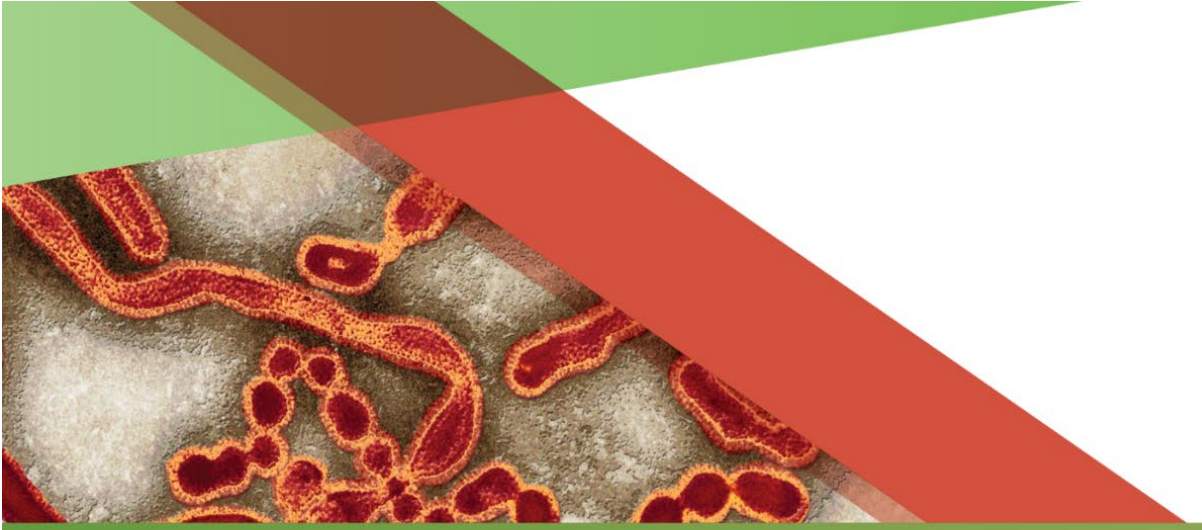

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, April 2021

Summary

This is the sixth report for the 2020-2021 influenza season. As of week 17/2021, only 865 influenza detections across the World Health Organization (WHO) European Region had been reported to The European Surveillance System (TESSy); 50% type A viruses, with A(H3N2) and A(H1N1)pdm09 being approximately equally represented, and 50% type B viruses, with only 16 having been ascribed to a lineage, 13 B/Victoria and three B/Yamagata. This represents a 99.5% drop in detections compared to the same period in 2020, probably due to the COVID-19 pandemic and measures introduced to combat it.

Since the March 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, little virus characterisation data has been generated. This report therefore focuses on genetic characterisation of the HA genes of seasonal influenza viruses with the most recent collection dates deposited and/or released in GISAID as of 30 April 2021, with collection date periods adjusted for each virus subtype/lineage to allow production of readable phylogenies. These data continue to show extremely low levels of influenza detections. 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.

Of the 49 seasonal A(H1N1) pdm09 HA sequences reported to GISAID from viruses collected in the course of the 2020-2021 season, two are group 6B.1A5B, one group 6B.1A5A, 44 subgroup 6B.1A5A+187V/A represented by A/Guangdong-Maonan/SWL1536/2019 (the vaccine virus for the northern hemisphere 2020-2021 season), and two subgroup 6B.1A5A+156K represented by A/Victoria/2570/2019 (the vaccine virus for the southern hemisphere 2021 and northern hemisphere 2021-2022 influenza seasons). The great majority of 6B.1A5A group and subgroup viruses were detected in West African countries.

Recently collected A(H3N2) viruses have HA sequences falling into two subgroups. 3C.2a1b+T135K-A subgroup viruses, represented by the reference virus A/Denmark/3264/2019, have been detected in countries in West Africa. 3C.2a1b+T131K-A subgroup viruses split into two antigenically distinguishable clusters originally defined by viruses from Cambodia (with HA1 amino acid substitutions of G186S, F193S, Y195F and S198P, many also having K171N) and

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

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Bangladesh (with HA1 amino acid substitutions of Y159N, T160I (loss of a glycosylation site), L164Q, G186D, D190N, F193S and Y195F); both clusters have shown greater geographic spread than the 3C.2a1b+T135K-A subgroup viruses. 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.

