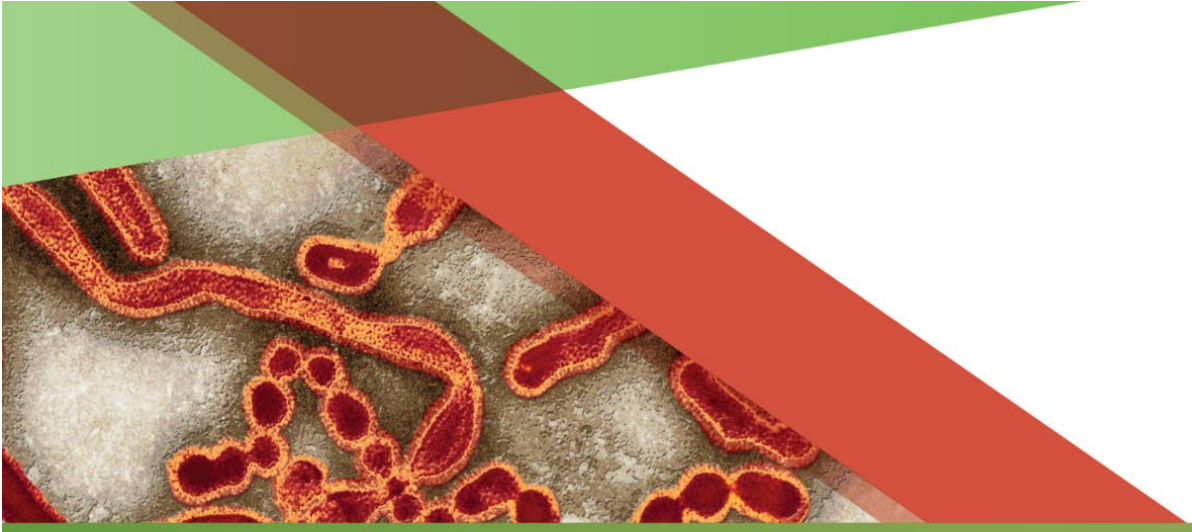


SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, October 2020

Summary

This is the first report for the 2020–2021 influenza season. As of week 44/2020, only 39 influenza detections across the WHO European Region had been reported to The European Surveillance System (TESSy); 59% type A viruses, with A(H3N2) prevailing over A(H1N1)pdm09, and 41% type B viruses with none having been ascribed to a lineage. This represents a 97% drop in detections compared with the same period in 2019, probably due to the COVID-19 pandemic and measures introduced to combat it.

Since the September 2020 characterisation report[1], no shipments of influenza-positive specimens from the European Union/European Economic Area (EU/EEA) countries, or elsewhere, have been received at the London WHO Collaborating Centre, the Francis Crick Worldwide Influenza Centre (WIC). Therefore, this report focuses largely on genetic characterisation of influenza viruses with collection dates prior to week 40, the start of weekly influenza surveillance reporting for the 2020-2021 influenza season.

The vast majority of A(H1N1)pdm09 viruses have continued to fall in genetic subclade 6B.1A5, mostly in the 6B.1A5A group with few in the 6B.1A5B group. 6B.1A5A viruses have continued to evolve and two subgroups have emerged designated 6B.1A5A+187V/A, representatives of which are recommended for use in the northern hemisphere 20202021 season, and 6B.1A5A+156K, an antigenically distinct group representatives of which are recommended for use in the southern hemisphere 2021 season. Following a rise in the number of 6B.1A5A+156K viruses detected, the two subgroups appear to be currently circulating in approximately equal proportions.

Recently circulating A(H3N2) viruses have continued to fall in clades 3C.2a and 3C.3a, with the vast majority of clade 3C.2a viruses being in the 3C.2a1b group which has now been divided into four subgroups designated 3C.2a1b+T131K-A, 3C.2a1b+T131K-B, 3C.2a1b+T135K-A and 3C.2a1b+T135K-B. Antisera raised in ferrets show high levels of clade/group specificity, though there is some subgroup cross-reactivity. Viruses representative of subgroup 3C.2a1b+T135K-B have been recommended for use in influenza vaccines for the northern hemisphere 2020-2021 season and the southern hemisphere 2021 season.

Of four antigenically distinct groups of viruses in the B/Victoria-lineage, only two have circulated recently, small numbers of that designated subclade 1A(△2) with a two amino acid deletion in HA1 and that designated subclade

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

This report was prepared by Rod Daniels, Burcu Ermetal, Aine Rattigan and John McCauley (Crick Worldwide Influenza Centre) for the European Centre for Disease Prevention and Control under an ECDC framework contract.

Suggested citation: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, October 2020. Stockholm: ECDC; 2020.

1A(△3)B with a three amino acid deletion in HA1 being hugely dominant. Viruses representative of subclade 1A(△3)B have been recommended for use in influenza vaccines for the northern hemisphere 2020-2021 season and the southern hemisphere 2021 season.

At the time of writing this report, genetic information for only 70 B/Yamagata-lineage viruses with collection dates in 2020 was available in GISAID. All 67 viruses for which full-length HA sequences were available belong to genetic clade 3 and contain at least two HA amino acid substitutions (HA1 L172Q and M251V) compared to B/Phuket/3073/2013-like viruses which have been recommended for use in quadrivalent influenza vaccines for the northern hemisphere 2020-2021 season and the southern hemisphere 2021 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 40-44/2020), compared with the same period (weeks 40-44/2019) 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 (~14 419, 25%), there has been a vast reduction in the number of samples testing positive for an influenza virus (1 099, 97%). This is probably due to three factors:

- the number of centres within the Region reporting over these periods has dropped from 47 to 34;
- significant numbers of samples taken from patients fulfilling ILI and/or ARI criteria are 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 impedes the spread of influenza viruses.

