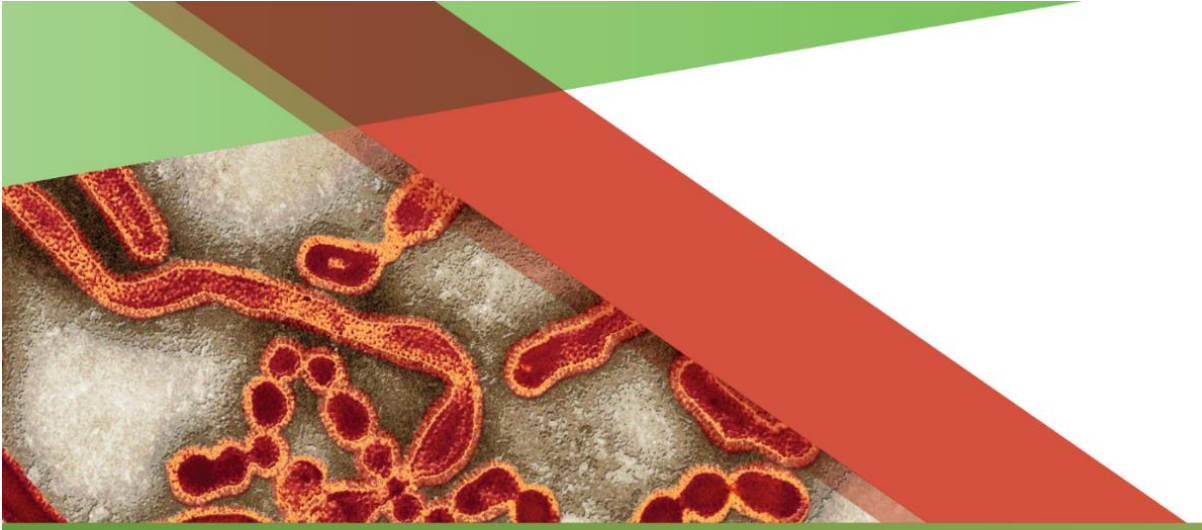

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, March 2021

Summary

This is the fifth report for the 2020-2021 influenza season. As of week 12/2021, only 758 influenza detections across the WHO European Region had been reported to The European Surveillance System (TESSy); 50% type A viruses, with A(H3N2) and A(H1N1)pdm09 being equally represented, and 50% type B viruses, with only 12 having been ascribed to a lineage, 10 B/Victoria and two B/Yamagata. This represents a 99.5% drop in detections compared with the same period in 2020, probably due to the COVID-19 pandemic and measures introduced to combat it.

Since the February 2021 characterisation report[1], no shipments from European Union/European Economic Area (EU/EEA) countries have been received at the London WHO Collaborating Centre, the Francis Crick Worldwide Influenza Centre (WIC): consequently, no new virus characterisation data have been generated. This report therefore focuses on genetic characterisation of the HA genes of seasonal influenza viruses based on sequences deposited and/or released in GISAID during March 2021, compared with the situation reported in the February 2021 characterisation report. The most recent data continues to show extremely low levels of influenza detections across North America and Europe. Globally, few or no detections of A(H1N1)pdm09 and B/Yamagata-lineage viruses have been reported while new variants of A(H3N2) and B/Victoria-lineage viruses have emerged, with the majority of detections reported by Asian and West African countries with evidence of wider geographic spread.

All HA sequences of newly reported A(H1N1)pdm09 viruses for the 2020-2021 season, detected in countries of West Africa, fall in the 6B.1A5A+187V/A subgroup represented by A/Guangdong-Maonan/SWL1536/2019, the vaccine virus for the northern hemisphere 2020-2021 season, but many had additional HA1 amino acid substitutions of I166T and A186T. A/Victoria/2570/2019-like viruses, in the antigenically distinct 6B.1A5A+156K subgroup, have been recommended for the southern hemisphere 2021 and northern hemisphere 2021-2022 influenza seasons.

A(H3N2) HA sequences of newly reported 2020-2021 season viruses detected in countries of West Africa fall in the 3C.2a1b+T135K-A subgroup represented by the reference virus A/Denmark/3264/2019. Other newly reported sequences fall in the 3C.2a1b+T131K-A subgroup which splits into two antigenically distinct clusters originally defined by viruses from Cambodia (with HA1 amino acid substitutions of G186S, F193S, Y195F and S198P, many also having K171N) and Bangladesh (with HA1 amino acid substitutions of Y159N, T160I (loss of a glycosylation site), L164Q,

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, February 2021. Stockholm: ECDC; 2020. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Influenza-characterisation-report-February-2021.pdf

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G186D, D190N, F193S and Y195F). A/Hong Kong/2671/2019-like viruses (subgroup 3C.2a1b+T135K-B) have been recommended for use in vaccines for the southern hemisphere 2021 season and A/Cambodia/e0826360/2020-like viruses (subgroup 3C.2a1b+T131K-A) for the 2021-2022 northern hemisphere season.

Of 19 B/Victoria-lineage HA sequences deposited and/or released in GISAID for viruses with collection dates in the 2020-2021 season, seven were from newly reported viruses and all fell within subclade 1A(△3)B. Of the seven viruses, one from the United States of America fell in the HA1 G133R substitution group, while six viruses from Niger, Nigeria, Senegal and Spain fell in the HA1 N150K, G184E, N197D (loss of a glycosylation site) and R279 substitution group and form a subgroup with additional HA1 substitutions of A127T, P144L, T182A, D197E, K203R and T221A. Antigenically, viruses in subgroups of the HA1 N150K, G184E, N197D (loss of a glycosylation site) and R279 substitution group differ and show some loss of reactivity with post-infection ferret antisera raised against the B /Washington/02/2019 vaccine virus which is recommended for inclusion in influenza vaccines for the 2020-2021 and 2021-2022 northern hemisphere seasons and 2021 southern hemisphere season.

No new B/Yamagata-lineage HA sequences were deposited and/or released in GISAID during March and those previously released with collection dates in 2020, all before 1 April, belong to genetic clade 3. In addition, all contain at least three HA1 amino acid substitutions (L172Q, D229N and M251V) compared with B/Phuket/3073/2013-like viruses which have been recommended for use in quadrivalent influenza vaccines for the 2020-2021 and 2021-2022 northern hemisphere seasons and 2021 southern hemisphere season. The antigenic effects of these amino acid substitutions have been minimal as assessed in earlier reports.

Table 1 shows a summary of influenza virus detections in the WHO European Region reported to The European Surveillance System (TESSy) database for the 2020-2021 season (weeks 40/2020-12/2021), compared with the same period for the 2019-2020 season. While there has been a significant reduction in the numbers of samples from patients fulfilling Influenza-Like Illness (ILI) and/or Acute Respiratory Infection (ARI) criteria being tested (~111 413, 15.9%), there has been a vast reduction in the number of samples testing positive for an influenza virus (157 356, 99.5%). This is probably due to a number of factors:

- the number of centres within the Region reporting over these periods having dropped from 52 to 45;
- significant numbers of samples taken from patients fulfilling ILI and/or ARI criteria being infected with other agents, possibly SARS-CoV-2, the virus responsible for the COVID-19 pandemic;
- restrictions on travel and social/work place gatherings imposed to help curtail the spread of SARS-CoV-2; also impeding the spread of influenza viruses;
- viral interference, with SARS-CoV-2 infection impeding infection by influenza viruses.

