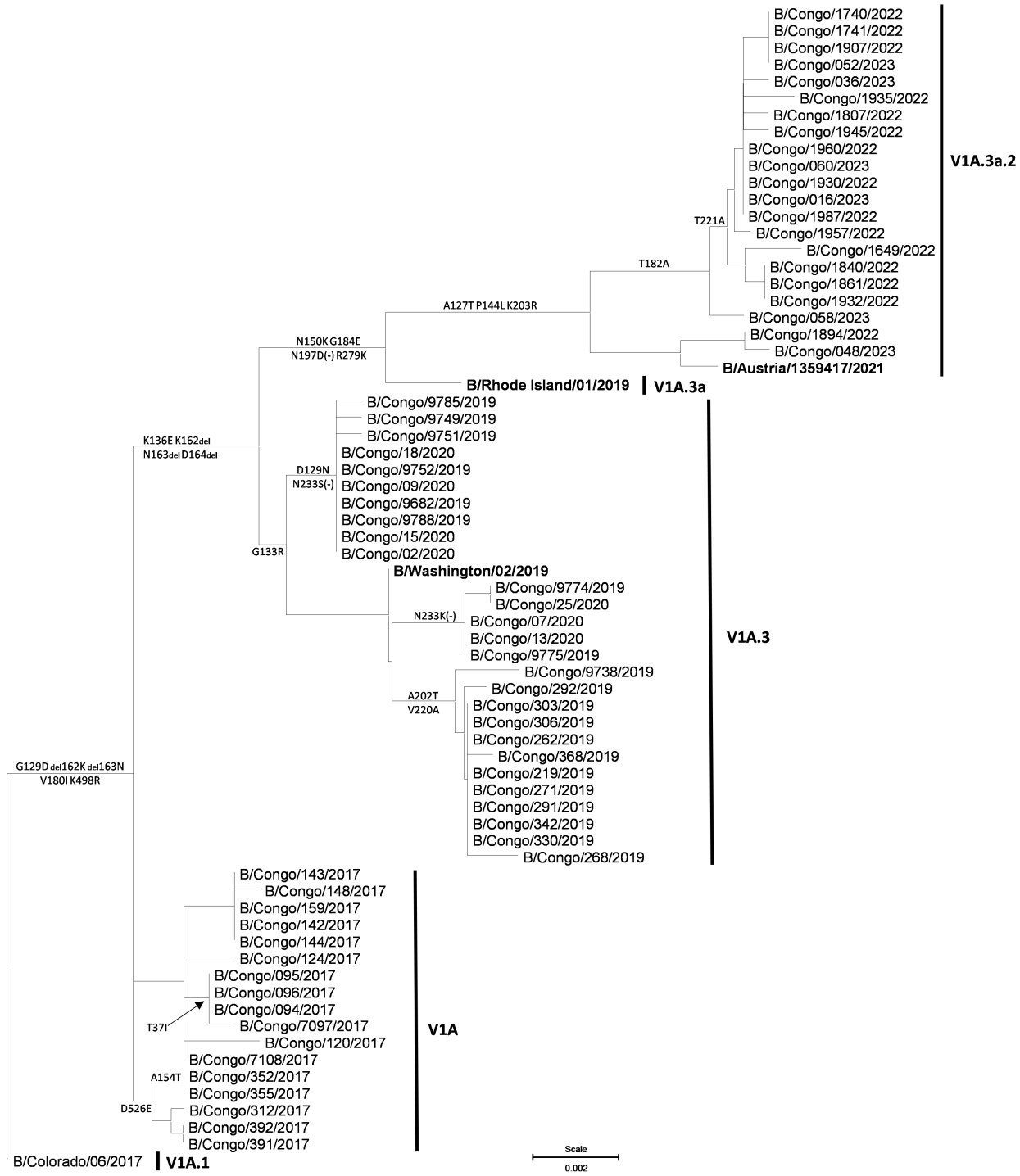

Figure 4. Phylogenetic tree of Influenza B (Victoria-lineage) hemagglutinin genes collected from the Democratic Republic of Congo between 2015 and 2022. The evolutionary history was inferred by using the Maximum Likelihood method and General Time Reversible model [18]. The tree with the highest log likelihood (−3186.66) is shown. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (6 categories (+*G*, parameter = 0.1000)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 70 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1710 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [19].

NA of the B/Victoria V1A or V4 genetic groups. These reassortant viruses were detected sporadically and remain antigenically like B/Phuket/3073/2013.

3.4.2. Genetic Characterization of HA from Flu B/Victoria

Our sequences of the gene which encodes the Influenza B/Victoria HA circulating in the DRC from 2017 and 2019-2022 were grouped into the V1A.3 clades, closely linked to the B/Brisbane/60/2008 vaccine strain, recommended by the WHO for the 2017 and 2018 seasons. A series of amino acid substitutions were observed in the HA protein of Influenza B Victoria during the evolution.