All B/Victoria-lineage HA sequences deposited and/or released in GISAID, for viruses with collection dates after 31 December 2020, fell within subclade 1A(△3)B. A single virus from the United States of America (US) fell in the HA1 G133R substitution group, while all other viruses fell in the HA1 N150K, G184E, N197D (loss of a glycosylation site) and R279 substitution group. The latter virus group splits into two subgroups, one of which, defined by HA1 substitutions V220M and P241Q, is dominant in China, while the other, defined by HA1 substitutions A127T, P144L and K203R (often with additional substitutions), shows wider geographic spread. 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.

A single B/Yamagata-lineage HA sequence from a virus collected in January 2020 was deposited and/or released in GISAID during April. Like previously released sequences from viruses with collection dates in 2020, it belongs to genetic clade 3 and carries three HA1 amino acid substitutions (L172Q, D229N and M251V) compared to 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 40/2020-17/2021), compared with the same period for the 2019-2020 season. While there has been a small reduction in the numbers of samples from patients fulfilling Influenza-Like Illness (ILI) and/or Acute Respiratory Infection (ARI) criteria being tested (~29 053, 3.5%), there has been a vast reduction in the number of samples testing positive for an influenza virus (161 447, 99.5%). This is probably due to a number of factors: (i) the number of centres within the Region reporting over these periods having dropped from 52 to 45; (ii) 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; (iii) 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 (iv) 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 31 weeks of the 2020-2021 season, the ratio of type A to type B detections is reduced compared to 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 appear again to be predominating over B/Yamagata lineage viruses, although only 16/420 (3.8%) of type B viruses detected in the 2020-2021 season have been ascribed to a lineage as of week 17/2021.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2020-21 season (weeks 40/2020-17/2021)[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	**28**	**417**	**445**	**51.4**	**1.1:1**	**11256**	**107634**	**118890**	**73.2**	**2.7:1**
A(H1N1)pdm09	13	28	41	43.6		6101	20105	26206	55.9	
A(H3N2)	6	47	53	56.4	1.3:1	4162	16474	20636	44.1	0.8:1
A not subtyped	9	342	351			993	71055	72048		
Influenza B	**13**	**407**	**420**	**48.6**		**6261**	**37161**	**43422**	**26.8**	
Victoria lineage	2	11	13	81.3	4.3:1	2388	2029	4417	98.1	50.8:1
Yamagata lineage	0	3	3	18.7		21	66	87	1.9	
Lineage not ascribed	11	393	404			3852	35066	38918		
Total detections (total tested)	**41 (39839)**	**824 (>752961)**	**865 (>792800)**			**17517 (50342)**	**144795 (>771511)**	**162312 (>821853)**		

[a] Numbers taken from Flu News Europe week 17/2021 and week 17/2020 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 April 2021 (Table 2). 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 therefore 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. Since the March 2021 report, a single A(H3N2) virus from Sweden was characterised genetically and phenotypically.

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	0	1	0				
NOVEMBER														
France	2										2	1		
DECEMBER														
France	2										2	1		
2021 JANUARY														
Austria	1										1	1		
Norway	1										1	1		
Sweden	3					1	1				2	0		
	20	0	0	1	0	8	2	0	1	0	10	5	0	0
5 Countries		0.00%		5.0%		40.0%			5.0%		50.0%		0.0%	
		45.0%							55.0%					

*** 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
Includes RNA extracts for which genetic characterisation only can be performed.

As of 2021-05-10

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 March 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 31 March 2021. The great majority were archival, i.e. from the 2019-2020 season, and of the 23 from the 2020-2021 season 22 HA sequences fell in the **6B.1A5A+187V/A** subgroup (with many having additional HA1 substitutions of I166T and A186T) and were detected in Niger and Nigeria, while a single group **6B.1A5B** virus (with **HA1 K130N**, **K160M**, **T216K**, **E235D**, **H296N** and **HA2 V193A** substitutions) was detected in the US (Figure 1a). As of 30 April 2021, complete HA sequences of just 51 A(H1N1)pdm09 viruses with collection dates after 31 August 2020 had been deposited in GISAID (Figure 1b). Of these, one from Ghana was **6B.1A5A** group, 44 were **6B.1A5A+187V/A** subgroup (41 from West Africa, two from Japan and one from US) and two were **6B.1A5A+156K** subgroup (one each from China and Ghana); two from the US were **6B.1A5B** group; and two were from zoonotic cases (swine subclade 1A.3.3.2) one of which, A/Denmark/1/2021, was detected in a human sample collected in January 2021 (Figure 1b). The virus recovered from the zoonotic case in Denmark was not recognised in HI assays performed with post-infection ferret antisera raised against any of the human seasonal A(H1N1)pdm09 vaccine viruses (results not shown).