With these caveats, and being mindful of the low number of detections over the first 26 weeks of the 2020-2021 season, the ratio of type A to type B detections is reduced compared with the 2019-2020 season (2.8:1 to 1:1), with similar proportions of influenza A subtypes while B/Victoria lineage viruses appear, again, to be predominating over B/Yamagata lineage viruses, although only 12/377 (3.2%) of type B viruses detected in the 2020-2021 season have been ascribed to a lineage as of week 12/2021.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2020-21 season (weeks 40/2020-12/2021)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40/2020-12/2021			Totals*		Cumulative number of detections for weeks 40/2019-12/2020			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**22**	**359**	**381**	**50.3**	**1:1**	**11109**	**105386**	**116495**	**73.7**	**2.8:1**
A(H1N1)pdm09	13	29	42	48.8		6030	19278	25308	55.6	
A(H3N2)	6	38	44	51.2	1:1	4085	16158	20243	44.4	0.8:1
A not subtyped	3	292	295			994	69950	70944		
Influenza B	**15**	**362**	**377**	**49.7**		**6061**	**35558**	**41619**	**26.3**	
Victoria lineage	2	8	10	83.3	5:1	2341	1960	4301	98.2	53:1
Yamagata lineage	0	2	2	16.7		21	60	81	1.8	
Lineage not ascribed	13	352	365			3699	33538	37237		
Total detections (total tested)	**37 (30829)**	**721 (>560658)**	**758 (>591487)**			**17170 (46859)**	**140944 (>656041)**	**158114 (>702900)**		

[a] **Numbers taken from Flu News Europe week 12/2021 and week 12/2020 reports**

*** Percentages are shown for total detections (types A & B [in bold type], and for viruses ascribed to influenza A subtype and influenza B lineage). Ratios are given for type A:B [in bold type], A(H3N2):A(H1N1)pdm09 and Victoria:Yamagata lineages.**

Since week 40/2020, six shipments of specimens (virus isolates and/or clinical specimens) were received at the Crick Worldwide Influenza Centre (WIC), none of which were received in March 2021. The packages contained 20 virus-related samples with collection dates after 31 August 2020 and were made up of nine type A viruses and 11 type B viruses.

Genetic and antigenic characterisation data generated at the WIC for viruses with collection dates after 31 August 2020 until 31 January 2021, up to 15 February 2021, contributed to the WIC virus characterisation report that was presented at the WHO influenza vaccine composition meeting (VCM) in February 2021 when recommendations were made for the northern hemisphere 2021-2022 season. Recommendations for the 2020-2021 northern hemisphere, the upcoming 2021 southern hemisphere and 2021-2022 northern hemisphere seasons, have been published [1, 2, 3].

Due to the low number of influenza-positive specimens detected and thereby available for sharing with WIC, recent influenza characterisation reports, and this one, have been based mainly on phylogenetic analyses of complete HA gene sequences submitted to the EpiFlu™ database of the Global Initiative on Sharing All Influenza Data (GISAID), inclusive of sequences generated at the WIC, with those from EU/EEA countries highlighted. No antigenic and antiviral susceptibility data were generated by the WIC, for viruses recovered from samples shared by EU/EEA countries, since the February 2021 report.

Influenza A(H1N1)pdm09 virus analyses

All recently circulating viruses have fallen into clade 6B.1A, defined by the amino acid substitutions **S74R**, **S84N**, **S162N** (introducing a potential N-linked glycosylation site), **S164T** (which alters the glycosylation motif at residues 162 to 164), **I216T** and **I295V** in **HA1**. Within clade 6B.1A, clusters of viruses (genetic groups) encoding a range of **HA** amino acid substitutions have emerged, with most recently circulating viruses carrying the substitution **S183P** in **HA1**, although this is not retained in all genetic groups. Figures 1a and 1b are annotated with **HA1 S183P** substitution groups assigned for the February 2019 WHO VCM (6B.1A/183P-1 to -7, abbreviated to 6B.1A1 to 6B.1A7) with updates introduced for the September 2020 WHO VCM. The recommended vaccine viruses for the northern hemisphere 2020–2021 (egg-based A/Guangdong-Maonan/SWL1536-like and cell-based A/Hawaii/70/2019-like) and southern hemisphere 2021 and northern hemisphere 2021-2022 (egg-based A/Victoria/5270/2019-like and cell-based A/Wisconsin/588/2019-like) influenza seasons are shown in red [1, 2, 3]. The seven subclades are defined by the following HA amino acid substitutions:

1. Subclade **6B.1A1** viruses, represented by the 2019-2020 vaccine virus **A/Brisbane/02/2018**, carry an HA gene mutation encoding **HA1 S183P** amino acid substitution.
2. Subclade **6B.1A2** viruses, represented by **A/Denmark/2728/2019**, carry HA gene mutations encoding **HA1 S183P** and **L233I** with **HA2 V193A** amino acid substitutions – a group within this subclade has emerged with additional **HA1** amino acid substitutions of **N129D, K130N, P137S, N156K** and **K211R** (e.g. **A/Hong Kong/110/2019**).
3. Subclade **6B.1A3** viruses, represented by **A/Norway/3737/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions.
4. Subclade **6B.1A4** represented by **A/Hungary/20/2018** carries HA gene mutations encoding **HA1 N129D, A144E** and **S183P** amino acid substitutions.
5. Subclade **6B.1A5** viruses carry HA gene mutations encoding **HA1 S183P** and **N260D** amino acid substitutions and splits into two groups designated **6B.1A5A** represented by **A/Norway/3433/2018** with additional **HA1** amino acid substitutions of **N129D** and **T185A**, and **6B.1A5B** represented by **A/Switzerland/3330/2017** with additional amino acid substitutions of **HA1 E235D** and **HA2 V193A**. Two subgroups within the **6B.1A5A** group have been defined based on **HA1** amino acid substitutions of **D187V/A** and **Q189E** (**6B.1A5A+187V/A**) or **K130N**, **N156K**, **L161I** and **V250A** (**6B.1A5A+156K**).
6. Subclade **6B.1A6** viruses, represented by **A/Ireland/84630/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions, like subclade **6B.1A3** viruses, but fall within a separate phylogenetic branch which is closer to subclade **6B.1A5** viruses.
7. Subclade **6B.1A7** viruses, represented by **A/Slovenia/1489/2019**, carry HA gene mutations encoding **HA1 K302T** and **HA2 I77M, N169S** and **E179D** amino acid substitutions sometimes with additional **HA1** substitutions of **E68D, S121N** and **L161I** (e.g. **A/Moscow/193/2019**). Note: a group within this subclade has emerged with **P183S** (reversion)**, T185I, I240V** and **I286L** substitutions in **HA1** (e.g. **A/Estonia/120012/2019**).

The two A(H1N1)pdm09 HA phylogenies have very similar profiles and vaccine viruses are shown in red. The first is repeated from the February 2021 report and was generated based on sequences from all A(H1N1)pdm09 viruses with collection dates from 1 April 2020 that were deposited and/or released in GISAID as of 28 February 2021. Just 27 viruses had collection dates after 31 August 2020 and these were detected in five countries: China (n = 1), Ghana (n =5), Japan (n = 2), Togo (n = 17) and US (n = 2) (Figure 1a). The majority (25/27; 93%) of recently circulating viruses had fallen into group **6B.1A5A** with one falling into the parent group and 22 and two, respectively, falling into the **6B.1A5A+187V/A** and **6B.1A5A+156K** subgroups. The two viruses from the US fell in subgroup **6B.1A5B** (with **HA1 K130N**, **K160M**, **T216K**, **E235D**, **H296N** and **HA2 V193A** substitutions). Of the HA sequences deposited and/or released in GISAID during March 2021, the great majority were archival, i.e. from the 2019-2020 season, and of the 23 from the 2020-2021 season two had been reported previously with different passage histories (Figures 1a and 1b). The remaining 21 HA sequences fell into the **6B.1A5A+187V/A** subgroup (with many having additional HA1 substitutions of I166T and A186T) and were detected in Niger and Nigeria (Figure 1b).

The great majority of viruses characterised antigenically by the WIC in the course of the 2019-2020 influenza season, with the exception of those in subgroup **6B.1A5A+156K**, were antigenically similar to A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like viruses (with **HA1 D187A** and **Q189E** amino acid substitutions), recommended for use in the northern hemisphere 2020-2021 influenza season [1], as assessed by haemagglutination inhibition (HI) assays with a panel of post-infection ferret antisera raised against vaccine and reference viruses. Results of HI assays for viruses detected in EU/EEA countries can be seen in previous influenza characterisation reports: https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/influenza-virus-characterisation [accessed 7 April 2021].

As was the case at the time of the February 2021 report: no A(H1N1)pdm09 viruses were characterised antigenically at the WIC during March 2021.