With these caveats, and being mindful of the low number of detections over the first five weeks of the 2020-2021 season, the ratio of type A to type B detections is reduced compared with the 2019–2020 season (4:1 to 1.4:1). Similar proportions of influenza A subtypes while no assessment can be made for type B viruses as none of the 16 detected over the course of the 2020-2021 season having been ascribed to a lineage.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2020-21 season (weeks 40-44/2020)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40-44/2020			Totals*		Cumulative number of detections for weeks 40-44/2019			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**1**	**22**	**23**	**59.0**	**1.4:1**	**49**	**861**	**910**	**80.0**	**4:1**
A(H1N1)pdm09	1	2	3	25.0		19	57	76	23.8	
A(H3N2)	0	9	9	75.0	3:1	29	214	243	76.2	3.2:1
A not subtyped	0	11	11			1	590	591		
Influenza B	**0**	**16**	**16**	**41.0**		**33**	**195**	**228**	**20.0**	
Victoria lineage	0	0	0			11	15	26	96.3	26:1
Yamagata lineage	0	0	0			0	1	1	3.7	
Lineage not ascribed	0	16	16			22	179	201		
Total detections (total tested)	**1 (1457)**	**38 (>42963)**	**39 (>44420)**			**82 (2677)**	**1056 (>56162)**	**1138 (>58839)**		

[a] **Numbers taken from Flu News Europe week 44/2020 and week 44/2019 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.**

No shipments of specimens (virus isolates and/or clinical specimens) relating to the 2020-2021 influenza season have been received at the Crick Worldwide Influenza Centre (WIC).

During the lockdown imposed by the UK government in March 2020 due to the COVID-19 pandemic, and since, WIC has been operating with reduced staff numbers. Consequently, only gene sequencing was performed to assess the emergence of any new genetic groups from March to May 2020. Virus isolation and propagation for phenotypic analyses was reinstated in June 2020 following the relaxation of lockdown restrictions in the UK and the introduction of real-time reverse transcriptase polymerase chain reaction (rtRTPCR) to screen all clinical specimens for presence of SARS-CoV-2. Consequently, recent influenza characterisation reports and those going forward have been/will be 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, together with antigenic and antiviral susceptibility data generated by the WIC.

Genetic and antigenic characterisation data generated at the WIC for viruses with collection dates after 31 August 2019 and until 31 January 2020 contributed to the WIC virus characterisation report (the deadline for the report was 21 February 2020) that was presented at WHO's influenza vaccine composition meeting (VCM) in February 2020. At this meeting, recommendations were made for the northern hemisphere 2020–2021 season [1]. Subsequently, data generated for viruses with collection dates after 31 January 2020 and until 31 August 2020 was used to inform the recent WHO VCM (held online from 16–24 September) to recommend viruses for inclusion in vaccines for the southern hemisphere 2021 influenza season. At this meeting, recommendations were made for the southern hemisphere 2021 season [2].

Influenza A(H1N1)pdm09 virus analyses

The first A(H1N1)pdm09 HA phylogeny is repeated from the September 2020 report and was generated based on sequences deposited in GISAID for recently circulating viruses, with collection dates from 1 March 2020, submitted to GISAID during August and September 2020 (Figure 1a). The second is based on viruses with collection dates from 1 March 2020, but with sequences deposited in GISAID during October 2020; a total of 112 sequences had been deposited (Figure 1b). All recently circulating viruses fell 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 (egg-based A/Victoria/5270/2019-like and cell-based A/Wisconsin/588/2019-like) influenza seasons are shown in red [1, 2]. 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** carry 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 phylogenies have very similar profiles and are largely made up of sequences from viruses detected in the later part of the 2019-2020 northern hemisphere season and early part of the 2020 southern hemisphere influenza season (Figures 1a and 1b). The vast majority of recently circulating viruses have fallen into group **6B.1A5A**, with approximately equal proportions falling into the parent group and the **6B.1A5A+187V/A** and **6B.1A5A+156K** subgroups. Relatively few viruses in subgroup **6B.1A5B** (with **HA1 K130N**, **K160M**, **T216K**, **E235D**, **H296N** and **HA2 V193A** substitutions) have also been detected and even fewer in subclade **6B.1A7**. Of the eight viruses with collection dates in April through June, two belong to group **6B.1A5B**, four to group **6B.1A5A** and two to subgroup **6B.1A5A+187V/A** (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**, remained antigenically similar to A/Brisbane/02/2018, the northern hemisphere 2019–2020 vaccine virus, and 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).

No A(H1N1)pdm09 viruses were characterised antigenically at the WIC since the September report.

Figure 1a. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, September 2020)