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 May 2021].

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

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

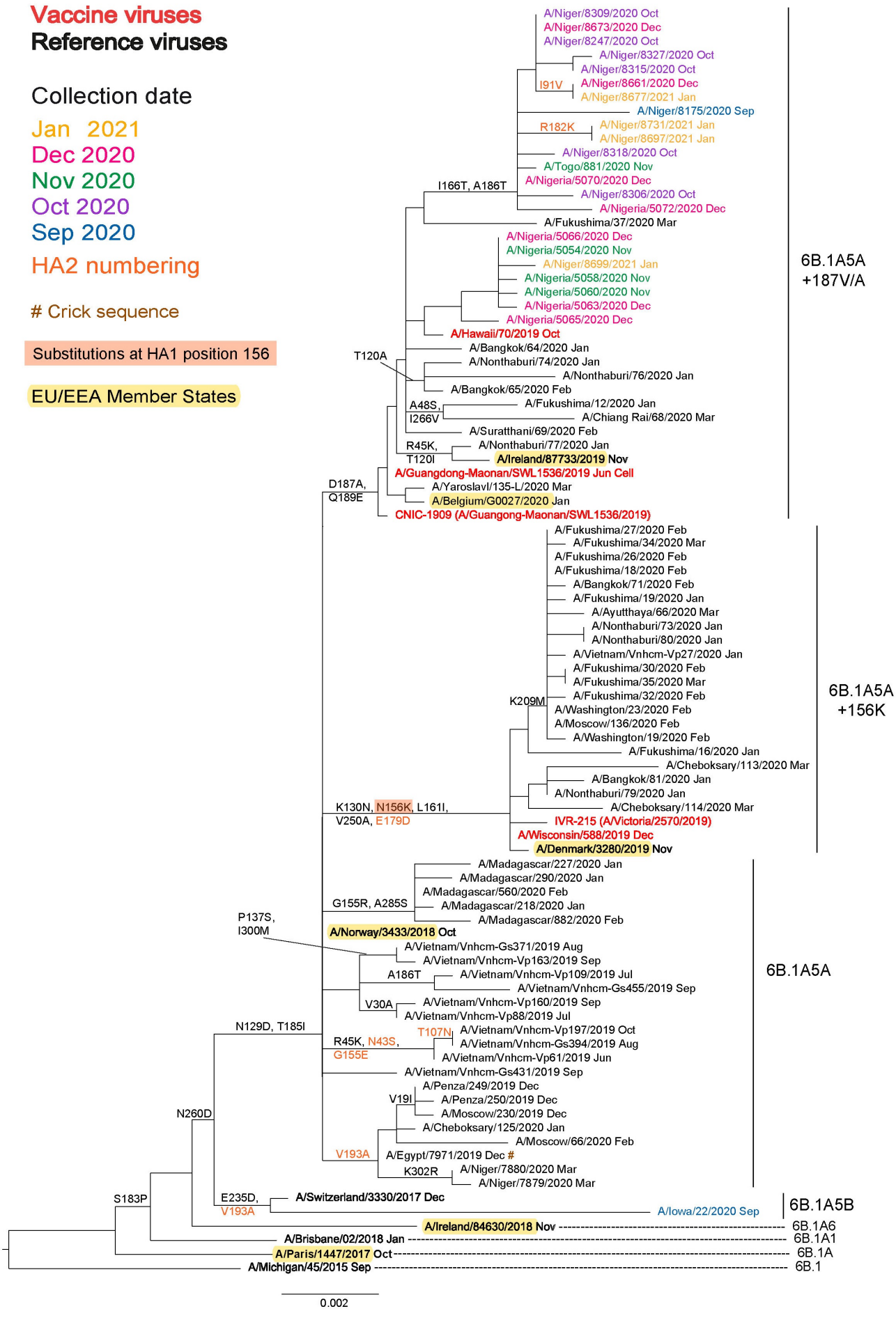
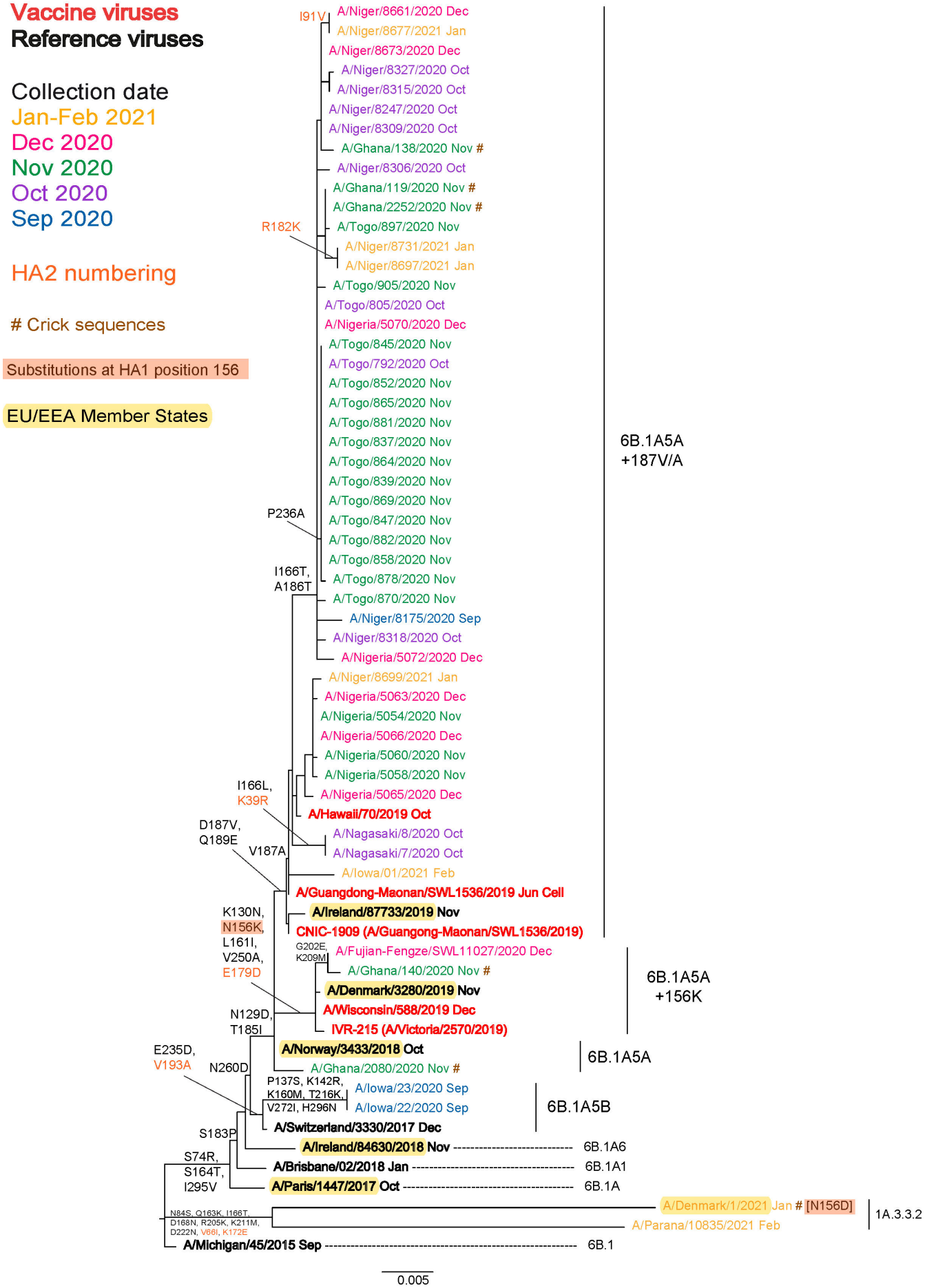