Figure 1a. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, February 2021)

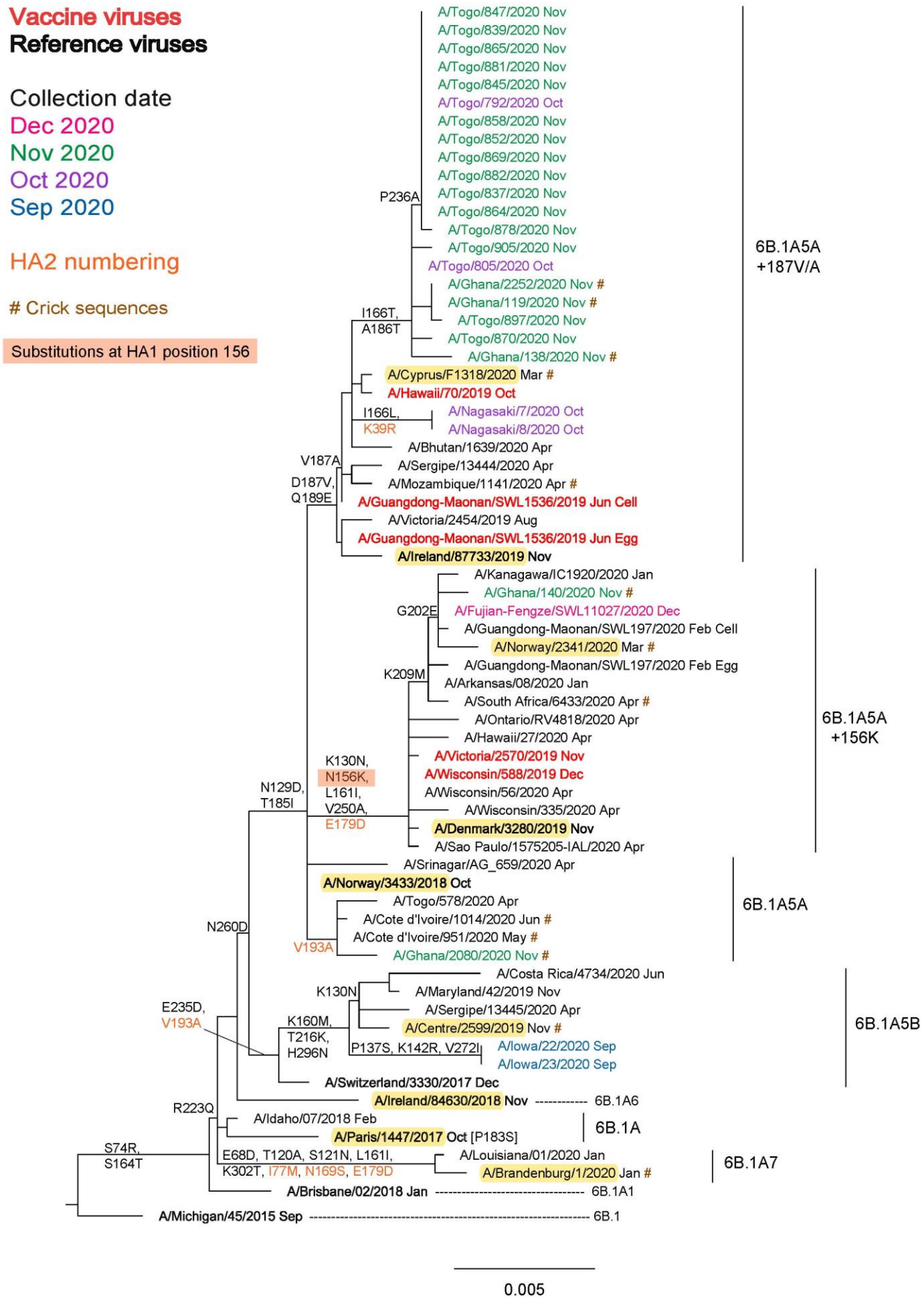
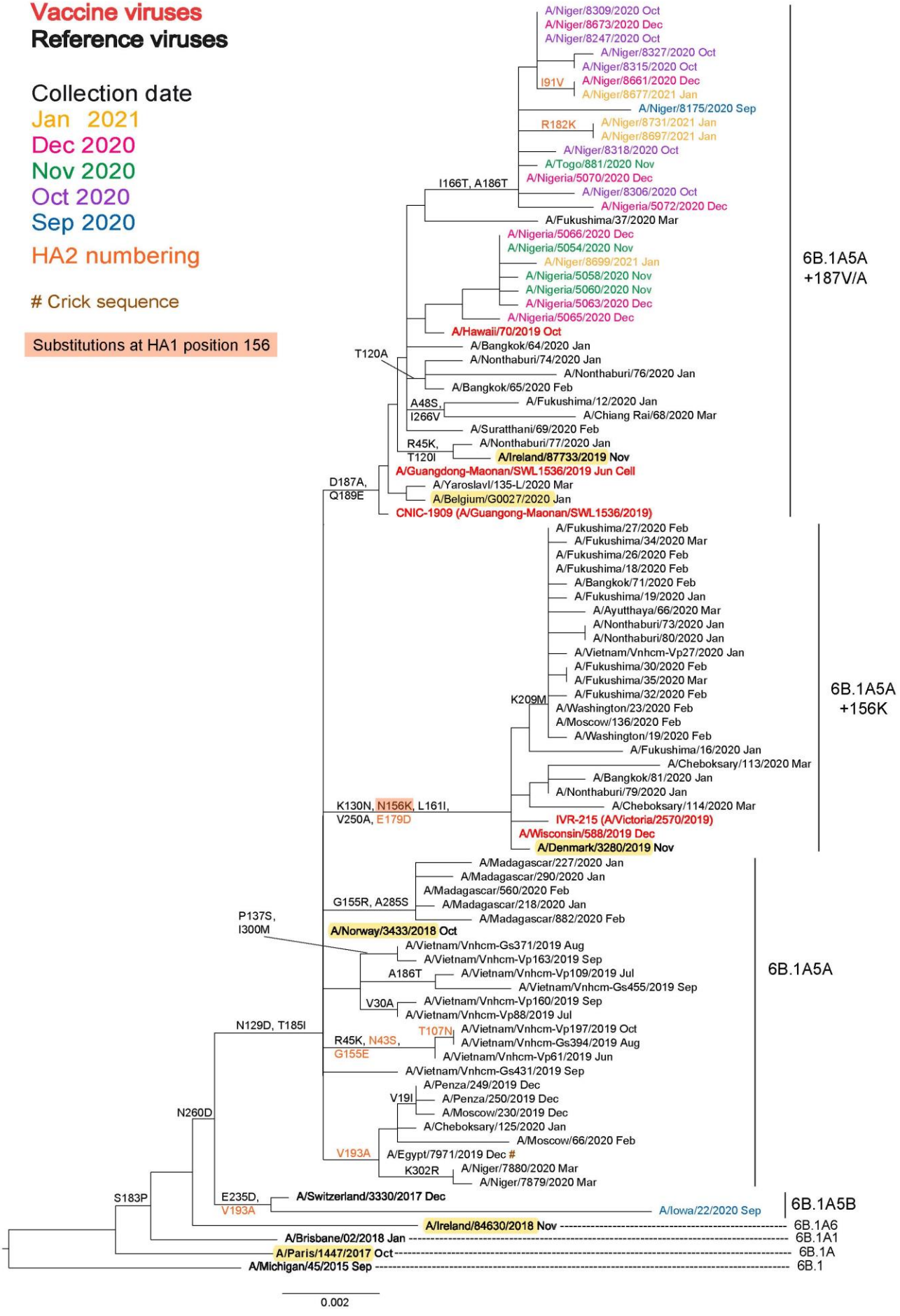


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, March 2021)



Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny is repeated from the February 2021 report and was generated based on sequences deposited in GISAID up to 28 February 2021 derived from viruses with collection dates from 1 September 2020 (Figure 2a). The second is based on all sequences deposited in GISAID during March 2021 with no limits on the collection dates of the viruses (Figure 2b).