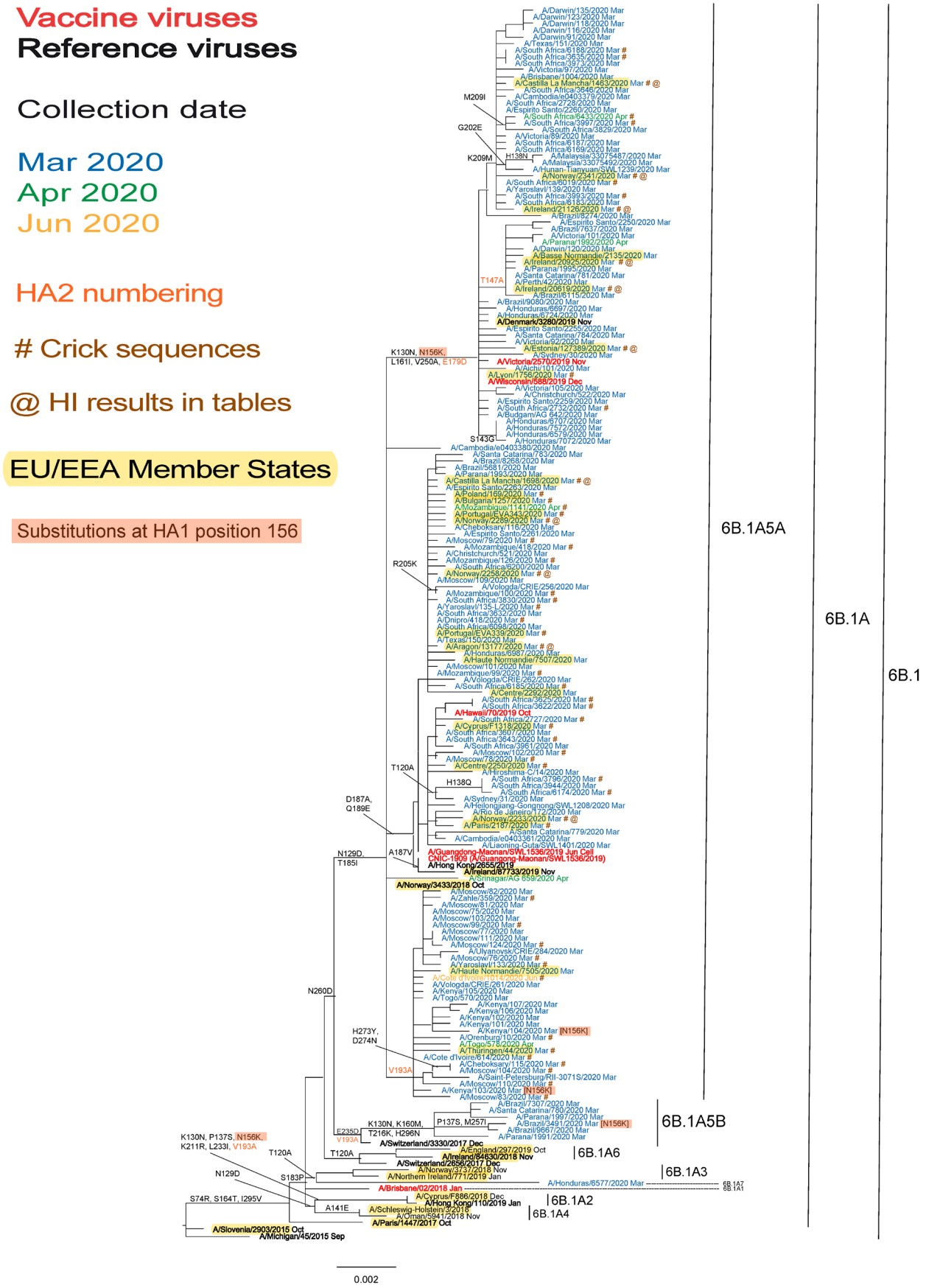
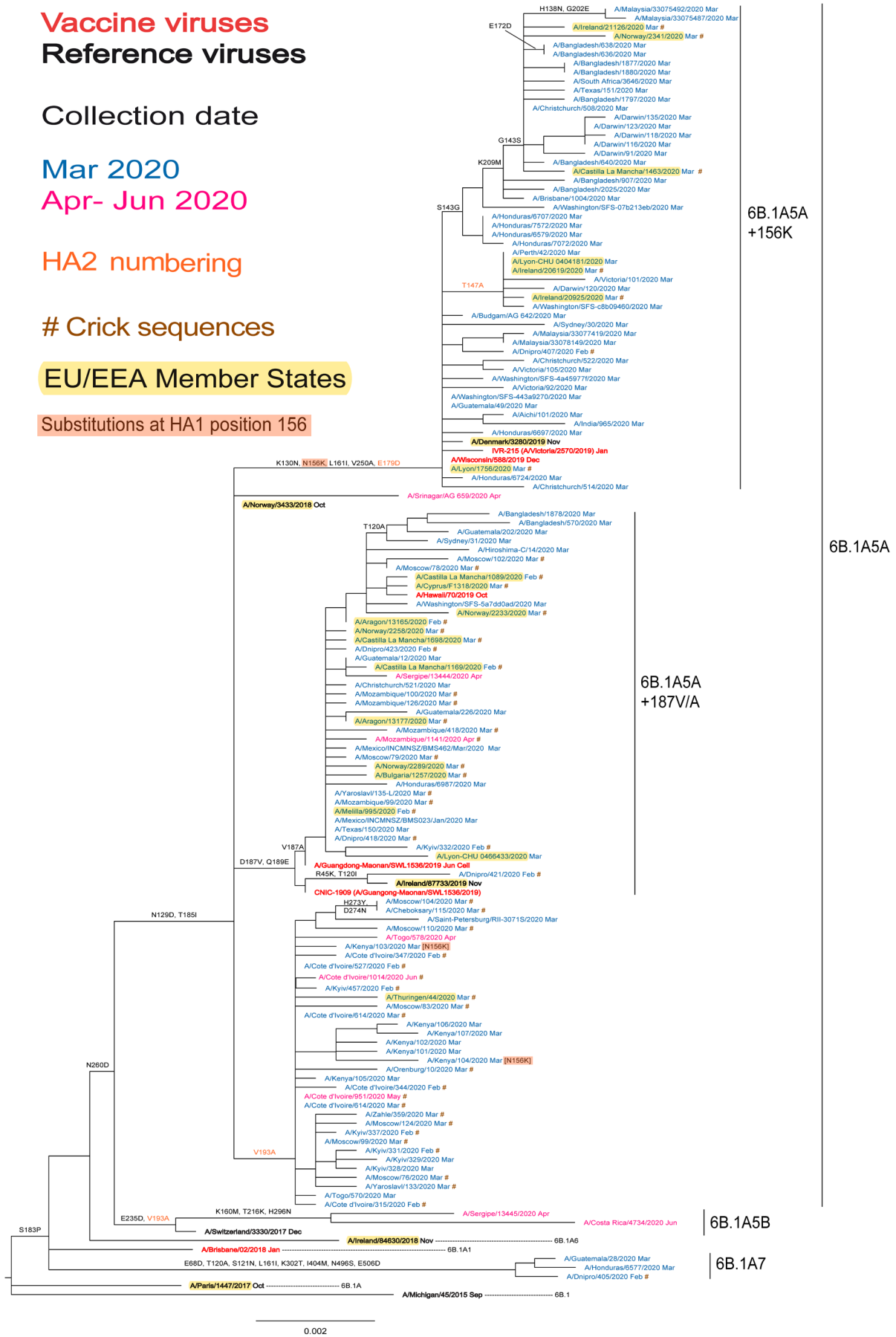


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, October 2020)



Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny is repeated from the September 2020 report and was generated based on sequences deposited in GISAID for recently circulating viruses, with collection dates from 1 February 2020, submitted to GISAID during August and September 2020 (Figure 2a). The second is again based on viruses with collection dates from 1 February 2020, but with a total of 99 sequences deposited in GISAID during October 2020.(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, apart from 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**, mostly 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 HA phylogeny generated for the September report, based on sequences recently deposited in GISAID, showed numbers of sequences to be approximately equally distributed between clades **3C.2a** (with group **3C.2a1b** being vastly dominant) and **3C.3a** viruses detected in EU/EEA countries, with the majority being in clade **3C.3a** and the subgroup **3C.2a1b+T131K-B** cluster, but with viruses having collection dates in February and March (Figure 2a). 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 updated phylogeny, for sequences deposited in October has fewer sequences from viruses detected in EU/EEA and other European countries, again with collection dates in February and March, with a markedly greater proportion of sequences derived from viruses detected in non-European countries, notably Australia, Bangladesh, Brazil, Cambodia, Honduras, India, Mexico, Nepal and Togo (Figure 2b). The profiles of the two phylogenies are very similar, but with sequences from only 16 viruses, with collection dates from April through September, with eight represented on both trees due to submissions relating to the same viruses but with different passage histories. Notably, 13 of these 16 viruses fall within the subgroup **3C.2a1b+T131K-A** cluster with eight viruses from Cambodia carrying additional **HA1** substitutions of **K171N**, **G186S**, **F193S**, **Y195F** and **S198P**, while three from Bangladesh have additional **HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N**, **F193S** and **Y195F**. Given the locations of these HA1 amino acid substitutions there is a need to assess the antigenic properties of such viruses and monitor their geographic spread.

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, recently 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 (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],

[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

this has been a significant problem for most viruses that fall in genetic clade **3C.2a**, though there has been 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 the ECDC website (https://www.ecdc.europa.eu/en/seasonal-influenza/surveillance-and-disease-data/influenza-virus-characterisation). 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.

No A(H3N2) viruses were characterised antigenically at the WIC since the September report.

Figure 2a. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, September 2020)

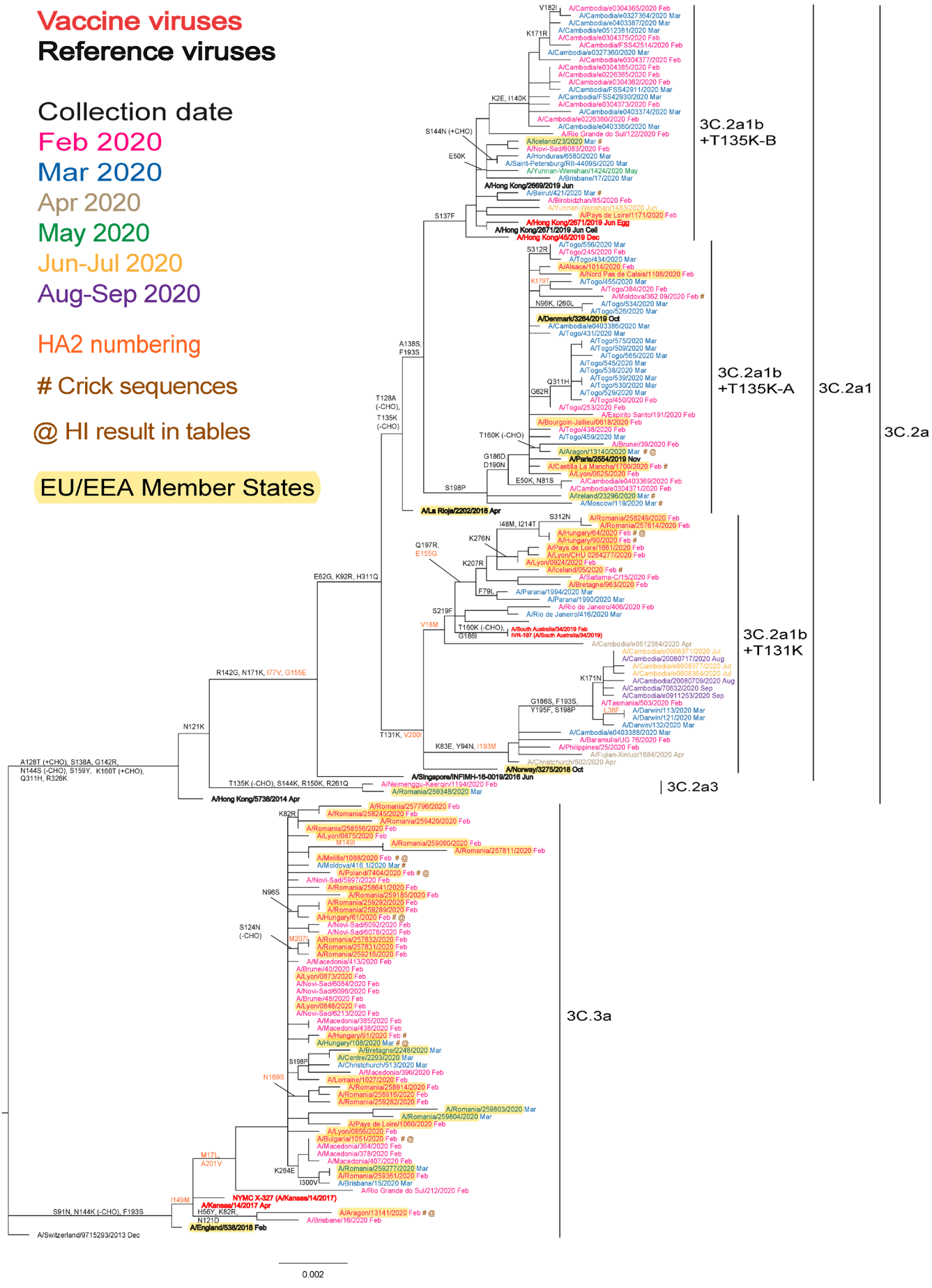
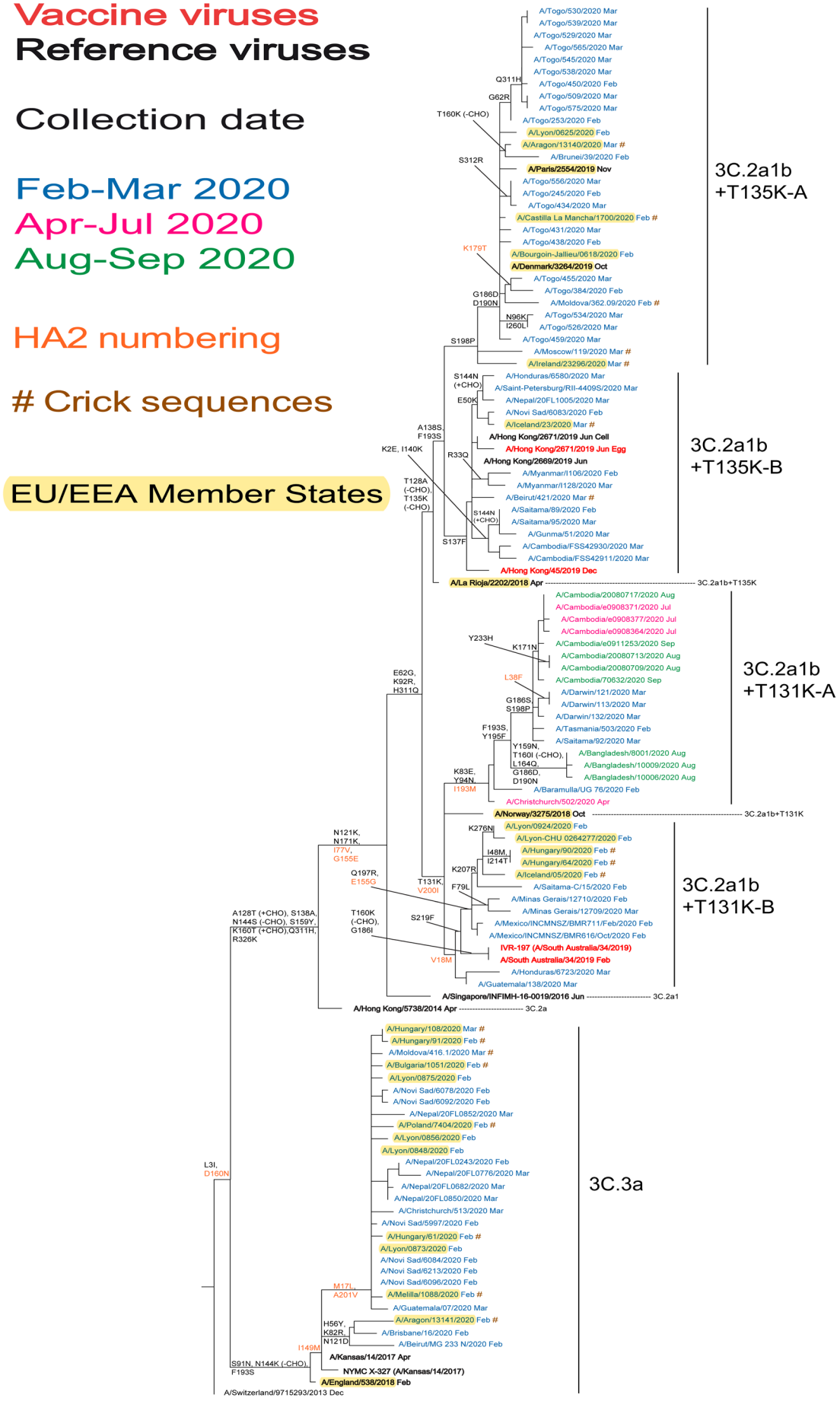


