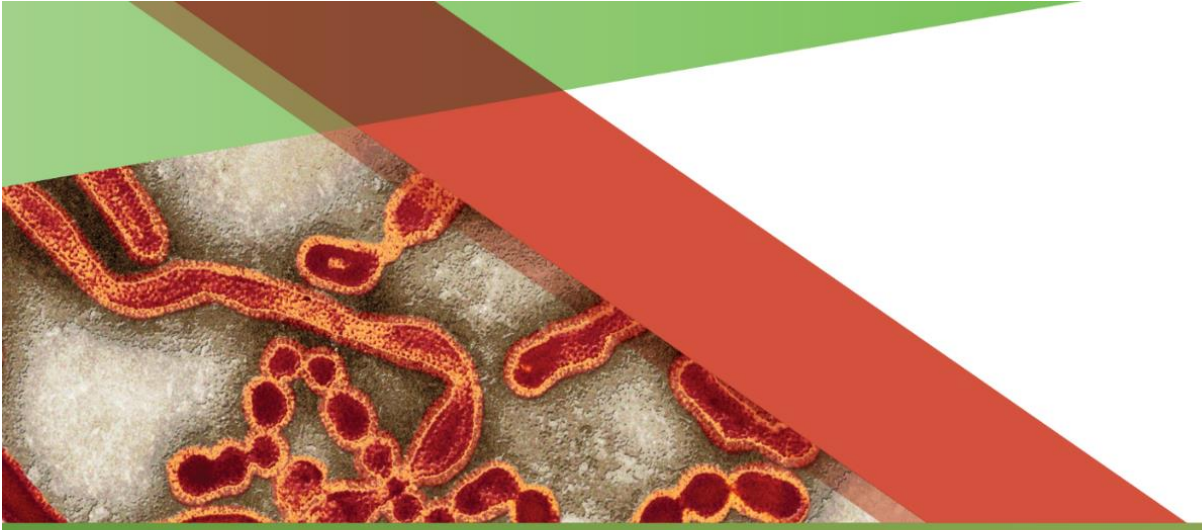

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, July 2021

Summary

This is the ninth report for the 2020-2021 influenza season. As of week 28/2021, only 943 influenza detections across the WHO European Region had been reported to the European Surveillance System (TESSy); 51% type A viruses, with A(H3N2) and A(H1N1)pdm09 being approximately equally represented, and 49% type B viruses, with only 16 having been ascribed to a lineage, 13 B/Victoria and three B/Yamagata. This represents a 99.4% drop in detections compared with the same period in 2020, probably due to measures introduced to combat the COVID-19 pandemic.

Since the June 2021 characterisation report[1], one shipment from an EU/EEA country (France) containing six virus isolates was received at the London WHO Collaborating Centre, the Francis Crick Worldwide Influenza Centre (WIC): virus characterisation of these six viruses is ongoing. This report therefore focuses on genetic characterisation of the HA genes of seasonal influenza viruses submitted and/or released in GISAID during July 2021, together with sequences recently determined at the WIC. The data continue to show extremely low levels of influenza detections globally.

The 60 A(H1N1)pdm09 HA sequences derived from viruses detected in 2021, as deposited/released in GISAID during July, all originated in Togo and were subgroup 6B.1A5A+187V/A, represented by the vaccine virus for the northern hemisphere 2020-2021 season, A/Guangdong-Maonan/SWL1536/2019. Most of these sequences (35) encoded additional HA1 amino acid substitutions of I166T and A186T.

All 57 HA sequences that became available in July for A(H3N2) viruses, detected in 2021, fell in subgroup 3C.2a1b+T131K-A. The viruses in this subgroup split into two antigenically distinguishable clusters originally defined by viruses from Cambodia (n = 13: with HA1 amino acid substitutions of G186S, F193S, Y195F and S198P, many also having K171N) and Bangladesh (n = 44: with HA1 amino acid substitutions of Y159N, T160I (loss of a glycosylation site), L164Q, G186D, D190N, F193S and Y195F); with Bangladesh-like viruses showing the greatest geographic spread. The seven viruses from EU/EEA countries were Bangladesh-like. An A/Cambodia/e0826360/2020-like virus (subgroup 3C.2a1b+T131K-A) has been recommended for use in the 2021-2022 northern hemisphere influenza season.

The 45 B/Victoria-lineage HA sequences derived from viruses collected in 2021 that became available in July were subclade 1A(△3)B. Of these, 10 from Kenya were equally split between groups defined by HA1 G133R substitution or

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

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HA1 K75E, E128K, T155A, G230N and I267V substitution. All other sequences were from N150K group viruses with HA1 amino acid substitutions of N150K, G184E, N197D (loss of a glycosylation site) and R279K. N150K group sequences split into two subgroups one of which, defined by HA1 substitutions V220M and P241Q, was confined to viruses detected in China (n = 2) while the second (n = 33 sequences), defined by HA1 substitutions A127T, P144L, and K203R (with two having additional substitutions of T182A, D197E and T221A), showed significant geographic spread. The four viruses detected in EU/EEA countries all fell in the latter subgroup but lacked the additional amino acid substitutions, as was the case for a set of 19 viruses collected in Singapore in June. Antigenically, viruses in subgroups of the N150K group differ and show some loss of reactivity with post-infection ferret antisera raised against the B /Washington/02/2019 vaccine virus (recommended for inclusion in influenza vaccines for the 2020-2021 and 2021-2022 northern hemisphere seasons and 2021 southern hemisphere season). This was clearly the case for the three N150K group viruses from Sweden characterised by HI in the June report.

No B/Yamagata-lineage HA sequences from clinical specimens collected in 2021, and none with collection dates after March 2020, were available. All of the 77 sequences from viruses detected in 2020, inclusive of 12 from EU/EEA countries, belong to genetic clade 3 and carry 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.

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 40/2020-28/2021), compared with the same period for the 2019-2020 season, is shown in Table 1. While there has been an increase in the numbers of samples from patients fulfilling Influenza-Like Illness (ILI) and/or Acute Respiratory Infection (ARI) criteria being tested (~87 570, 9.3%), there has been a vast reduction in the number of samples testing positive for an influenza virus (163 944, 99.4%). This is probably due to a number of factors: (i) 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; (ii) 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, and; (iii) 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 42 weeks of the 2020-2021 season, the ratio of type A to type B detections is reduced compared with the 2019-2020 season (2.7:1 to 1:1), with a reversal in the proportions of influenza A subtypes, while B/Victoria lineage viruses again predominate over B/Yamagata lineage viruses, although only 16/461 (3.5%) of type B viruses detected in the 2020-2021 season have been ascribed to a lineage as of week 28/2021.