In 2019 and 2020, the analysis of the sequences of the gene which encodes the HA of Influenza B viruses circulating in the DRC was in favor of the V1A.3 clade having undergone evolutions marked by deletions of the 3 AAs (K162, N163 and D164) in the HA protein that emerged in Asia and Africa and were closely related to the B/Washington/02/2019 strain that the WHO had recommended for the influenza vaccine for the 2020-2021 NH season.

In its evolution, the V1A.3 clade with 3 deletions then emerged to give the V1A.3a subclade (represented by the strain B/Rhode Island/01/2019). The latter then emerged to give two subclades V1A.3a.1 and V1A.3a2, the first of which had limited circulation in China, and the second, V1A.3a2, had a more global distribution, represented by strain B /Austria/359417/2021. (**Figure 4**)

In 2022, the analysis of VIB sequences circulating in the DRC reported the V1A.3a.2 subclades closely linked to the vaccine strain B/Austria/359417/2021, recommended by the WHO for the influenza season in the HN.

3.5. Analysis of Amino Acid Sequences of Flu B Strains Circulating in the DRC

The alignment and comparison of the reference sequences showed deletions (deletions) of amino acids (AA) in the HA protein of several Victoria/Influenza B Viruses sequences which circulated in the DRC between 2019 and 2020. These sequences were characterized by the loss of three amino acids: K162, N163, and D164.

In 2022, the V1A.3a.2 subclades from the V1A.3 clade of Influenza B viruses/Victoria, circulated in the DRC in 2022. These sequences are characterized by additional substitutions in position A127T, P144L and K203R, represented by strain A /Austria/359417/2021)

4. Discussion

Limited data are available on the epidemiology and genetic evolution of Influenza B viruses circulating in the DRC while the molecular epidemiology of Influenza A has already been carried out [20]. Our study focused on the epidemiology and evolutionary dynamics of Influenza B viruses having circulated in the DRC, 2015-2022.

During these influenza seasons, Influenza B viruses co-circulated with Influenza A viruses, with an average prevalence of 39% of positive cases of Influenza B

between 2015 and 2022. These results are similar to those from other countries over the world [21] [22]. However, the prevalence of Influenza A viruses was greater than that of Influenza B viruses in our study, except in 2018 and 2019, during which the prevalence of Influenza A (53%) and Influenza B (47%) were comparable. This confirms the important role of the Influenza B virus in the spread of infection within the population and its impact similar to that of Influenza A during intense activity [1]. The more intense activity of Influenza B during these two seasons coincided with the lineage change of Influenza B, passing from Victoria (2017) to Yamagata/Y3 (2018) and then from Yamagata to Victoria clade V1A.3 (2019), confirming thus the great variability of Influenza B across the world [23] [24].

Indeed, the viral activity of Influenza B viruses would be closely dependent on that of Influenza A because, when there is an extensive and lasting epidemic of Influenza A, Influenza B viruses will necessarily suffer from a major bottleneck at the level of population. The interaction with the Influenza A virus could, therefore, be essential in shaping the evolutionary dynamics of the Influenza B virus and therefore would facilitate the change in lineage dominance or codominance between the Victoria and Yamagata lineages [25]. Indeed, the prevalence of the Influenza B virus fluctuates each year, sometimes very low due to severe population bottlenecks, but it always remains associated with a high prevalence of Influenza A. These two viruses (Influenza A and Influenza B) are clearly in competition to occupy the same target, represented by the susceptible human population, and it is probable that infection by one type of virus would inhibit either the infection or the replication of the other [26].

The detection of Influenza B lineages showed an alternating circulation of B/Yamagata and B/Victoria lineages in the DRC, with a predominance of the Influenza B/Victoria lineage. Influenza B/Yam was predominant in 2015, 2016 and 2018 while Influenza B/Vic in 2017 and 2019-2022. Similar observations have been observed in other countries, such as Senegal [21], Australia and New Zealand [27], Kenya [28], Italy [29] and Cameroon [30]. Since 2020, no confirmed cases of Influenza B/Yamagata lineage have been reported in the Democratic Republic of the Congo (DRC) or globally. Some experts suggest that the absence of reported cases may indicate the potential extinction of the B/Yamagata lineage following the emergence of SARS-CoV-2 [31]. This could prompt health authorities to revise influenza vaccine composition, possibly moving from quadrivalent to trivalent vaccines that exclude the B/Yamagata strain.

However, different profiles of Victoria and Yamagata lineage B viruses were observed in our study regarding their distribution in relation to the age groups of the population studied. The two Influenza B lineages, Victoria and Yamagata, were more likely to infect patients under 5 years old, followed by those aged 5 - 15 years (60%), with similar results previously reported by several authors in Senegal (78%), in Australia (63%), Finland (52%) and Thailand (67%) where the majority of cases of influenza due to Influenza B were detected in the pediatric population

under 15 years of age [32] [33].

Young children are thought to be at greater risk of influenza infection than older people due to a naive immune system in early childhood [34]. Furthermore, other studies have shown that Influenza B lineages tend to vary significantly depending on age; the Victoria lineage predominates in children under 10 years old and the Yamagata lineage in those over 10 years old, with a significant predominance in adults over 25 years old, preferably adults over 64 years old, as in other countries [35].