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, April 2021)



Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny is repeated from the March 2021 report and was based on all sequences deposited in GISAID during March 2021 with no limits on the collection dates of the viruses (Figure 2a). The second is based on sequences from viruses with collection dates after 31 October 2020 available in GISAID as of 30 April 2021, together with sequences from viruses recently characterised at the WIC (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 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 March report, based on sequences deposited and/or released in GISAID during March 2021, contained many sequences from viruses with collection dates before the start (week 40/2020) of the 2020-2021 influenza season (Figure 2a). It showed a huge preponderance of viruses in group **3C.2a1b** over those in clade **3C.3a**. Of those viruses with collection dates after 31 August 2020, those in the subgroup **3C.2a1b+T135K-A** cluster (n = 29) were detected in countries of West Africa while those in the subgroup **3C.2a1b+T131K-A** cluster (n = 34) were split between 'Cambodia-like' viruses (n = 26) carrying additional **HA1** substitutions of **G186S**, **F193S**, **Y195F** and **S198P** with 23 also having **K171N** (detected in Australia, Cambodia, Japan, Thailand, the US and Vietnam) and 'Bangladesh-like' viruses (n = 8) carrying additional **HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N**, **F193S** and **Y195F** (detected in Bahrain, Bangladesh, India and United Arab Emirates). The updated phylogeny, largely based on HA sequences from viruses with collection dates after 31 October 2020, shows similar distributions of the subgroup **3C.2a1b+T135K-A** cluster and the **3C.2a1b+T131K-A** cluster 'Cambodia-like' viruses, while the **3C.2a1b+T131K-A** cluster 'Bangladesh-like' viruses have now been reported by additional countries (Australia, Sweden and the US) (Figure 2b).

While the number of detections of seasonal influenza viruses remains low, compared to 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. A single **3C.2a1b+T131K-A** cluster 'Bangladesh-like' virus, A/Stockholm/1/2021, has been antigenically characterised by HI at the WIC with a small panel of post-infection ferret antisera: while generally well recognised, titres were 8-fold reduced compared to homologous titres with antisera raised against egg-propagated vaccine viruses, A/Hong Kong/2671/2019 and A/Cambodia/e0826360/2020, for use in the 2020-2021 and 2021-2022 northern hemisphere influenza seasons, respectively (Table 3).

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 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, March 2021)