Table 1. Influenza virus detections in the WHO European Region for the 2020-21 season from the start of reporting (weeks 40/2020-28/2021)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40/2020-28/2021			Totals*		Cumulative number of detections for weeks 40/2019-30/2020			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**29**	**453**	**482**	**51.1**	**1:1**	**11302**	**108950**	**120252**	**72.9**	**2.7:1**
A(H1N1)pdm09	13	29	42	41.2		6126	20302	26428	56.0	
A(H3N2)	8	52	60	58.8	1.4:1	4174	16593	20767	44.0	0.8:1
A not subtyped	8	372	380			1002	72055	73057		
Influenza B	**17**	**444**	**461**	**48.9**		**6325**	**38310**	**44635**	**27.1**	
Victoria lineage	2	11	13	81.3	4.3:1	2449	2030	4479	98.1	50.3:1
Yamagata lineage	0	3	3	18.7		23	66	89	1.9	
Lineage not ascribed	15	430	445			3853	36214	40067		
Total detections (total tested)	**46 (45 513)**	**897 (>983 608)**	**943 (>1 029 121)**			**17 627 (52 452)**	**147 260 (>889 099)**	**164 887 (>941 551)**		

[a] Numbers taken from Flu News Europe to week 28/2021 and week 30/2020 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, nine shipments of specimens (virus isolates and/or clinical specimens) were received at the Crick Worldwide Influenza Centre (WIC), one of which was received in July 2021 from France, containing six virus isolates, five of which were recovered from specimens taken in Abidjan (Table 2). Overall, the nine packages contained 37 virus-related samples with collection dates after 31 August 2020 and were made up of 22 type A viruses and 15 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 a report deadline of 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 viruses from EU/EEA countries have been characterised genetically and antigenically since the June 2021 report.

Table 2. Summary of seasonal influenza clinical samples and virus isolates*, with collection dates from 1 September 2020, contained in packages received from EU/EEA Member States since week 40/2020

MONTH / Country	TOTAL RECEIVED Seasonal viruses	A		H1N1pdm09		H3N2			B		B Victoria lineage		B Yamagata lineage	
		Number received	Number propagated[1]	Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]	Number received	Number propagated[1]
2020 SEPTEMBER														
Slovakia	6			1	0	5	0							
OCTOBER														
France	3					1	1				2	1		
Slovakia	2					1	0		1	0				
NOVEMBER														
France	2										2	1		
DECEMBER														
France	2										2	1		
2021 JANUARY														
Austria	1										1	1		
Norway	2			1	1						1	1		
Sweden	4					2	2				2	0		
FEBRUARY														
Norway	1			1	1									
Sweden	1					1	1							
March														
Sweden	4					1	1				3	3		
April														
Norway	1					1	1							
Sweden	2					2	2							
May														
June														
France	2			2	in process									
July														
France	4			2	in process	1	in process				1	in process		
5 Countries	37	0	0	7	2	15	8	0	1	0	14	8	0	0
		0.00%		18.9%		40.5%			2.7%		37.8%		0.0%	
		59.5%							40.5%					

*** Note: Where clinical sample and a virus isolate from the same patient were received, this is counted as one in the Total Received and following columns.**

1. Propagated to sufficient titre to perform HI assay (the totalled number does not include any from batches that are in process)
2. Propagated to sufficient titre to perform HI assay in the presence of 20nM oseltamivir (the totalled number does not include any from batches that are in process)
Numbers in red indicate viruses recovered but with insufficient HA titre to permit HI assay (H3N2 only)
Includes RNA extracts for which genetic characterisation only can be performed.
Samples from Abidjan

As of 2021-08-06

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 show similar profiles. The first is repeated from the June 2021 report and was generated based on sequences from viruses collected in the course of 2021 as available in GISAID at the end of June and/or generated at the WIC: just 79 in total with none having collection dates after April (Figure 2a). Of these 79, two viruses detected in Norway were subclade **6B.1A7**, a single virus from the US was subgroup **6B.1A5A+156K** but with **HA1 K156Q** amino acid substitution (A/Iowa/02/2021, H1N1v - swine 1A.3.3.2 subclade), and 76 were subgroup **6B.1A5A+187V/A** viruses with the great majority (n = 68) being detected in Togo and 45 of these viruses having additional **HA1 I166T** and **A186T** substitutions. The remaining eight subgroup **6B.1A5A+187V/A** viruses were detected in Belgium (n = 1), India (n = 1), Niger (n =4) and the US (n = 2). Of the HA sequences deposited/released in GISAID during July, there were 60 with collection dates in 2021, all from Togo, collected in January through March (Figure 2b). Again, these 60 sequences were derived from subgroup **6B.1A5A+187V/A** viruses and the majority (35) had additional **HA1 I166T** and **A186T** substitutions.

The great majority of A(H1N1)pdm09 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 10 August 2021].