Viruses in clade 3C.2a have been dominant since the 2014-15 influenza season with group 3C.2a1b viruses predominating over the course of the 2019-2020 season in most WHO-defined regions of the world but for the European Region where there was equivalence of clade 3C.3a viruses. The HA gene sequences of viruses in both clades 3C.2a and 3C.3a continue to diverge. Notably, clade 3C.3a viruses have evolved to carry **HA1** amino acid substitutions of **L3I, S91N**, **N144K** (loss of a N-linked glycosylation motif at residues 144-146), **F193S** and **K326R**, and **D160N** in **HA2**, compared with cell culture-propagated A/Stockholm/6/2014. Greater variation has been observed among clade 3C.2a viruses, resulting in the designation of new subclades/groups/subgroups. Amino acid substitutions that define these subclades/groups/subgroups are:

- Subclade **3C.2a1**: Those in clade **3C.2a** plus **N171K** in **HA1** and **I77V** and **G155E** in **HA2**, most also carry **N121K** in **HA1**, e.g. **A/Singapore/INFIMH-16-0019/2016** (a former vaccine virus).
- Group **3C.2a1a**: Those in subclade **3C.2a1** plus **T135K** in **HA1**, resulting in the loss of a potential glycosylation site, and **G150E** in **HA2**, e.g. **A/Greece/4/2017.**
- Group **3C.2a1b**: Those in subclade **3C.2a1** plus **E62G, R142G** and **H311Q** in **HA1**, often with additional amino acid substitutions – notably **HA1 T131K** and **HA2 V200I**, the **3C.2a1b+T131K** subgroup (e.g. **A/Norway/3275/2018**) or **HA1 T135K** (resulting in the loss of a potential glycosylation site) commonly with **T128A** (resulting in the loss of a potential glycosylation site), the **3C.2a1b+T135K** subgroup (e.g. **A/La Rioja/2202/2018**). Distinct clusters of viruses within both these subgroups have emerged defined by specific **HA1** and/or **HA2** amino acid substitutions: **3C.2a1b+T131K-A** with additional amino acid substitutions of **HA1 K83E** and **Y94N** with **HA2 I193M** (e.g. **A/Christchurch/502/2020**); **3C.2a1b+T131K-B** with **HA2 V18M** substitution, often with additional **HA1** substitutions (e.g. A/South Australia/34/2019); **3C.2a1b+T135K-A** with additional amino acid substitutions of **HA1 A138S**, **F193S** and **S198P**, many also with **G186D** and **D190N** (e.g. **A/Denmark/3284/2019**); and **3C.2a1b+T135K-B** with additional amino acid substitutions of **HA1 S137F, A138S** and **F193S** (e.g. **A/Hong Kong/2671/2019**).
- Clade **3C.3a**: represented by a former vaccine virus, **A/Switzerland/9715293/2013**, with recently circulating clade **3C.3a** viruses carrying additional substitutions of **S91N, N144K** (resulting in the loss of a potential glycosylation site)**, and F193S** in **HA1** and **D160N** in **HA2**, e.g. **A/England/538/2018** and **A/Kansas/14/2017**, the A(H3N2) vaccine virus for the 2019-2020 northern hemisphere influenza season.

The significant geographic spread of viruses in the antigenically distinct **3C.2a1b+T135K-B** cluster influenced the selection of an A/Hong Kong/2671/2019-like virus as the A(H3N2) component of vaccines for the 2020-2021 northern hemisphere and 2021 southern hemisphere influenza seasons [1, 2].

The HA phylogeny generated for the February report, based on sequences derived from viruses with collection dates from 1 September 2020 and deposited in GISAID up to 28 February, showed a huge preponderance of viruses in group **3C.2a1b** over those in clade **3C.3a** (Figure 2a). A single virus from Bangladesh fell in the subgroup **3C.2a1b+T135K-B** cluster, 24 in the subgroup **3C.2a1b+T135K-A** cluster (23 from West African countries and one from France) and 137 in the subgroup **3C.2a1b+T131K-A** cluster. Of those in the subgroup **3C.2a1b+T131K-A** cluster: 40 'Cambodia-like' viruses carried additional **HA1** substitutions of **G186S**, **F193S**, **Y195F** and **S198P** with 39 also having **K171N** (22 from Cambodia, one from Cote d'Ivoire, 12 from Lao People's Democratic Republic, four from Viet Nam and one from US) and 96 'Bangladesh-like' viruses had additional **HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N**, **F193S** and **Y195F** (84 from Bangladesh, 4 from Bahrain, one from Oman, two from Sweden, three from the United Arab Emirates and two from US). The phylogeny for sequences deposited and/or released in GISAID during March 2021 shows a similar pattern for viruses detected during the 2020-2021 season with evidence of geographic spread (Figure 2b). All 29 viruses (26 newly reported and three with a different passage history) in the subgroup **3C.2a1b+T135K-A** cluster were detected in countries of West Africa and detections of viruses in the subgroup **3C.2a1b+T131K-A** cluster were largely confined to Asian countries but with reports of new 'Cambodia-like' virus detections in Australia, Cambodia, Japan, Thailand and Vietnam, and 'Bangladesh-like' viruses in India. While the number of detections of seasonal influenza viruses remain low, compared with previous seasons, the WHO Collaborating Centres for Influenza have shown viruses in these recently emerged virus clusters to be antigenically distinct from one another and other A(H3N2) virus subgroups.

The locations of HA sequences for A/Hong Kong/2671/2019 (**3C.2a1b+T135K-B**) and its cell culture-equivalent A/Hong Kong/45/2019, recommended for egg- and cell culture-generated vaccines to be used in the 2020-2021 northern hemisphere [1] and 2021 southern hemisphere [2] seasons, are indicated on the phylogenies as are the recently recommended egg- and cell culture-propagated cultivars of A/Cambodia/e0826360/2020 (**3C.2a1b+T131K-A**) recently recommended for use in northern hemisphere 2021-2022 vaccines [3] (Figures 2a and 2b).

As described in many previous reports[2], influenza A(H3N2) viruses have continued to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells (RBCs) from guinea pigs, turkeys, and humans, often with the loss of ability to agglutinate any of these RBCs. As was highlighted first in the November 2014 report[3], this has been a significant problem for most viruses that fall in genetic clade **3C.2a**, although there was some alleviation of this over the course of the 2019-2020 influenza season.

Results of HI assays with panels of post-infection ferret antisera raised against A(H3N2) vaccine and reference viruses for viruses detected in EU/EEA countries can be seen in previous influenza characterisation reports on ECDC's website. Overall, these data show strong clade/subclade-specific recognition of test viruses by post-infection ferret antisera raised against cell culture-propagated reference viruses, with limited cross-clade/subclade recognition and further reductions in recognition of cell culture-propagated recently circulating viruses by antisera raised against A(H3N2) egg-propagated vaccine viruses.

[2] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2013. Stockholm: ECDC; 2013. Available from: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/influenza-virus-characterisation-sep-2013.pdf

[3] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, November 2014. Stockholm: ECDC; 2014. Available from: https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/ERLI-Net%20report%20November%202014.pdf

Figure 2a. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, February 2021)