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, October 2020)



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.

The HA phylogeny generated for the September report showed continued dominance of **subclade 1A(△3)B** viruses having **HA1 K136E,** often with **G133R** substitution, and several virus clusters had emerged defined by specific amino acid substitutions, e.g. **HA1 N126K** or **E128K** or **D129N** with **N233K** (loss of a glycosylation site) or **N150K** with **G184E** or **N233K** (loss of a glycosylation site) or **HA2 T120A**, and relatively few **subclade 1A(△2)** viruses had been detected (Figure 3a). The updated phylogeny for sequences deposited in GISAID during October is largely made up of viruses detected in Europe and countries of the southern hemisphere with the great majority having been detected in February and March, and only 10, all in **subclade 1A(△3)B,** detected in April through June (Figure 3b); the phylogeny profile is very similar to that of Figure 3a.

Following the spread of **1A(Δ2)** viruses a representative, B/Colorado/06/2017, was recommended for use in trivalent influenza vaccines for the 2019–2020 northern hemisphere season, but recent predominance of **1A(Δ3)B** viruses led to the recommendation of a representative (B/Washington/02/2019) for use in trivalent influenza vaccines for the northern hemisphere 2020–2021 [1] and 2021 southern hemisphere [2] seasons.

Since the September 2020 report a batch of 12 B/Victoria-lineage viruses from Cyprus were analysed by HI assay (Table 2). Test viruses are sorted by date of collection (February to March 2020): 10 were subclade **1A(Δ3)B** and two subclade **1A(Δ2)**.

Poor test virus reactivity with ferret antisera raised against viruses in **clade 1A** (n=4) was observed. Antisera raised against three **subclade 1A(△2)** viruses, tissue culture-propagated B/Norway/2409/2017, tissue culture- and egg-propagated cultivars of B/Colorado/06/2017, recognised both **subclade 1A(△2)** at titres within fourfold of their respective homologous titres, while at least eightfold reductions were seen with **subclade 1A(△3)B** viruses. Antisera raised against two **subclade 1A(△3)B** viruses, tissue culture- and egg-propagated cultivars of B/Washington/02/2019, recognised only 9/10 **subclade 1A(△3)B** viruses at titres within fourfold of their corresponding homologous titres. The **subclade 1A(△3)B** test virus that was poorly recognised, B/Cyprus/F1359/2020, fell within a cluster of viruses defined by **HA1 D129N**, **G133R**, **N233S** (loss of a glycosylation site) and **K272R** amino acid substitutions (Table 2 and Figure 3b).

Influenza B/Yamagata-lineage

No B/Yamagata-lineage viruses were characterised at the WIC since the September report. The HA phylogeny, for viruses with collection dates from 1 January 2020, has been updated from the September report to contain three sequences submitted to GISAID in October, one each from France, Honduras and Norway with collection dates in January, March and February respectively (Figure 4). As for other recently detected B/Yamagata-lineage viruses, the 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. Some sub-clustering of sequences from recently collected viruses, 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, which is recommended for inclusion in quadrivalent vaccines for the 2020–2021 northern hemisphere and 2021 southern hemisphere seasons [1, 2].