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

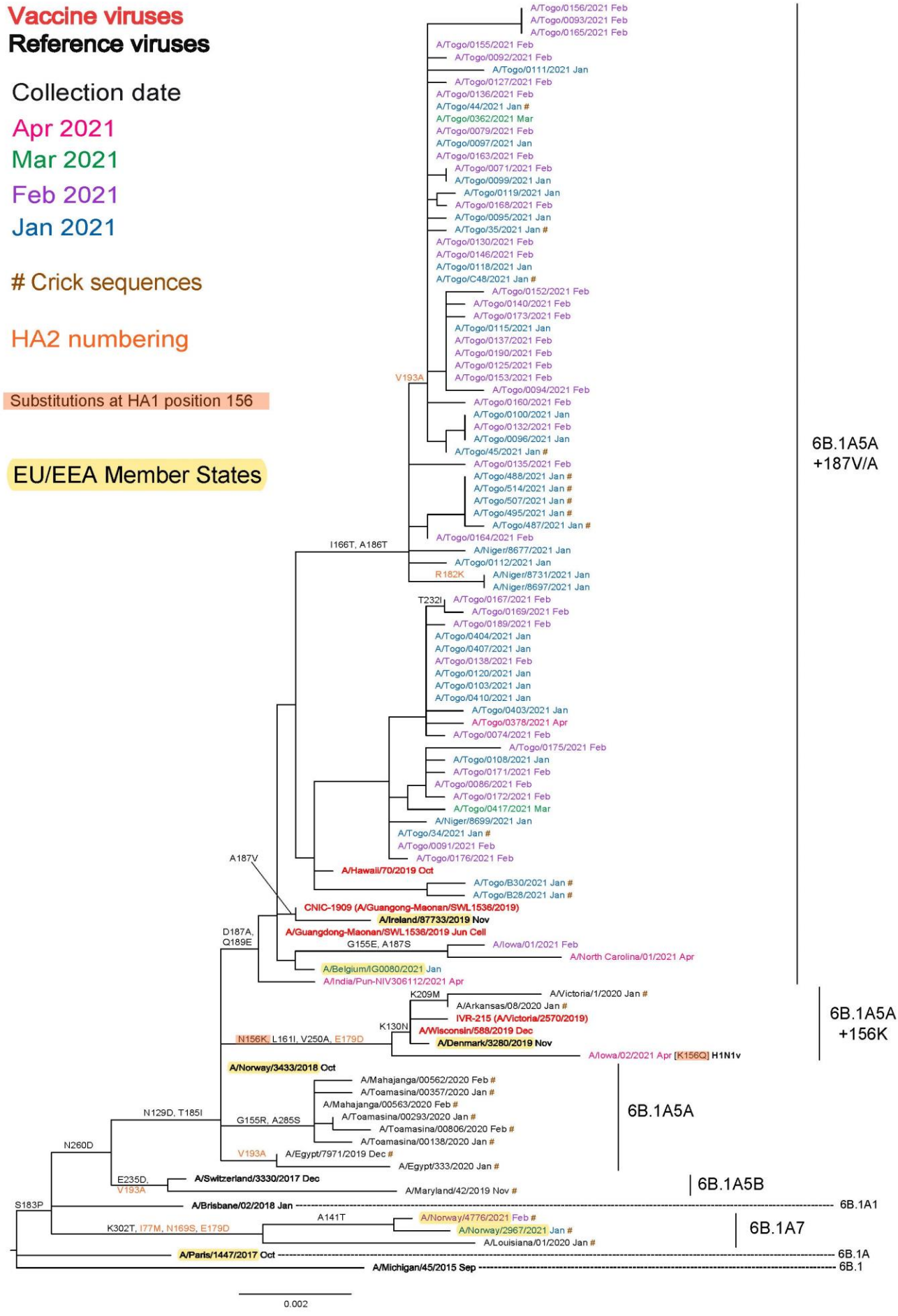
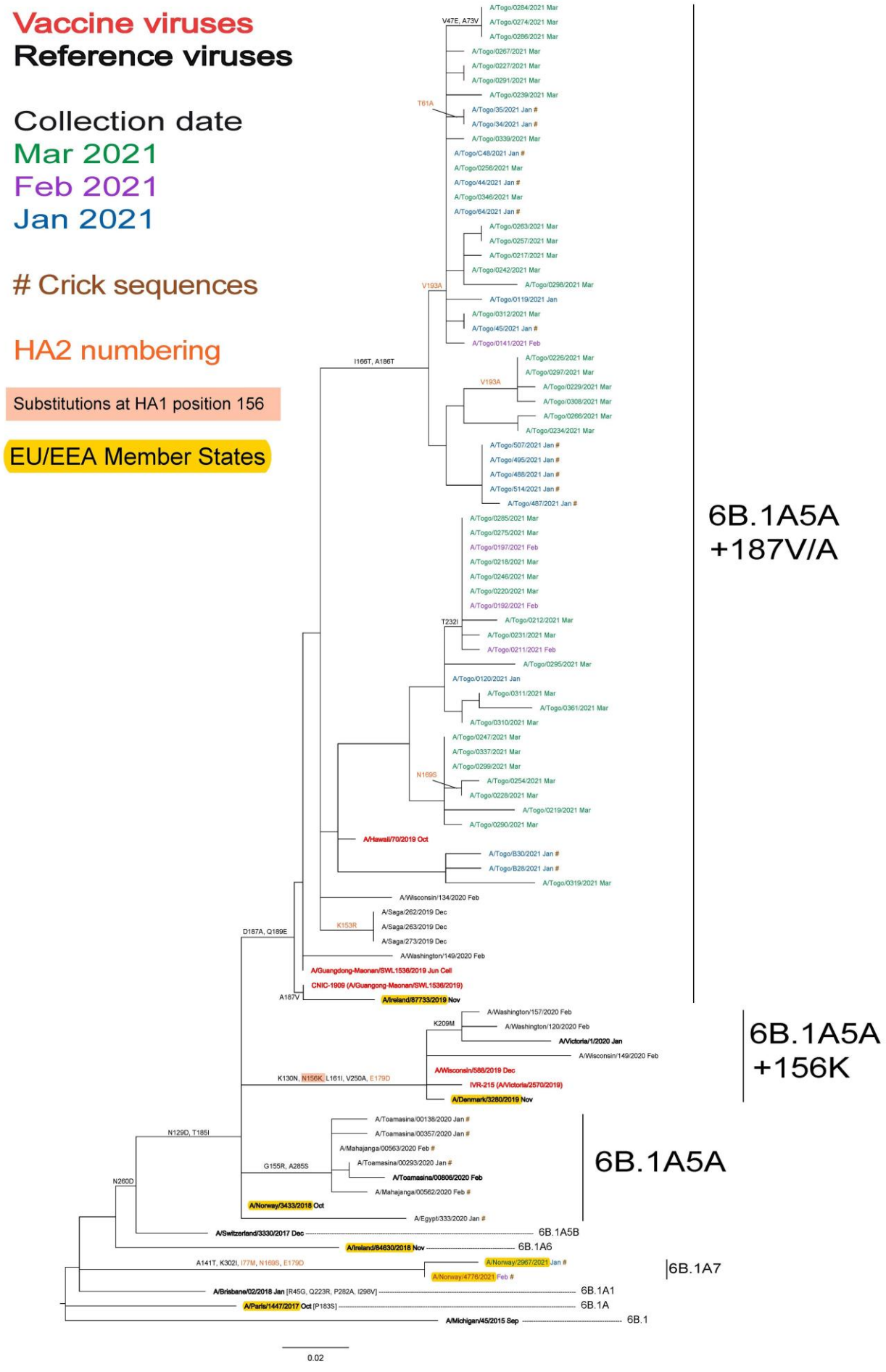


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, July 2021)



Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny is repeated from the June 2021 report and was based on sequences from viruses collected in the course of 2021 as available in GISAID at the end of June and/or generated at the WIC: just 74 in total. (Figure 2a). The second is based on sequences that became available in GISAID during July (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, except 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 June report was based on sequences with collection dates in 2021 deposited and/or released in GISAID as of the end of June 2021 (Figure 2a). It contained a single **3C.2a1b+T135K-B** virus (A/Laos/418/2021), 13 **3C.2a1b+T135K-A** viruses (all from Africa: Congo (n =5), Niger (n =3) and Togo (n = 5)), 13 'Cambodia-like' **3C.2a1b+T131K-A** viruses (carrying additional **HA1** substitutions of **G186S**, **F193S**, **Y195F** and **S198P**) from Australia (n =3), Japan (n = 2) and Laos (n = 8), and 47 'Bangladesh-like' **3C.2a1b+T131K-A** viruses (carrying additional **HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N**, **F193S** and **Y195F)** showing wider geographic spread with eight being detected in EU/EEA countries (Netherlands (n = 1), Norway (n = 1) and Sweden (n = 6)). All eight viruses from EU/EEA countries carried additional **HA1 L164Q** and **D190N** substitutions. Of the 57 HA sequences from viruses with collection dates in 2021 that became available in July, 13 were 'Cambodia-like' (one from Australia, two from Japan and 10 from Timor-Leste) and 44 were 'Bangladesh-like' (four from Australia, four from India, 21 from Kenya, one from Nepal, one from Norway, three from Singapore, six from Sweden and four from the United Arab Emirates) (Figure 2b).

While the number of detections of seasonal influenza viruses remains low, compared with previous seasons, the WHO Collaborating Centres for Influenza have shown viruses in these recently emerged virus clusters to be antigenically distinguishable from one another and other A(H3N2) virus subgroups. Antigenic characterisation of five A(H3N2) viruses from Sweden was presented in the June report and all gave HI-reactivity profiles most similar to 'Bangladesh-like' reference viruses.

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 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 during 2019-2020, with continuation into the 2020-2021 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] For example, the September 2013 report: 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, June 2021)

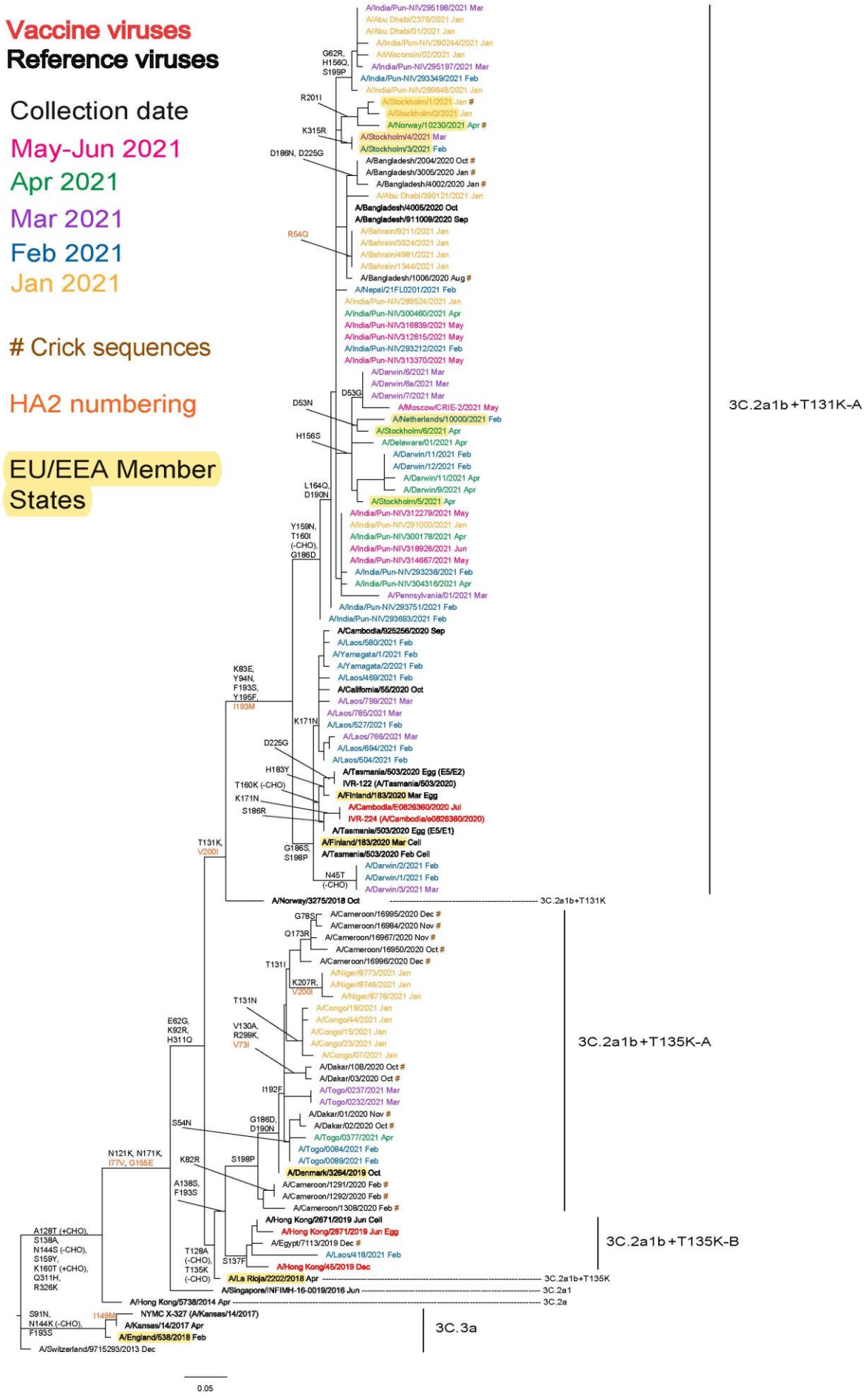
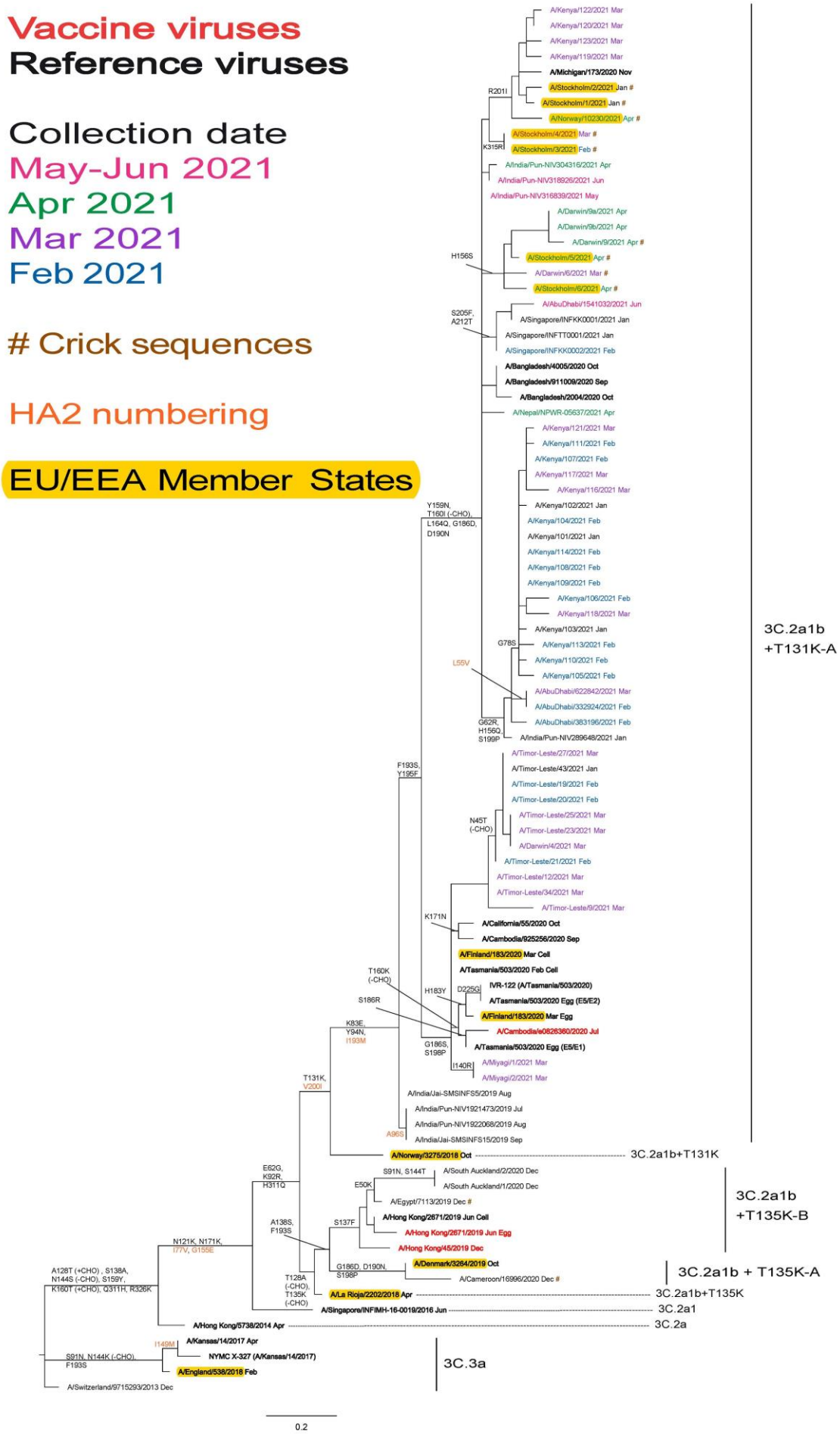