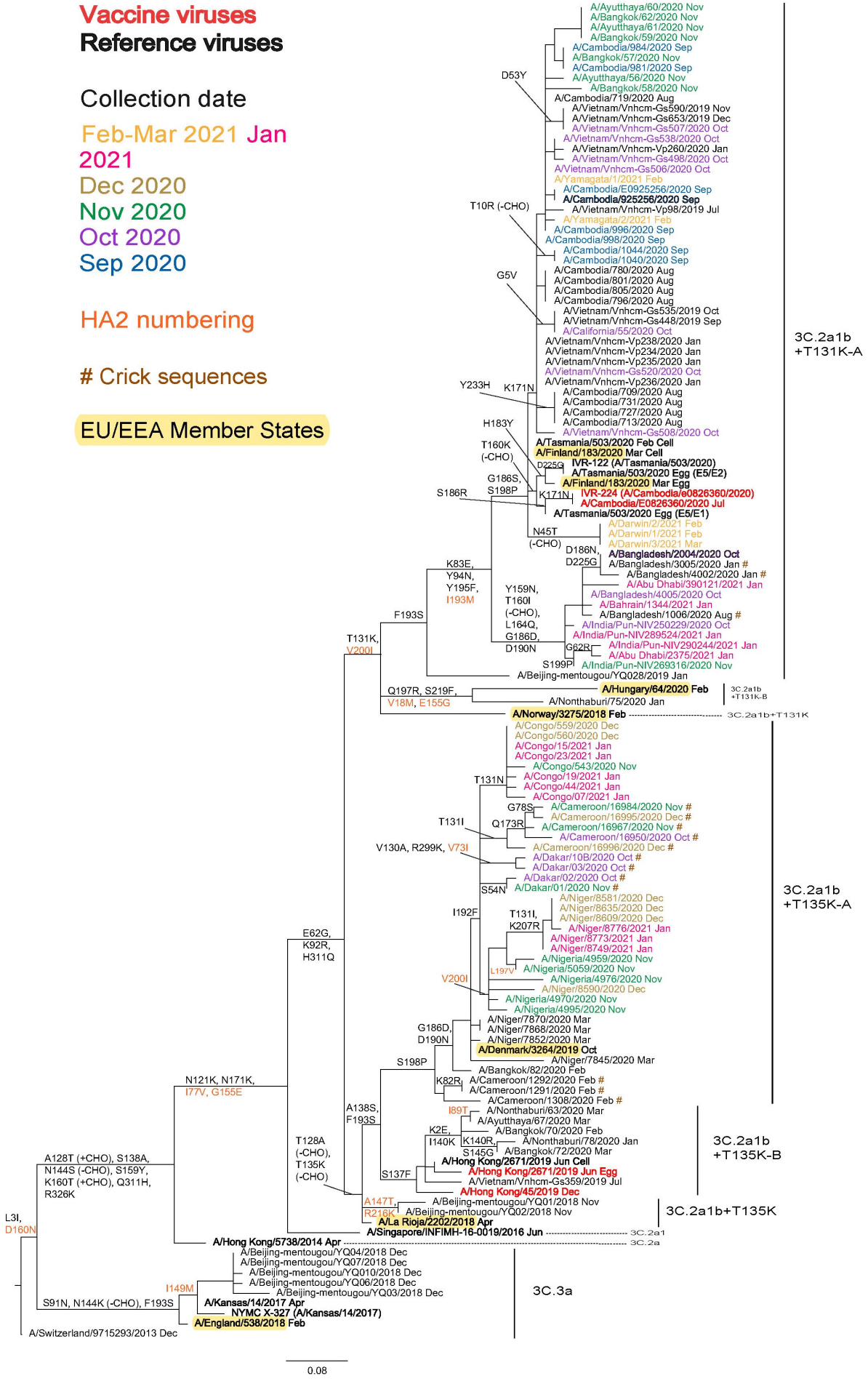


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, April 2021)