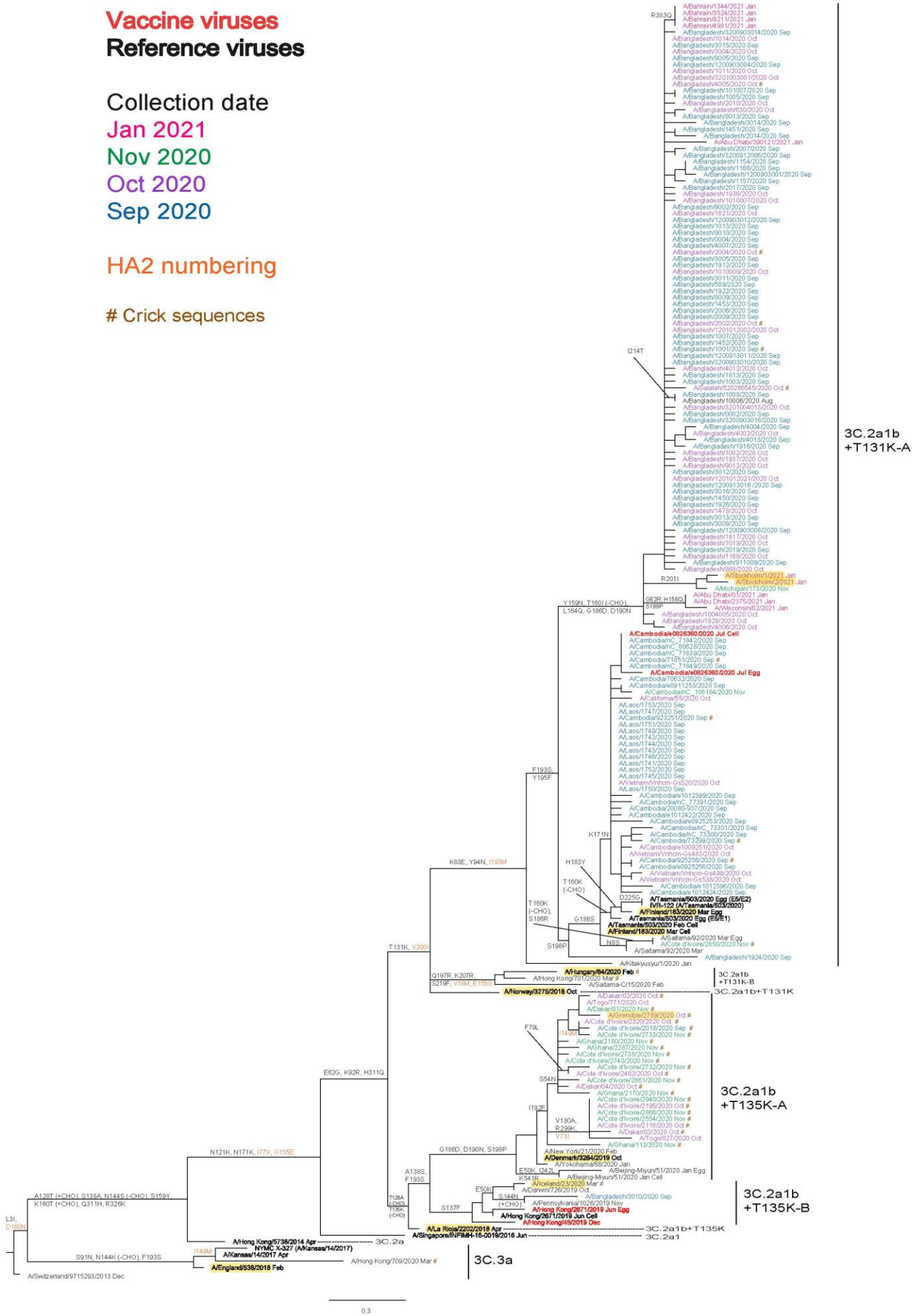
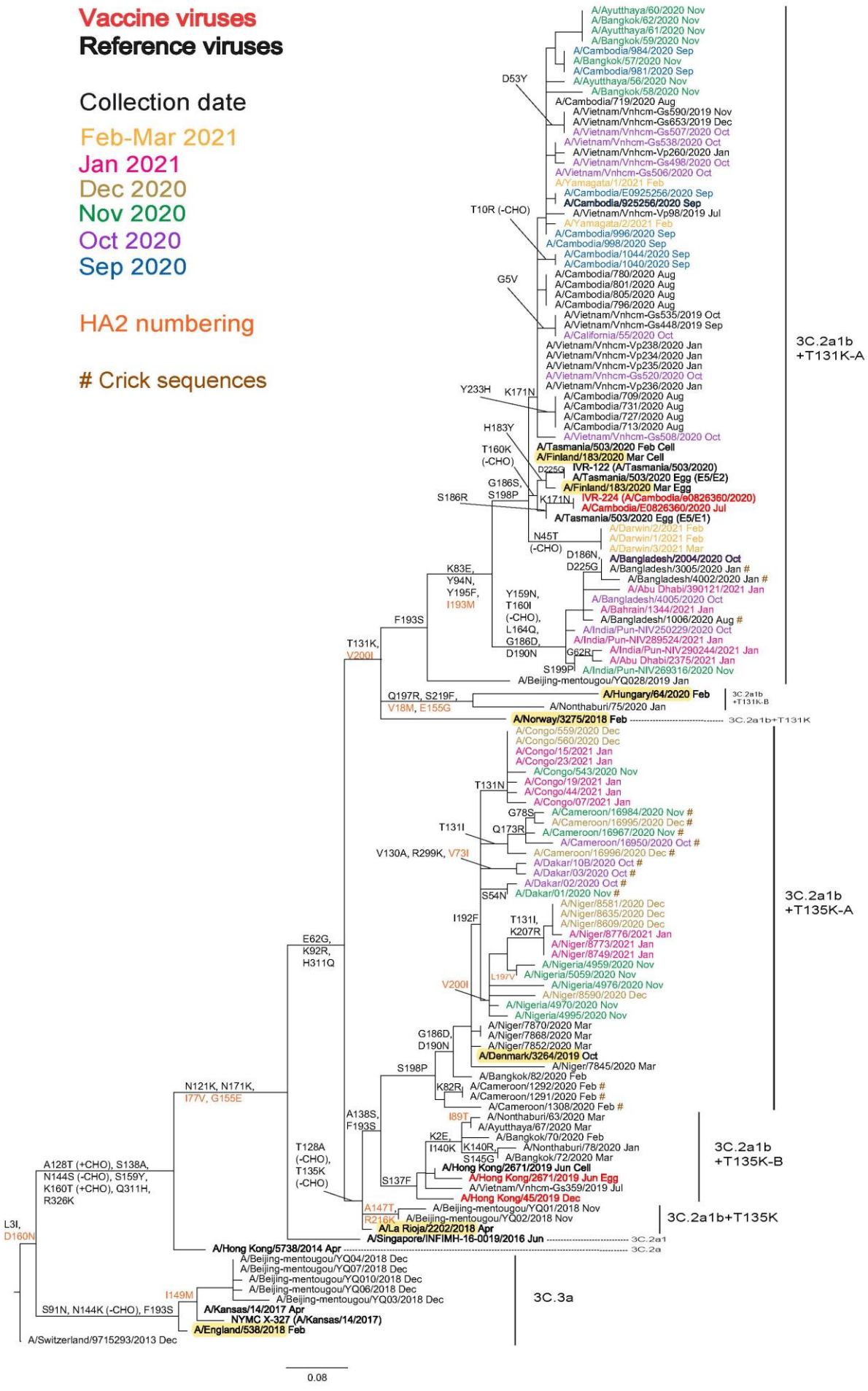


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, March 2021)



Influenza B virus analyses

Influenza B/Victoria-lineage

All recently circulating B/Victoria-lineage viruses have fallen in genetic **clade 1A**, represented by **B/Brisbane/60/2008**, a former vaccine virus, but with additional **HA1** amino acid substitutions of **I117V, N129D** and **V146I** (e.g. **B/Ireland/3154/2016**). Viruses retaining full-length HAs have remained similar antigenically to B/Brisbane/60/2008. However, three genetic groups (described below with amino acid substitutions/deletions relative to B/Brisbane/60/2008 indicated) containing deletions of HA gene codons have emerged and the viruses in these groups are antigenically distinct from B/Brisbane/60/2008 and each other (as noted in the September 2018 characterisation report[4] and earlier ones), such that four antigenically distinguishable groups had been circulating:

- A group with double deletion of **HA1** residues **162** and **163** (**subclade △162-163** or **1A(△2)**) with amino acid substitutions of **D129G** and **I180V**, and **HA2 R151K** that spread worldwide and is represented by a previous vaccine virus, **B/Colorado/06/2017**.
- A group with triple deletion of **HA1** residues **162** to **164** (**subclade △162-164A** or **1A(△3)A**) first detected in Asia, with amino acid substitutions of **I180T** and **K209N** that showed limited geographic spread (with no detections having been made recently), represented by **B/Hong Kong/269/2017**.
- A group with triple deletion of **HA1** residues **162** to **164** (**subclade △162-164B** or **1A(△3)B**) first detected in Africa, with amino acid substitution **K136E** often with **G133R** that showed geographic spread and dominance in recent months, represented by **B/Washington/02/2019** the vaccine virus recommended after WHO VCMs in February and September 2020, and February 2021 [1, 2, 3].