As for the analysis of the amino acid sequences of the Influenza B HA gene, their alignment showed a deletion of three amino acids in the Victoria line. Similar strains were detected in different regions and clustered with the B/Washington/02/2019 virus belonging to the B/Vic 1A subclade [21] [36]. Deletions in the HA1 region of Influenza B hemagglutinin can significantly impact virus transmission, vaccine escape, and genetic diversity. They may alter receptor binding, potentially enhancing or reducing transmissibility and affecting the virus's adaptability to human populations. By modifying major antigenic sites, these deletions can facilitate immune escape, reducing vaccine effectiveness and necessitating frequent updates. Additionally, they contribute to clade evolution, influencing the dynamics and dominance of B/Yamagata and B/Victoria lineages based on immune pressure from vaccines or prior infections.

Analysis of the HA phylogenetic tree showed the circulation in the DRC of two clades of the Yamagata lineage (clades Y2 and Y3) and 1 clade of the Victoria lineage (clades 1A) from 2015 to 2022; also reported in the countries cited above [29] [30] and elsewhere in Uruguay [20], Malaysia [37] and China [34]. However, the dynamics of the evolution of dominance of clades may differ from one country to another.

Our phylogenetic analysis indicates that the evolution of the Influenza B virus is marked by the continuous replacement of antigenically distinct lineages. Each dominant antigenic group tends to be short-lived, persisting only briefly before being supplanted by a new group of viruses. This process, similar to antigenic drift, is clearly driven by selective pressures, as indicated by the rapid evolutionary changes in HA and the identification of multiple amino acid sites undergoing positive selection (likely underestimated due to challenges in detecting site-specific positive selection). Therefore, immune selection on HA proteins, and perhaps also on NA proteins, plays a major role in determining patterns of evolutionary change in Influenza B virus.

The importance of immune selection is also reflected in the alternation of dominance between the Vic 87 and Yam 88 lineages. This model of alternating dominance is compatible with a model in which one of these two lineages dominates the viral population until sufficient herd immunity is generated against it, either by recovery from an infection or by vaccination. At this point, the other lineage can increase in frequency.

We have, moreover, observed a B/Yamagata-B/Victoria inter-lineage reassort-

ment (Yamagata HA lineage and Victoria NA lineage) among the Influenza B viruses circulating in the DRC in 2016, as reported elsewhere, in Senegal in 2015-2016, in Thailand in 2014-2015, in Cameroon between 2014 and 2017, in China during the 2014-2015 season [38] and in Kenya in 2016 [30] [38] [39]. However, GISAID sequence analyses showed that these Y3 reassorting viruses had circulated in several other African countries, including Burkina Faso (2015), Congo (2016), Ivory Coast (2015), Ghana (2015-2016), Mali (2015-2016), Mozambique (2015), Niger (2015-2017), Nigeria (2015), Rwanda (2016), South Africa (2015), Tanzania (2015-2016), Togo (2016), Uganda (2016) and Zambia (2014-2015).

However, some studies have shown that reassortment is one of the evolutionary mechanisms of Flu B, allowing it to adapt to selection pressure [40]. Nevertheless, despite the antigenic variability of Flu B in the DRC, all strains of Influenza B circulating in the DRC were suitable for the influenza vaccines validated by the WHO for each specific influenza season.

Finally, our study highlights an alternation between B/Yamagata clades (Y2, Y3, Y3V1A) and B/Victoria clades (V1A, V1A.3) with vaccine compatibility. However, the deletions observed in HA1 emphasize the necessity for continuous genetic surveillance to anticipate mutations that could impact transmissibility and vaccine efficacy.

The weaknesses of this study include its limited geographic scope, as it covers the Democratic Republic of the Congo but does not incorporate a comparative analysis with other regions or countries, which could restrict the understanding of regional versus global trends. Additionally, there is a risk of sampling bias if the samples are not representative of all regions within the country, potentially affecting the generalizability of the findings.

The strengths of the study include its extended 8-year period, offering valuable insights into the evolution and temporal dynamics of Influenza B viruses. The large dataset, with 8,497 samples, enhances the reliability and generalizability of the findings. The study's comprehensive surveillance covers both outpatients and hospitalized patients, providing a broad view of virus circulation. Molecular techniques, such as qRT-PCR and sequencing, enable detailed genetic and antigenic analysis, deepening the understanding of virus evolution. The evaluation of vaccine strain alignment is crucial for assessing vaccine effectiveness. Additionally, the identification of reassortment events and the use of phylogenetic analysis contribute to understanding the genetic diversity and adaptation of the viruses.

5. Conclusion

This study highlights the genetic variability and evolutionary dynamics of Influenza B viruses circulating in the DRC from 2015 to 2022. The detection of genetic reassortants and amino acid deletions within the Victoria lineage emphasizes the importance of molecular surveillance to identify mutations that may affect transmissibility, vaccine efficacy, and public health. Understanding the interaction between influenza viruses and WHO-recommended vaccine strains is crucial for

predicting their impact and improving vaccine design. The findings also underline the need for genomic surveillance to detect emerging strains that could pose significant health risks.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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