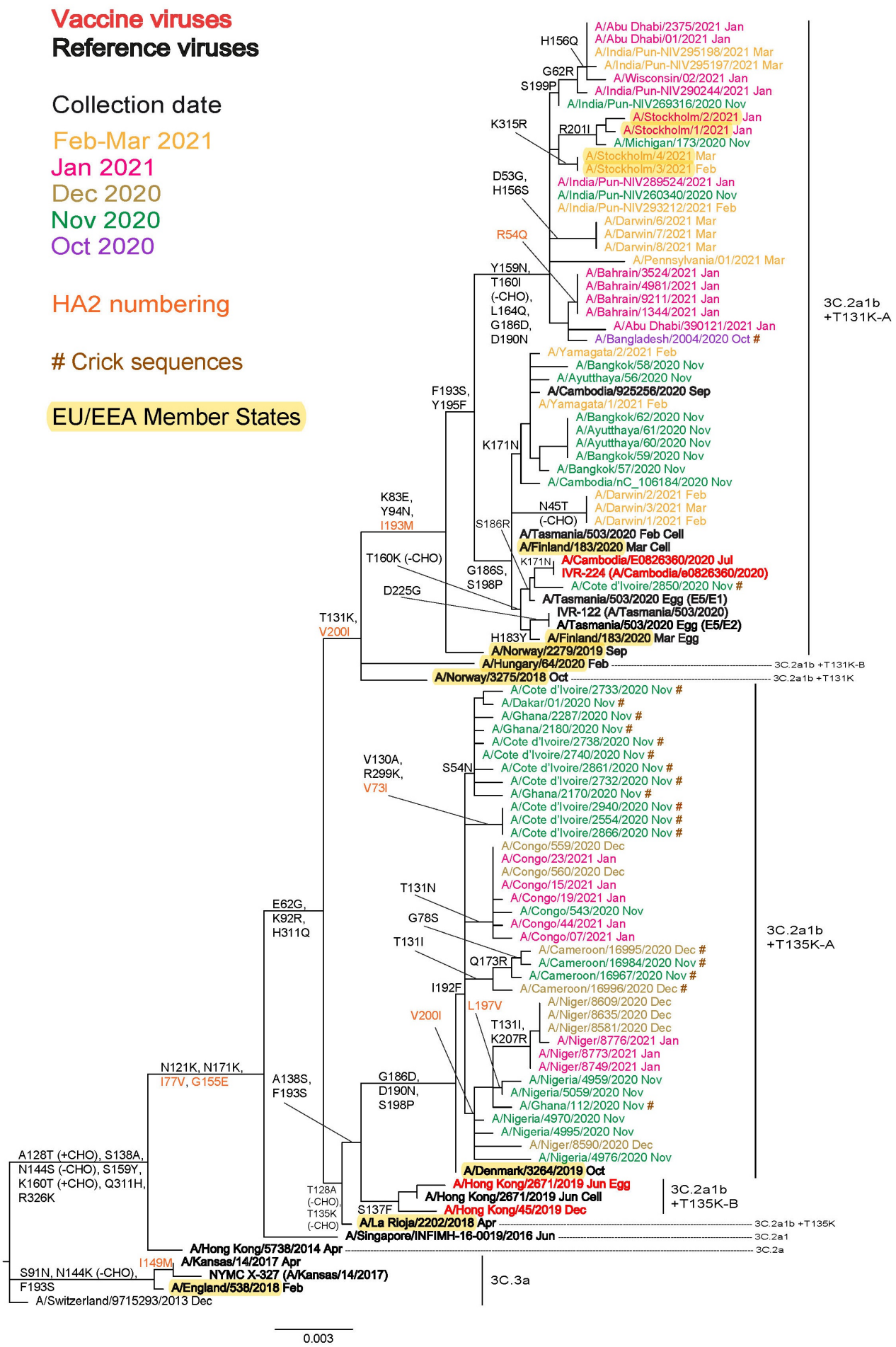


Table 3. Antigenic analysis of A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre								
				Post-infection ferret antisera								
	Passage history			A/HK 5738/14 MDCK	A/Singapore 0019/16 Egg 10^{-4}	A/Denmark 3264/19 SIAT	A/HK 2671/19 Egg	A/HK 2671/19 Cell	A/Camb e0826360/20 Egg	A/Eng 538/18 SIAT	NYMC X-327 A/Kansas/14/17 Egg	A/Kansas 14/17 SIAT
	Ferret number			St Judes F60/17*1	F13/19*1	F19/20*1	F44/19*1	St Judes F21/20*1	F10/21*1	F31/18*1	F16/19*1	F17/19*1
	Genetic group			3C.2a	3C.2a1	3C.2a1b+T135K-A	3C.2a1b+T135K-B	3C.2a1b+T135K-B	3C.2a1b+T131K-A	3C.3a	3C.3a	3C.3a
REFERENCE VIRUSES												
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK1/MDCK2/SIAT2	160	320	<	<	40	80	160	320	160
A/Singapore/INFIMH-16-0019/2016	3C.2a1	2016-04-14	E5/E3	80	320	80	80	<	40	40	40	<
A/Denmark/3264/2019	3C.2a1b+T135K-A	2019-10-25	SIAT5	160	320	320	160	320	320	160	320	160
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	2019-06-17	E8/E3	<	160	160	1280	320	320	160	640	160
A/Hong Kong/2671/2019	3C.2a1b+T135K-B	2019-06-17	MDCK1/SIAT4	160	160	320	320	320	320	160	80	80
A/Cambodia/e0826360/2020	3C.2a1b+T131K-A	2020-07-16	E5/E2	40	160	160	160	<	2560	320	160	160
A/England/538/2018	3C.3a	2018-02-26	MDCK1/SIAT4	<	40	<	<	<	40	320	160	320
NYMC X-327 (A/Kansas/14/17)	3C.3a	2017-12-14	Ex/E1	<	40	<	160	<	80	160	1280	320
A/Kansas/14/2017	3C.3a	2017-12-14	SIAT3/SIAT2	40	80	40	40	40	80	640	320	640
TEST VIRUSES												
A/Stockholm/1/2021	3C.2a1b+T131K-A	2021-01-08	SIAT0/SIAT1	40	160	160	160	80	320	320	320	320
					Vaccine NH 2018-19 SH 2018		Vaccine NH 2020-21 SH 2021		Vaccine NH 2021-22		Vaccine NH 2019-20	

* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)