The phylogeny generated for the February report showed HA genes of viruses with collection dates from 1 April 2020 as deposited in GISAID up to 28 February 2021 (Figure 3a). Several virus groups had emerged defined by specific amino acid substitutions, e.g. **HA1 E128K** or **D129N** with **N233S** (loss of a glycosylation site) or **N150K** with **G184E**, **N197D** (loss of a glycosylation site) and **R279K** or **N233K** (loss of a glycosylation site), with no **subclade 1A(△2)** viruses having been detected. Viruses with collection dates up to March 2020 mainly fell in virus groups defined by **HA1** substitutions **N126K** or **E128K** or **D129N** with **N233S** (loss of a glycosylation site) or **N233K** (loss of a glycosylation site). However, four viruses from France, two each with collection dates in October and November 2020, fell in the **N150K** with **G184E** group with additional **HA1** substitutions of **A127T**, **P144L** and **K203R**, with three of the viruses having further **HA1** substitutions of **T128A**, **D197E** and **T221A**. All 77 viruses with collection dates from 1 September 2020 fell in **subclade 1A(△3)B**: seven fell in the **HA1 G133R** group (four from China and 3 from Kenya); 38 from China fell in the **N150K** with **G184E** group of viruses with additional **HA1 V220M** and **P241Q** substitutions; and 32 fell in the **N150K** with **G184E** group with additional **HA1 A127T**, **P144L** and **K203R** substitutions and 21 of these had further **HA1 T128A**, **D197E** and **T221A** substitutions. The latter 32 viruses were detected in 12 countries in Europe (Austria, France, Norway and Sweden), the Middle East (Bahrain, Oman and the United Arab Emirates), US and West Africa (Cote d'Ivoire, Ghana, Senegal and Togo).

All 19 viruses with collection dates from the start of the 2020-2021 season deposited and/or released in GISAID during March fell within **subclade 1A(△3)B,** however, 12 were for viruses reported previously but with different passage histories (Figure 3b). Of the seven new viruses, the HA of one, B/Florida/01/2021, fell in the **HA1 G133R** group and was B/Washington/02/2019-like, while the other six (five from West Africa and one from Spain) all fell in the **N150K** with **G184E** group of viruses with additional **HA1 A127T**, **P144L**, **K203R**, **T128A**, **D197E** and **T221A** substitutions. The WHO Collaborating Centres for Influenza have shown the **HA1 N150K** with **G184E** group viruses with additional HA1 substitutions to be antigenically distinct from one another and, despite the low number of B/Victoria-lineage viruses detected, there is indication of geographic spread of viruses in these recently emerged virus subgroups, notably those with **HA1 A127T**, **P144L** and **K203R** substitutions.

Influenza B/Yamagata-lineage

As was the case at the time of the February report: no B/Yamagata-lineage viruses from WHO European Region countries were characterised at the WIC during March. Further, no B/Yamagata-lineage virus sequences were deposited and/or released in GISAID during March 2021, so the phylogeny is repeated from the February report (Figure 4). HA genes fall in genetic **clade 3**, the B/Wisconsin/1/2010–B/Phuket/3073/2013 clade, within a subgroup defined by **HA1 L172Q** and **M251V** amino acid substitutions compared to B/Phuket/3073/2013 which is recommended for inclusion in quadrivalent vaccines for the 2020-2021 northern hemisphere, 2021 southern hemisphere and 2021-2022 northern hemisphere seasons [1, 2, 3]. Some sub-clustering of sequences, defined by specific amino acid substitutions (e.g. **HA1 N164K, K211R, D229N** or **D232N** [introducing a potential N-linked glycosylation site] sometimes with **R48K**), has occurred. As noted in previous characterisation reports, none of these amino acid substitutions have any obvious antigenic effects based on HI assays using post-infection ferret antisera raised against egg-propagated B/Phuket/3073/2013.

[4] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2018. Stockholm: ECDC; 2018. Available from: https://ecdc.europa.eu/sites/portal/files/documents/ECDC-Flu-Characterisation-Report-Sep-2018.pdf

Figure 3a. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, February 2021)

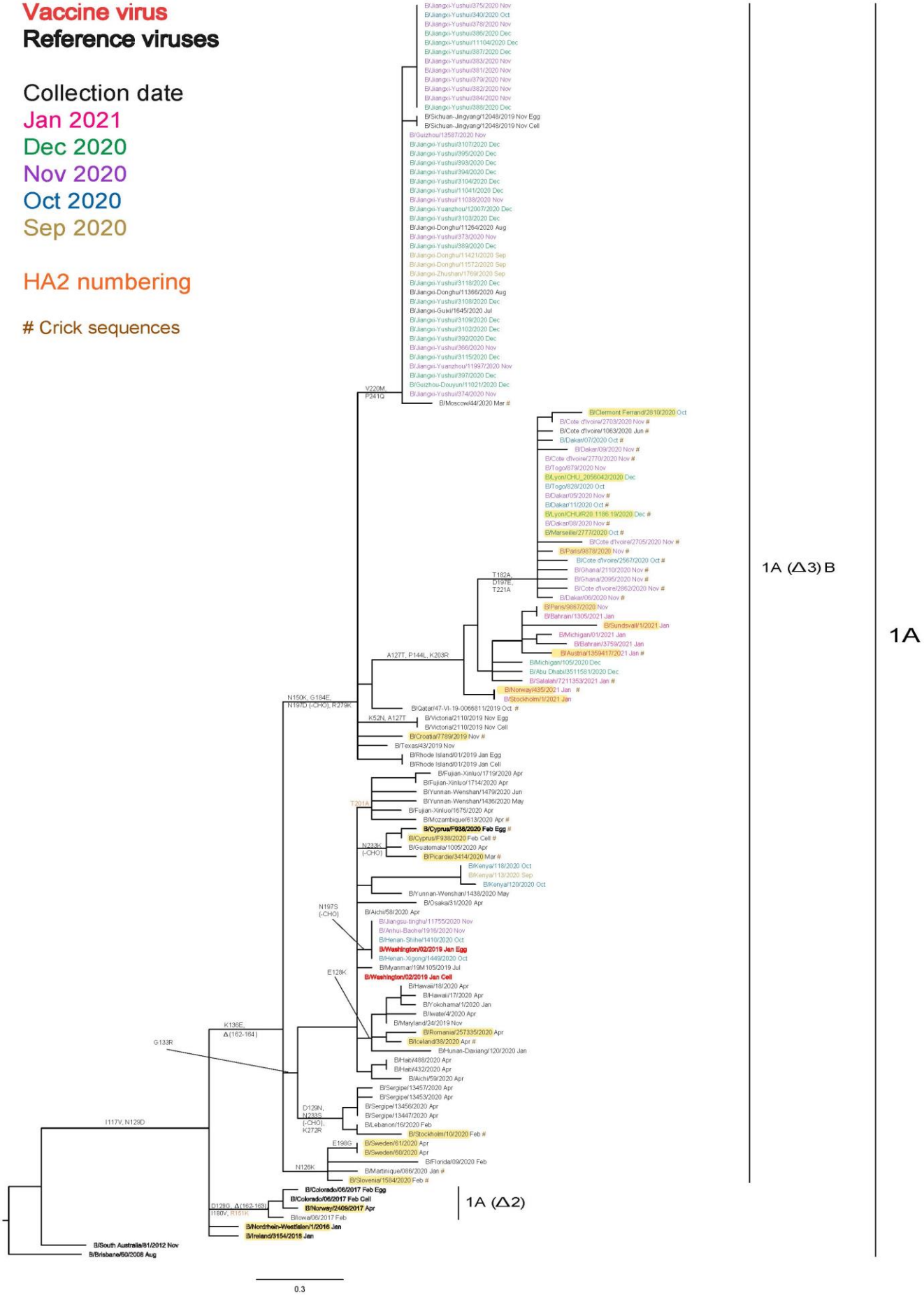


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, March 2021)