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, July 2021)



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** and **N129D** (e.g. **B/Ireland/3154/2016**). Viruses retaining full-length HAs had 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. 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 became dominant, 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 June report was based on sequences from viruses collected in the course of 2021 as available in GISAID at the end of June and/or generated at the WIC: just 91 in total, all belonging to **subclade 1A(△3)B** (Figure 3a). Of these, four detected in the US belonged to the current **B/Washington/02/2019** vaccine group and carried **HA1 G133R** substitution, 58 (all from China with collection dates in January) belonged to the **N150K group,** defined by **HA1 N150K**, **G184E**, **N197D** (loss of a glycosylation site) and **R279K** amino acid substitutions, with additional **HA1 V117I** and **V220M** substitutions, and 29 (detected in Africa, Asia, Europe, Middle East and North America, with 8 reported by EU/EEA countries; Austria (n =1), Norway (n = 1), Spain (n =1) and Sweden (n = 5)) belonged to the **N150K group** with additional **HA1 A127T**, **P144L** and **K203R** substitutions. Of these 29, eight also carried **HA1 T182A**, **D197E** and **T221A** substitutions, while a group of six viruses from China carried **HA1 H122Q** substitution. The updated phylogeny includes more sequences from viruses detected in China falling in the two **N150K subgroups** identified above, but with an additional change in the **H122Q subgroup** involving an **HA1** amino acid insertion (**ins167N**), and further illustrates the diversity of B/Victoria-lineage viruses in circulation globally (Figure 3b). Among a set of 13 sequences derived from viruses detected in Kenya during February to May, five fell in the **B/Washington/02/2019** vaccine group and carried **HA1 G133R** substitution and a further five fell in a group defined by **HA1** substitutions **K75E**, **E128K**, **T155A**, **G230N** and **I267V**, while three of four viruses detected in May fell in the **N150K subgroup** with additional **HA1 A127T**, **P144L** and **K203R** substitutions. A set of 19 sequences derived from viruses detected in Singapore in June also fell in the latter subgroup but carried an additional **A202V** substitution in **HA1**.

The WHO Collaborating Centres for Influenza have shown the **N150K 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. In the June report, three **N150K group** viruses from Sweden, with **HA1 A127T**, **P144L** and **K203R** substitutions, were characterised antigenically with a panel of post-infection ferret antisera. All three showed poor reactivity with antisera raised against the current vaccine virus.

Influenza B/Yamagata-lineage

It is assumed that no B/Yamagata-lineage viruses have been detected after March 2020 as no sequences for such viruses with collection dates after this had been released is GISAID as of 31 July 2021. Figure 4 is repeated from the June report and was generated based on the 77 HA sequences from viruses with collection after 31 December 2019 to 31 March 2020 available in GISAID. All sequences fell 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 with 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, June 2021)