1 < = <40, ND = Not Done
In phylogentic tree

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 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 March report (Figure 3a) was based on HA genes deposited/released in GISAID in March 2021, many of which were from viruses with collection dates from before the start (week 40/2020) of the 2020-2021 season. No recently collected **subclade 1A(△2)** viruses had been detected and all other viruses fell in **subclade 1A(△3)B**. Of the **subclade 1A(△3)B** viruses with collection dates during the 2020-2021 season, a single virus fell in a group defined by **HA1 G133R** amino acid substitution, while all others fell in a group defined by **HA1 N150K**, **G184E**, **N197D** (loss of a glycosylation site) and **R279K** (**N150K group**), and additional **A127T**, **P144L** and **K203R** substitutions, with many viruses also having **T182A**, **D197E** and **T221A** substitution.

The phylogeny for this report focuses on HA sequences available in GISAID up to 30 April 2021, for viruses with collection dates after 31 December 2020, together with viruses characterised recently at the WIC (Figure 3b). Only **subclade 1A(△3)B** detections have been reported during 2021, significantly by China where **N150K group** viruses with additional **HA1** substitutions of **V220M** and **P241Q** have dominated. However, China – together with countries in Europe, the Middle East, West Africa, and the US – have also reported the detection of **N150K group** viruses with additional HA1 substitutions of **A127T**, **P144L** and **K203R**, with some viruses carrying additional **HA1** substitutions – notably those from China with **H122Q**.