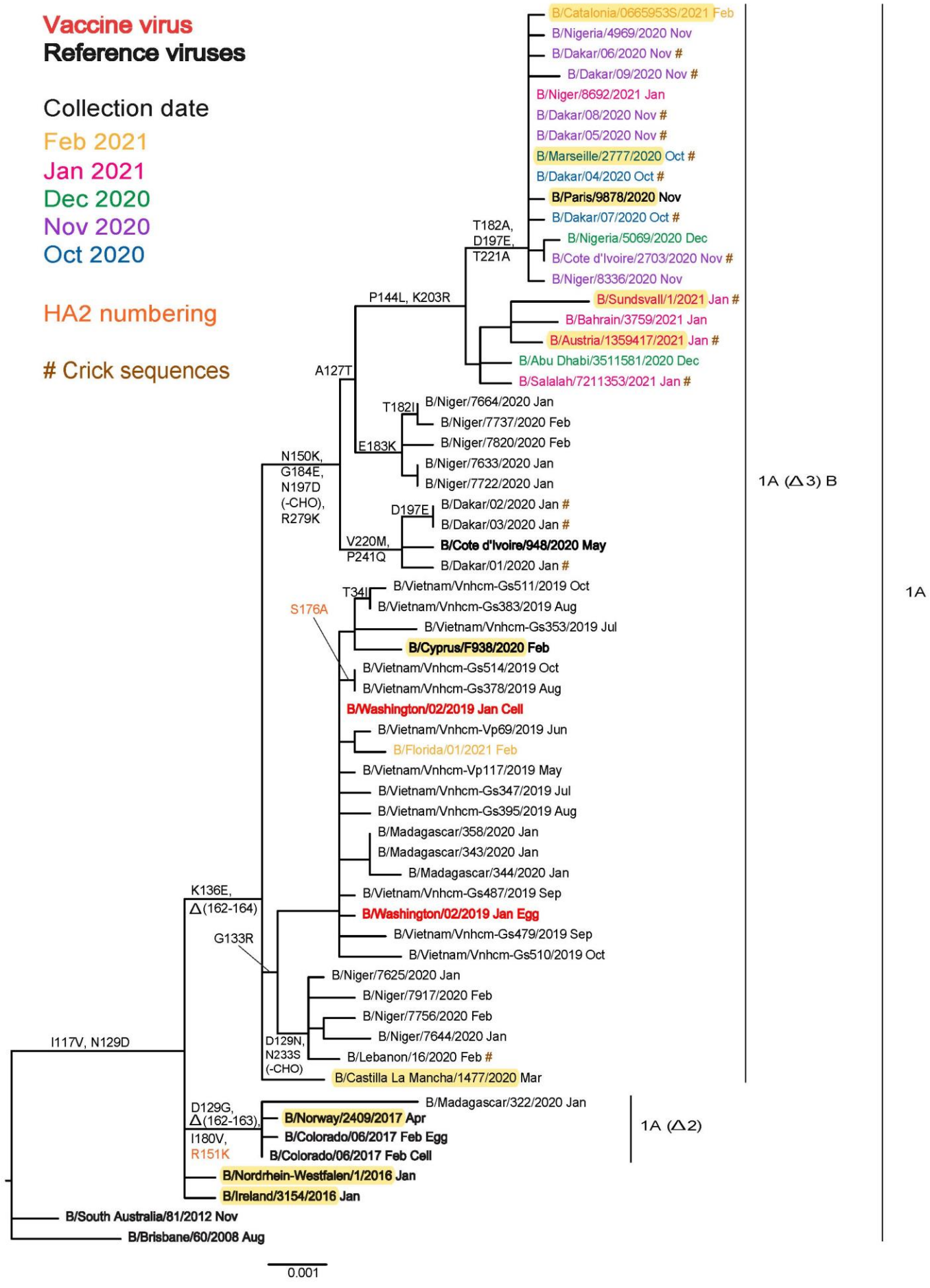
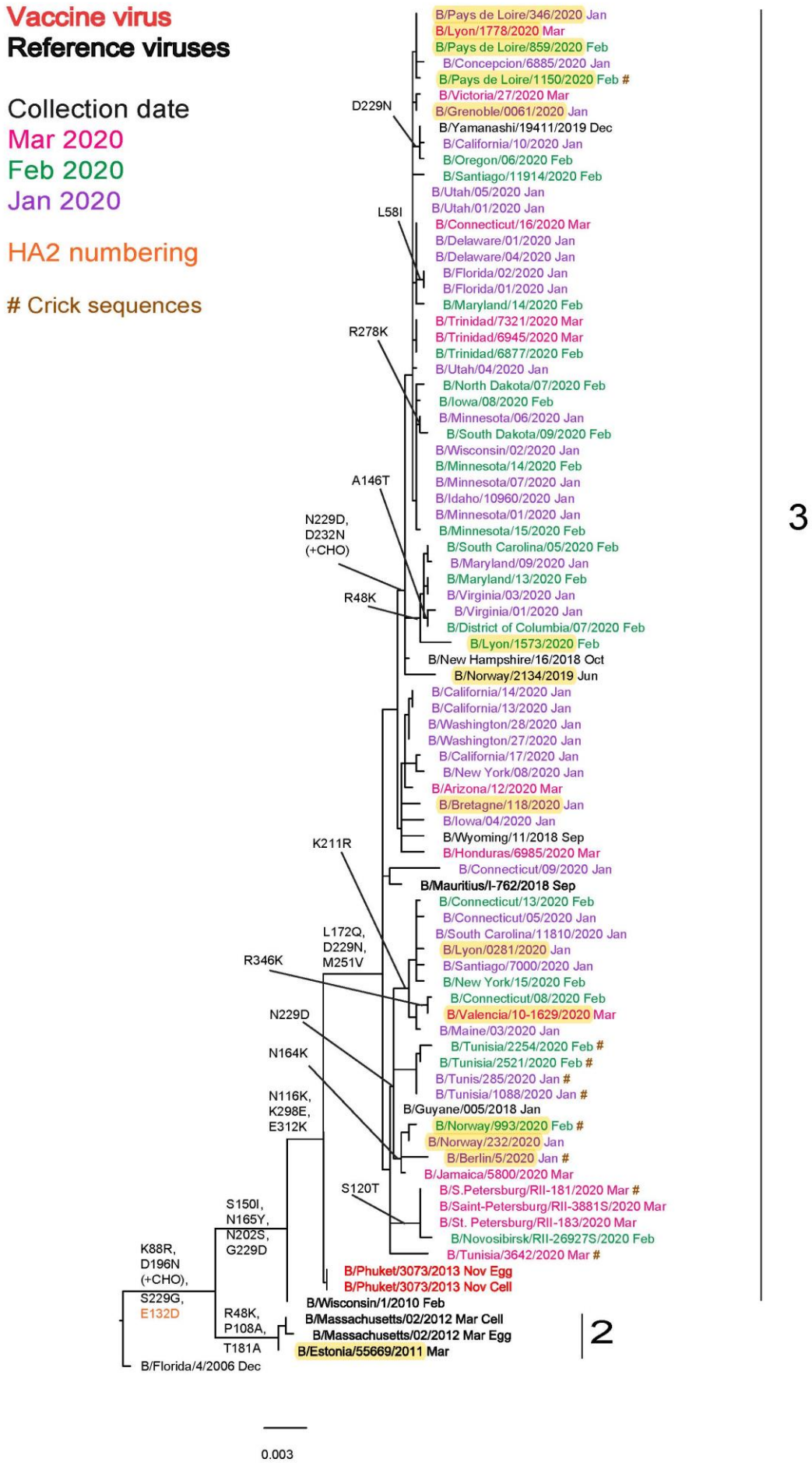


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, February 2021)



Summaries of data submitted to TESSy

Genetic characterisation

Eleven viruses detected over the course of the 2020-2021 season (weeks 40/2020-12/2021) have been genetically characterised:

- One A(H1N1)pdm09 virus attributed to the 6B.1A5A+187V/A group represented by A/Guangdong-Maonan/SWL1536/2019.
- Four A(H3N2) viruses with two attributed to subgroup 3C.2a1b+T131K-A represented by A/Slovenia/1637/2020, one attributed to subgroup 3C.2a1b+T135K-A represented by A/Denmark/3264/2019 and one attributed to subgroup 3C.2a1b+T135K-B represented by A/Hong Kong/2671/2019.
- Six B/Victoria-lineage viruses, four of which were not ascribed to a clade, while two were attributed to subclade 1A(Δ3)B represented by B/Washington/02/2019.