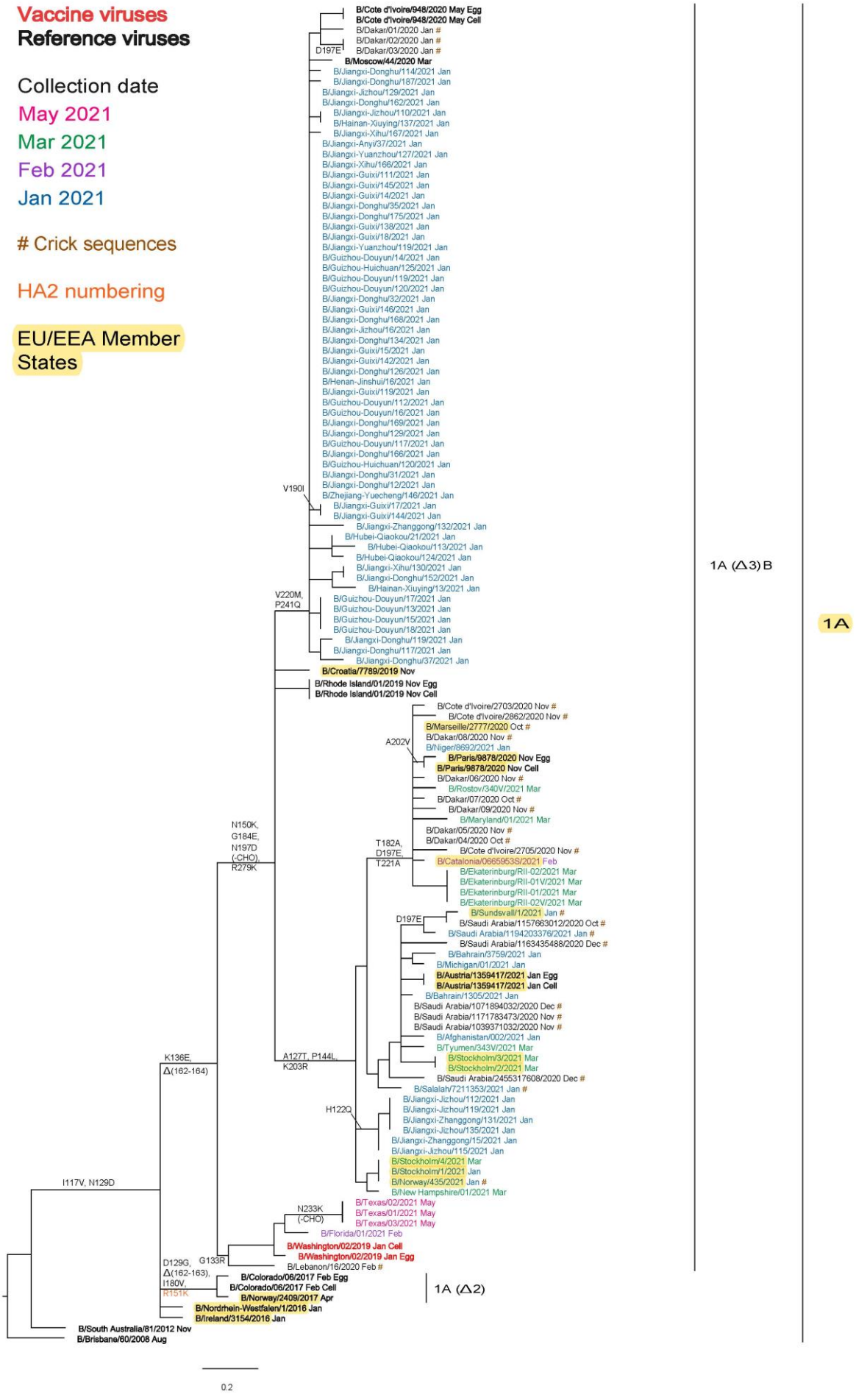


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, July 2021)