[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, September 2020)

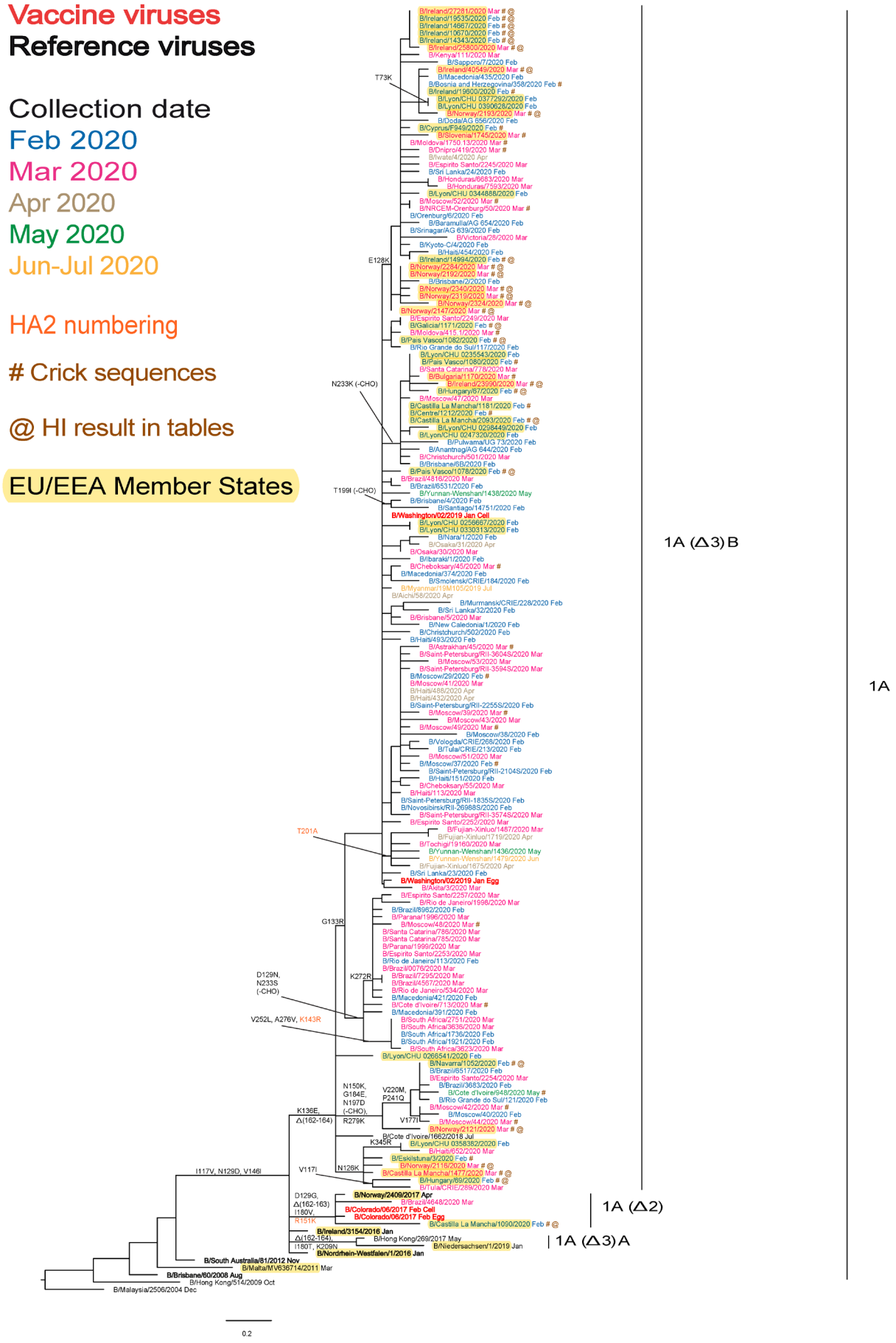


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, October 2020)