For the 2019-20 season, 2 752 viruses were characterised genetically and ascribed to a genetic clade up to week 20/2020 (no additional characterisations were reported during weeks 21–39/2020).

- In total, 982 were A(H1N1)pdm09 viruses, with 945 being subclade 6B.1A5 (904 subgroup 6B.1A5A represented by A/Norway/3433/2018 and 41 subgroup 6B.1A5B represented by A/Switzerland/3330/2018), 19 being subgroup 6B.1A7 represented by A/Slovenia/1489/2019, 11 being subgroup 6B.1A1 represented by A/Brisbane/02/2018 and seven attributed to a known group not listed in the 2019-20 reporting categories.
- There were 1 048 A(H3N2) viruses, with 342 being subgroup 3C.2a1b+T131K represented by A/South Australia/34/2019, 560 being clade 3C.3a represented by A/Kansas/14/2017, 81 being subgroup 3C.2a1b+T135K-B represented by A/Hong Kong/2675/2019, 64 being subgroup 3C.2a1b+T135K-A represented by A/Denmark/3264/2019 and one attributed to a known group not listed in the 2019-20 reporting categories.
- A total of 26 were B/Yamagata-lineage clade 3, represented by the vaccine virus B/Phuket/3073/2013, with a further two attributed to a known group not listed in the 2019-20 reporting categories.
- There were 694 B/Victoria-lineage viruses, with 630 being subclade 1A(Δ3)B represented by B/Washington/02/2019, 19 being subclade 1A(Δ2) represented by the vaccine virus B/Colorado/06/2017, five being subclade 1A(Δ3)A represented by B/Hong Kong/269/2017 and 40 attributed to a known group not listed in the 2019-20 reporting categories.

Antiviral susceptibility

Four influenza viruses detected within the WHO European Region during the 2020-2021 season have been tested for susceptibility to neuraminidase inhibitors (NAIs: oseltamivir and zanamivir): two each A(H3N2) and B/Victoria-lineage viruses. All four viruses showed normal inhibition (NI) by both NAIs.

Over the course of the 2019-2020 influenza season, of 2 292 viruses assessed for susceptibility to NAIs, only nine (0.39%) showed either reduced or highly reduced inhibition (RI/HRI) by at least one NAI.

At the WIC, five influenza viruses detected within EU/EEA countries during the 2020-2021 season have been assessed phenotypically against oseltamivir and zanamivir: one A(H3N2) and four B/Victoria-lineage. All showed NI by both NAIs.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response System [4] reported that the China Health and Family Planning Commission had notified WHO of three cases of human infection with influenza A(H7N9). A description of the characteristics of H7N9 viruses can be found on WHO's website [5]. Increased numbers of cases were reported over the course of the following seasons, and cases were reported in 2017, including the fifth (2016-17) and largest wave to date, which included the emergence of highly pathogenic avian influenza (HPAI) strains that have caused some zoonoses, although few human cases were reported during the 2017-18 season [6]. WHO posted an analysis of information on A(H7N9) viruses on 10 February 2017 [7], and ECDC published a rapid risk assessment on the implications of A(H7N9) for public health on 3 July 2017 [8]. Current risk assessments are included in WHO's monthly summary and assessment of influenza at human-animal interface (accessed 7 April 2021). The assessment published on 29 January 2021 indicated that there have been no publicly available reports from animal health authorities in China or other countries on influenza A(H7N9) virus detections in animals in recent months [9]. However, the H7N9 situation update published by the Food and Agricultural Organization of the United Nations on 3 February 2021 indicated there had been 14 detections in chickens in Shandong province, China during October 2020, but the report published on 7 April 2021 indicated that there have been no additional detections since then [10]. The most recent human case was detected in mid-March 2019 [11]. The latest overview of avian influenza by ECDC in collaboration with the European Food Safety Authority and the EU Reference Laboratory for Avian Influenza was published on 26 February 2021 and can be found on ECDC's website [12].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 29 January 2021. Since the previous risk assessment on 9 December 2020, four laboratory-confirmed H5N6 cases, all with exposure to poultry, had been reported by China: two in adults both of whom remain hospitalised and two in children, one of whom recovered while the other died [9]. According to reports received by the World Organisation for Animal Health (OIE), various influenza A(H5Nx) subtypes continue to be detected in wild and/or domestic birds in Africa, Europe and Asia. The latest human case of A(H5N1) infection was reported on 31 October 2020 by Lao People's Democratic Republic and was the first reported to WHO since the case in Nepal in March 2019 [13]. On 30 September 2020, ECDC published an alert related to outbreaks of avian influenza viruses in Europe [14]. The latest collaborative report from ECDC and the European Food Safety Authority (EFSA), reports 1 022 highly pathogenic avian influenza (HPAI) A(H5) detections between 8 December 2020 and 23 February 2021 [12]. Detections occurred in 25 EU/EEA countries and the UK, with the majority being associated with 589 poultry outbreaks, predominantly in France (n=446), Germany (n=51) and Poland (n=37). Detections in wild birds (n=421) were predominantly in Germany (n=207), Denmark (n=63) and Ireland (n=20). While a variety of HPAI virus subtypes and different genotypes were detected, suggesting the occurrence of multiple virus introductions into Europe, the great majority of recent detections were subtype A(H5N8).

Influenza A(H9N2) virus

Since the previous WHO update on 9 December 2020 eight laboratory-confirmed human cases of influenza A(H9N2) virus infection in China have been reported [9]. Six cases were in children and two in adults both aged 52, with all but one of the child cases reporting exposure to poultry. All eight cases had mild illnesses and recovered. Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa. The latest ECDC/EFSA report had included mention of China having recently reported 10 cases of human infection with H9N2 viruses [12].

Other influenza zoonotic events

Since the previous WHO update on 9 December 2020, six zoonotic events with Eurasian avian-like swine influenza viruses, A(H1N1)v, were reported to WHO, one by the Netherlands and five by China [9]. All patients recovered, and while the Dutch patient had no exposure to animals before disease onset, the swine exposure status of the Chinese patients was unknown.

Two further zoonoses with swine influenza variant viruses were reported in the latest WHO report [9]. A four-year-old female was infected in November 2020 with an A(H1N2)v virus in Brazil, with the H1 component being derived from an A(H1N1)pdm09-like virus. A child in the US was infected with an A(H3N2)v virus in January 2021 which was similar to viruses circulating in swine in mid-western US during 2019-2020. Both children made a full recovery, had been exposed to swine and neither were hospitalised.

WHO Collaborating Centre reports

A description of results generated by the London WHO Collaborating Centre at the WIC and used at the February 2021 WHO vaccine composition meeting (held online: 17-25 February 2021 for seasonal influenza viruses), and previous ones, can be found at https://www.crick.ac.uk/partnerships/worldwide-influenza-centre/annual-and-interim-reports (accessed 7 April 2021).

Note on the figures

The phylogenetic trees were constructed using RAxML, drawn using FigTree, and annotated using Adobe Illustrator. The bars indicate the proportion of nucleotide changes between sequences. Reference strains are viruses to which post-infection ferret antisera have been raised. The colours indicate the month(s) of sample collection. Isolates from WHO NICs in EU/EEA countries are highlighted in yellow. Sequences for most viruses from non-EU/EEA countries were recovered from the GISAID EpiFlu database. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from the GISAID EpiFlu database, which were downloaded for use in the preparation of this report (all submitters of data may be contacted directly via the GISAID website), along with all laboratories who submitted sequences directly to WHO CC London.

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