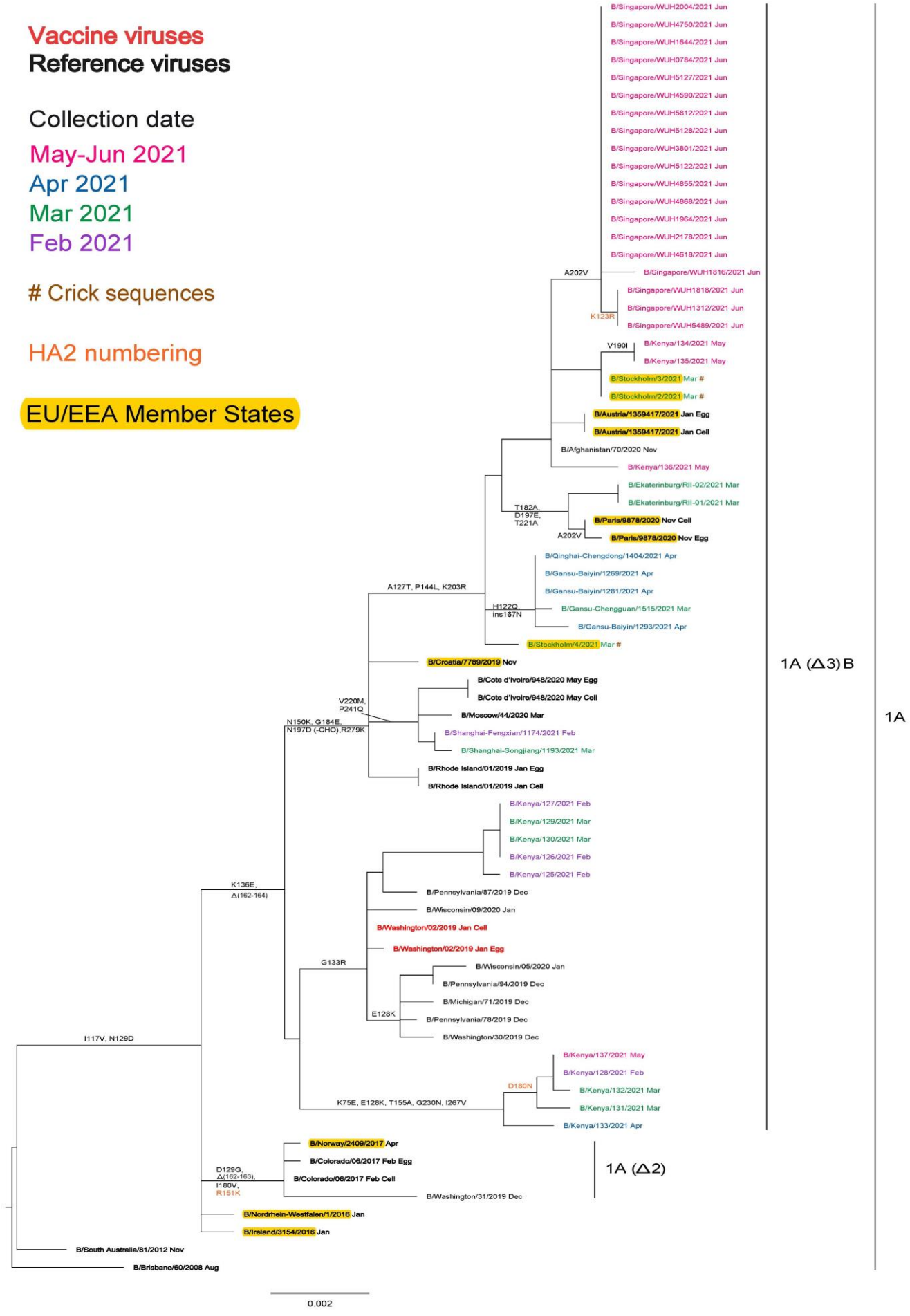
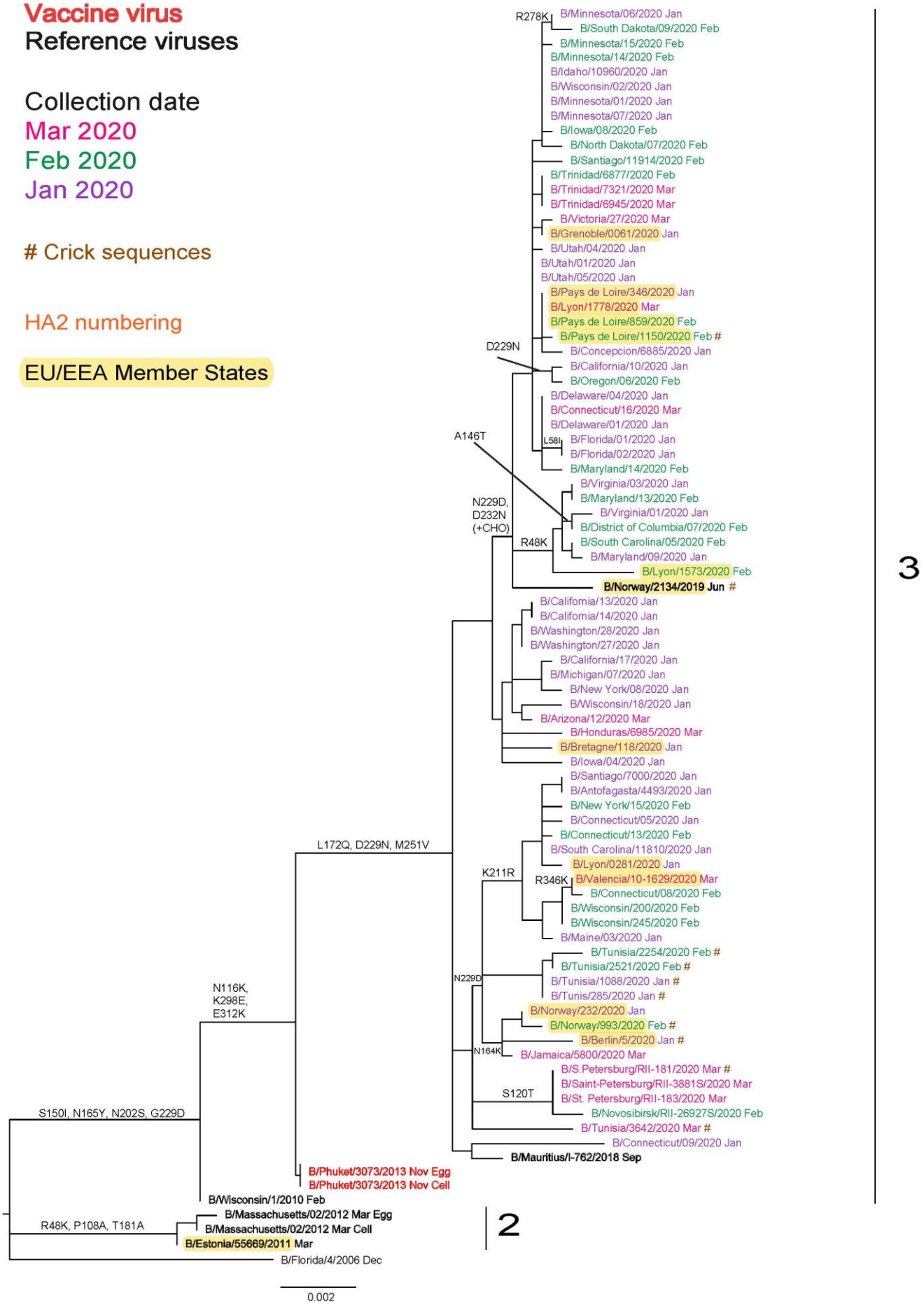


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, July 2021)



Summaries of data submitted to TESSy

Genetic characterisation

Fifteen viruses detected over the course of the 2020-2021 season (weeks 40/2020-28/2021) have been genetically characterised:

- Two A(H1N1)pdm09 viruses, one attributed to the 6B.1A5A subclade represented by A/Norway/3433/2018 and one attributed to the 6B.1A5A+187V/A group represented by A/Guangdong-Maonan/SWL1536/2019.
- Eight A(H3N2) viruses with six 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+T131K-B represented by A/Bretagne/1323/2020.
- Five B/Victoria-lineage viruses, all of which were ascribed 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

Very few influenza viruses, just four as of week 15/2021 (two each A(H3N2) and B/Victoria-lineage viruses), have been tested for susceptibility to neuraminidase inhibitors (NAIs) and sequence analysis has indicated normal inhibition (NI) by both oseltamivir and zanamivir.

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, 16 influenza viruses detected within EU/EEA countries during the 2020-2021 season have been assessed phenotypically against oseltamivir and zanamivir: two A(H1N1)pdm09, seven A(H3N2) and seven 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). 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 [5]. WHO posted an analysis of information on A(H7N9) viruses on 10 February 2017 [6], and ECDC published a rapid risk assessment on the implications of A(H7N9) for public health on 3 July 2017 [7]. Current risk assessments are included in WHO's https://www.who.int/teams/global-influenza-programme/avian-influenza/monthly-risk-assessment-summary (accessed 11 August 2021). The assessment published on 8 August 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 [8]. 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 July 2021 indicated that there have been no additional detections since then [9]. The most recent human case was detected in mid-March 2019 [10]. 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 31 May 2021 and can be found on ECDC's website [11].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human-animal interface was published by WHO on 8 August 2021. Since the previous risk assessment on 22 June 2021, one human case of infection with an avian influenza A(H5N1) virus and six human cases of infection with avian influenza A(H5N6) viruses were officially reported [8]. The source of exposure to A(H5N1) in India of a male child with underlying immunodeficiency was unknown, but he was also infected with an influenza B/Victoria-lineage virus, and the outcome was fatal. This latest human case of known A(H5N1) infection is the first since that reported on 31 October 2020 by Lao People's Democratic Republic [12]. The six cases of A(H5N6) infection were all related to sporadic infections in China, and at the time of reporting, one case was fatal, four were critical and one showed mild symptoms; the cases involved adults in the age range 51-66 and all reported exposure to poultry [8].