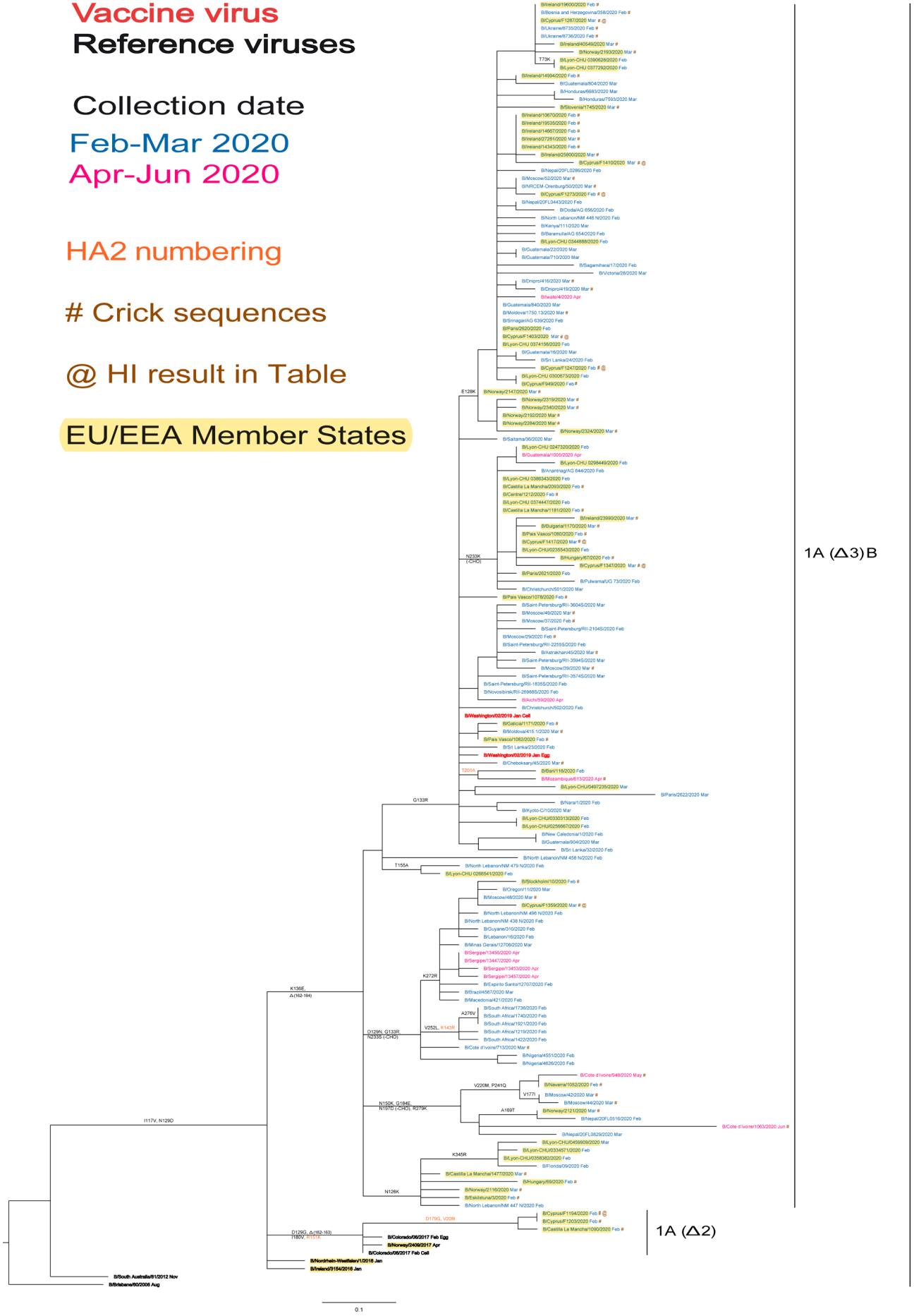
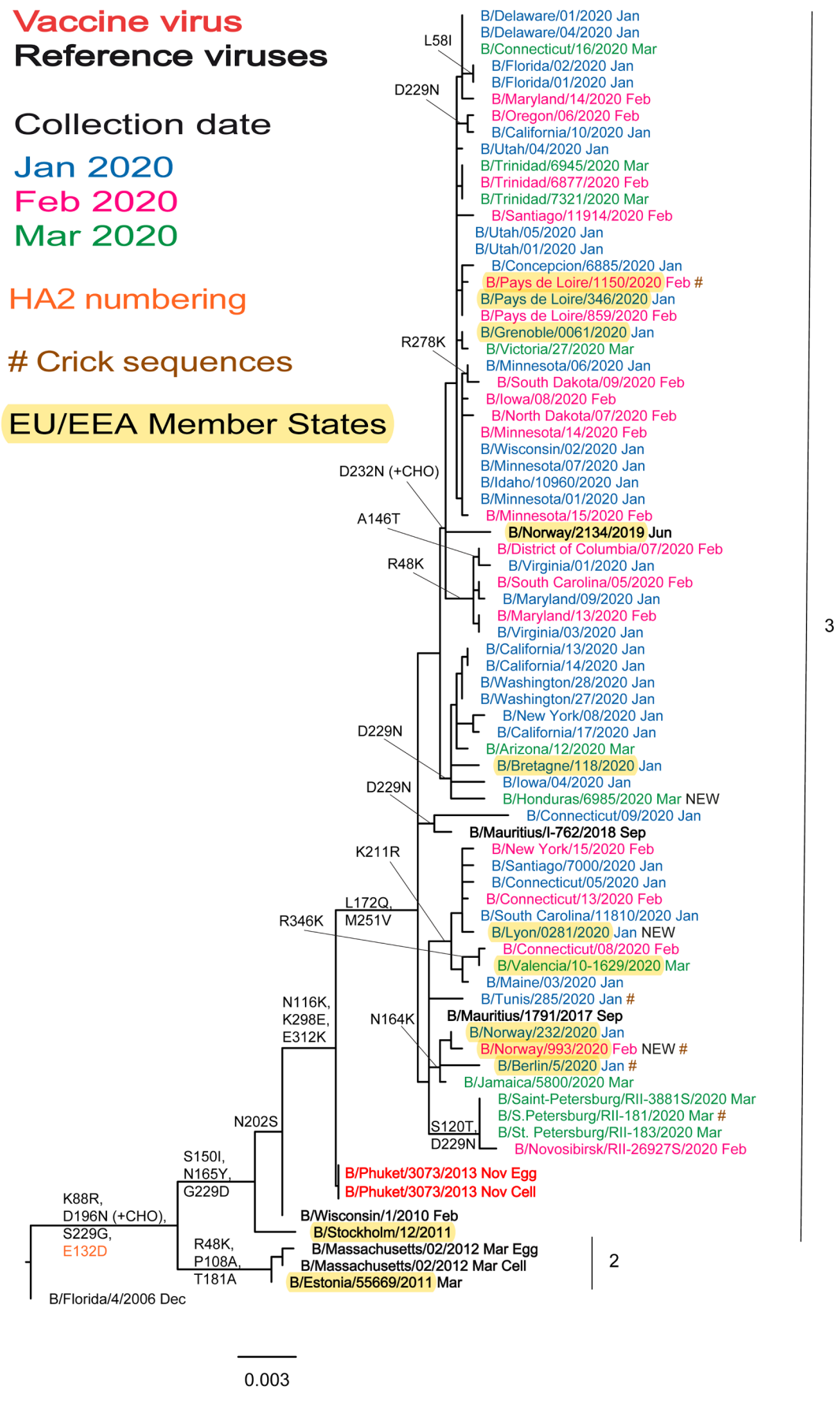