On 30 September 2020, ECDC published an alert related to outbreaks of avian influenza viruses in Europe [13]. The latest collaborative report from ECDC and the European Food Safety Authority (EFSA) reports 1 672 highly pathogenic avian influenza (HPAI) A(H5) detections between 24 February and 14 May 2021, 580 in poultry, 1 051 in wild birds and 41 in domestic birds [11]. Detections occurred in 24 EU/EEA countries and the UK. Of the poultry detections, 297 were reported by Poland and 168 by Germany, and of the wild bird detections 603 were reported by Germany, 167 by Denmark and 56 by Poland. A second peak of HPAI-associated wild bird mortality in north-west Europe was recorded in February to April 2021. 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). According to reports compiled by the Food and Agricultural Organization of the United Nations (FAO) as of 28 July 2021, various influenza A(H5Nx) subtypes continued to be detected in wild and/or domestic birds in Africa, Asia and Europe, and since 23 June 2021, a total of 84 (all HPAI) outbreaks had been reported [14].

Influenza A(H9N2) virus

Since the previous WHO update on 22 June 2021 no laboratory-confirmed human cases of influenza A(H9N2) virus infection were reported [8]. Public Health England recently published and updated a risk assessment of avian influenza A(H9N2) [15]. Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa. The latest ECDC/EFSA report includes mention of earlier human infections with H9N2 viruses, following on from the previous report up to 23 February 2021 [11].

Other influenza zoonotic events

Since the previous WHO update on 22 June 2021, no zoonotic events with swine-related variant influenza A viruses (H1N1v, H1N2v or H3N2v) had been reported to WHO [8].

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 8 August 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.

References

1. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2020-2021 northern hemisphere influenza season. Wkly Epidemiol Rec. 2020 Mar 20;95(12):105-116. Available from: http://extranet.who.int/iris/restricted/bitstream/handle/10665/331503/WER9512-eng-fre.pdf
2. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2021 southern hemisphere influenza season. Wkly Epidemiol Rec. 2020 Oct 16;95(42):497-508. Available from: https://apps.who.int/iris/bitstream/handle/10665/336144/WER9542-eng-fre.pdf
3. World Health Organization. Recommended composition of influenza virus vaccines for use in the 2021-2022 northern hemisphere influenza season. Wkly Epidemiol Rec. 2021 Mar 19;96(11):77-88. Available from: https://reliefweb.int/sites/reliefweb.int/files/resources/WER9611-eng-fre.pdf
4. World Health Organization. Human infection with influenza A(H7N9) virus in China. 1 April 2013 [Internet]. Geneva: WHO; 2013 [accessed 11 August 2021]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2013_04_01-en
5. World Health Organization. Human infection with avian influenza A(H7N9) virus – China [Internet]. Geneva: WHO; 2017 [accessed 11 August 2021]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/26-october-2017-ah7n9-china-en
6. World Health Organization. Influenza at the human–animal interface. Summary and assessment, 26 January to 2 March 2018. Geneva: WHO; 2018. Available from: https://cdn.who.int/media/docs/default-source/influenza/human-animal-interface-risk-assessments/influenza_summary_ira_ha_interface_02_03_2018.pdf?sfvrsn=f96c970a_5&download=true
7. European Centre for Disease Prevention and Control. Influenza A(H7N9) virus in China - implications for public health - 7th update, 3 July 2017. Stockholm: ECDC; 2017. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/2017-07-03-RRA-Disease-China_H7N9_0.pdf
8. World Health Organization. Influenza at the human-animal interface. Summary and assessment, from 23 June to 8 August 2021. Geneva: WHO; 2021. Available from: https://cdn.who.int/media/docs/default-source/influenza/human-animal-interface-risk-assessments/influenza_summary_ira_ha_interface_08_08_2021.pdf?sfvrsn=5aa110_5&download=true
9. Food and Agricultural Organization of the United Nations. H7N9 situation update, 7 July 2021. Rome: FAO; 2021 [accessed 7 July 2021]. Available from: http://www.fao.org/ag/againfo/programmes/en/empres/h7n9/situation_update.html
10. World Health Organization. Influenza at the human–animal interface. Summary and assessment, 13 February to 9 April 2019. Geneva: WHO; 2019. Available from: https://cdn.who.int/media/docs/default-source/influenza/human-animal-interface-risk-assessments/influenza_summary_ira_ha_interface_09_04_2019.pdf?sfvrsn=7f2bf2a2_11&download=true
11. European Centre for Disease Prevention and Control, European Food Safety Authority, European Union Reference Laboratory for Avian Influenza. Avian influenza overview February - May 2021. Parma and Stockholm: EFSA, ECDC; 2021. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Avian-influenza-overview-February-May-2021_1.pdf
12. World Health Organization. Cumulative number of confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003–2021. Geneva: WHO; 2021. Available from: https://cdn.who.int/media/docs/default-source/influenza/h5n1-human-case-cumulative-table/2021_june_tableh5n1.pdf?sfvrsn=839e65a9_10&download=true
13. European Centre for Disease Prevention and Control. Avian influenza: EU on alert for new outbreaks. 30 September 2020 [internet]. Stockholm: ECDC; 2020 [accessed 11 August 2021]. Available from: https://www.ecdc.europa.eu/en/news-events/avian-influenza-eu-alert-new-outbreaks
14. Food and Agricultural Organization of the United Nations. Global AIV with zoonotic potential situation update, 28 July 2021. Rome: FAO; 2021 [accessed 11 August 2021]. Available from: https://mcusercontent.com/dc0b96ca6646c8eedf16a2216/files/3972edf2-7ac9-50ee-93ec-97500c798375/Global_update_zoonoticAIV_2021_07_28.pdf
15. Public Health England. Guidance, risk assessment of avian influenza A(H9N2) update, 9 August 2021. UK.gov: PHE; 2021 [accessed 19 August 2021]. Available from: https://www.gov.uk/government/publications/risk-assessment-of-avian-influenza-ah9n2/risk-assessment-of-avian-influenza-ah9n2