Table 2. Antigenic analysis of influenza B/Victoria-lineage viruses by HI

Viruses	Other information	Passage history / Ferret number / Genetic group	Collection date	Passage history	Haemagglutination inhibition titre												
					Post-infection ferret antisera												
					B/Bris 60/08 Egg Sh 539, 540, 543, 544, 570, 571, 574[1,3]	B/Bris 60/08 Egg F44/17[-4]	B/Sth Aus 81/12 Egg F25/16[-4]	B/Ireland 3154/16 MDCK F12/18[-2]	B/Nord-West 1/16 MDCK F38/17[-2]	B/Norway 2409/17 MDCK F40/17[-2]	B/Colorado 06/17 MDCK F21/18[-2]	B/Colorado 06/17 Egg F11/18[-2]	B/Wash'ton 02/19 MDCK F37/19[-2]	B/Wash'ton 02/19 Egg F38/19[-1]
		Passage history			1A	1A	1A	1A	1A	1A(Δ2)	1A(Δ2)	1A(Δ2)	1A(Δ3) B	1A(Δ3) B
		Genetic group												
REFERENCE VIRUSES														
B/Brisbane/60/2008		1A	2008-08-04	E4/E4	1280	640	320	80	80	<	<	160	<	160
B/South Australia/81/2012		1A	2012-11-28	E4/E2	1280	320	320	20	80	<	<	40	<	80
B/Ireland/3154/2016		1A	2016-01-14	MDCK1/MDCK4	1280	40	20	80	160	<	<	<	<	<
B/Nordrhein-Westfalen/1/2016		1A	2016-01-04	C2/MDCK2	1280	<	20	80	160	<	<	<	<	<
B/Norway/2409/2017		1A(Δ2)	2017-04-27	MDCK1/MDCK3	40	<	<	<	<	40	40	80	<	<
B/Colorado/06/2017		1A(Δ2)	2017-02-05	MDCK1/MDCK2	80	<	<	<	<	40	40	80	<	<
B/Colorado/06/2017		1A(Δ2)	2017-02-05	E5/E2	1280	160	40	<	10	40	40	320	<	160
B/Washington/02/2019		1A(Δ3) B	2019-01-19	C2/MDCK3	320	<	80	<	<	<	<	80	20	160
B/Washington/02/2019		1A(Δ3) B	2019-01-19	E3/E2	640	40	40	<	<	<	<	160	20	320
TEST VIRUSES														
B/Cyprus/F1188/2020		1A(Δ3) B	2020-02-23	MDCK1	40	<	<	<	<	<	<	<	10	160
B/Cyprus/F1194/2020		1A(Δ2)	2020-02-24	MDCK1	80	<	<	<	10	40	40	80	<	<
B/Cyprus/F1222/2020		1A(Δ2)	2020-02-25	MDCK1	80	<	<	<	<	40	20	80	<	<
B/Cyprus/F1273/2020		1A(Δ3) B	2020-02-27	MDCK1	320	<	<	<	<	<	<	40	20	160
B/Cyprus/F1247/2020		1A(Δ3) B	2020-02-27	MDCK2	80	<	<	<	<	<	<	<	20	160
B/Cyprus/F1296/2020		1A(Δ3) B	2020-03-03	MDCK1	80	<	<	<	<	<	<	20	20	160
B/Cyprus/F1287/2020		1A(Δ3) B	2020-03-03	MDCK1	80	<	<	<	<	<	<	20	40	160
B/Cyprus/F1359/2020	D129N, G133R, N233S (-CHO), K272R	1A(Δ3) B	2020-03-04	MDCK1	160	<	<	<	<	<	<	40	<	<
B/Cyprus/F1347/2020		1A(Δ3) B	2020-03-04	MDCK1	80	<	<	<	<	<	<	20	20	160
B/Cyprus/F1403/2020		1A(Δ3) B	2020-03-12	MDCK1	160	<	<	<	<	<	<	40	40	160
B/Cyprus/F1417/2020		1A(Δ3) B	2020-03-13	MDCK1	160	<	<	<	<	<	<	40	20	160
B/Cyprus/F1410/2020		1A(Δ3) B	2020-03-13	MDCK1	320	<	<	<	<	<	<	40	40	320
												Vaccine SH 2019 NH 2019-20		Vaccine SH 2020 NH 2020-21 SH 2021

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used):
[1] < = <40; [2] < = <10; [3] hyperimmune sheep serum; [4] < = <20; ND = Not Done
Sequences in Phyogenetic tree (Fig. 3b)

Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, October 2020)



Summaries of data submitted to TESSy

Genetic characterisation

No viruses detected over the course of the 2020-2021 season (weeks 40-44/2020) have been genetically characterised. 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/La Rioja/2202/2018 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

No 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). Over the course of the 2019-2020 influenza season where 2 292 viruses were 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, no viruses detected within EU/EEA countries during the 2020-2021 season have been assessed phenotypically against NAIs oseltamivir and zanamivir. Over the course of the 2019-2020 influenza season, of 1 030 viruses assessed for susceptibility to NAIs, only five (0.49%) showed either RI or HRI by at least one NAI.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [3] reported that the China Health and Family Planning Commission 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 [4]. 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 monthly summary and assessment of influenza at human-animal interface (accessed 9 November 2020). The assessment published on 23 October 2020 indicates 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 most recent human case was detected in mid-March 2019 [9]. 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 30 September 2020 and can be found on ECDC's website [10].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human–animal interface was published by WHO on 23 October 2020. While no new human cases were reported, according to reports received by the World Organisation for Animal Health (OIE), various influenza A(H5) subtypes continue to be detected in birds in Africa, Europe and Asia [8]. No new human cases of A(H5N1) infection have been detected since the case in Nepal in March 2019, the first human case of A(H5N1) infection reported to WHO since 2017. There have, however, been reports of A(H5N1) infection in domestic birds since February 2019 [11]. On 30 September 2020, ECDC published an alert related to outbreaks of avian influenza viruses in Europe [12] with a link to the latest collaborative report from ECDC and the European Food Safety Authority, which can be found on ECDC's website [10].

Influenza A(H9N2) virus

Since the previous update on 10 July 2020, one new laboratory-confirmed human case of influenza A(H9N2) virus infection in China has been reported [8]. The case was in a child in Guangdong province who developed mild symptoms

on 3 August 2020 and was admitted to hospital on 4 August 2020, making a full recovery thereafter. Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa.

Other influenza zoonotic events

Since the previous update on 10 July 2020, one zoonotic event with a swine influenza A(H3N2)v virus was reported by the USA on 25 July [8]. The infection was detected in a child in Hawaii who developed influenza-like illness on 30 June 2020. No exposure to swine was reported and the child recovered without need for hospitalisation.

WHO Collaborating Centre reports

A description of results generated by the London WHO Collaborating Centre at the WIC and used at the most recent WHO vaccine composition meeting (held online: 16-24 September 2020 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 9 November 2020).

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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