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

No B/Yamagata-lineage viruses from WHO European Region countries were characterised at the WIC during April 2021 and a single HA sequence, from B/Antofagasta/4493/2020 collected 06 January 2020, was deposited and/or released in GISAID during this month (Figure 4). No HA sequences from viruses with collection dates after March 2020 have been deposited in GISAID and 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 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, March 2021)

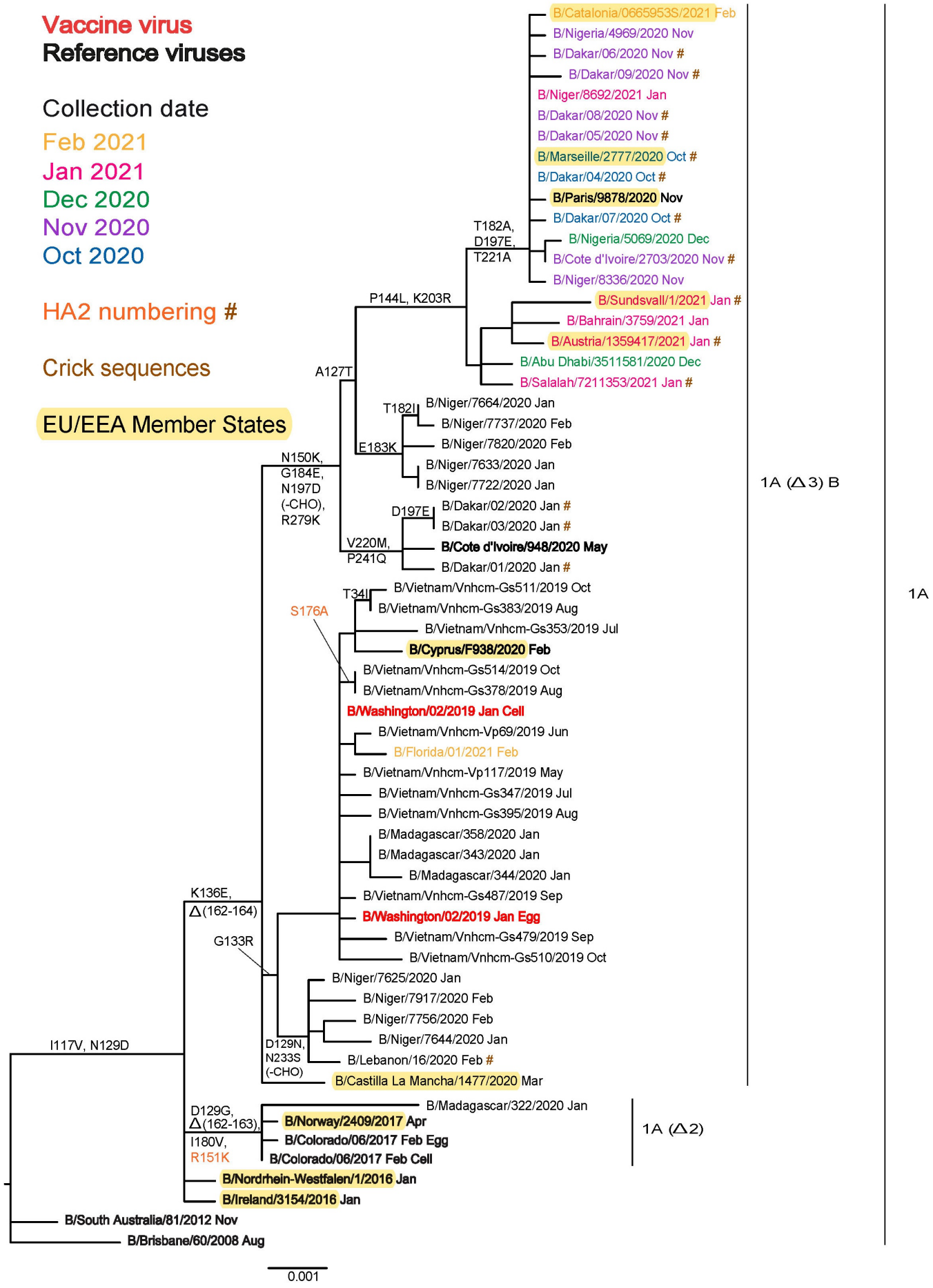


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, April 2021)

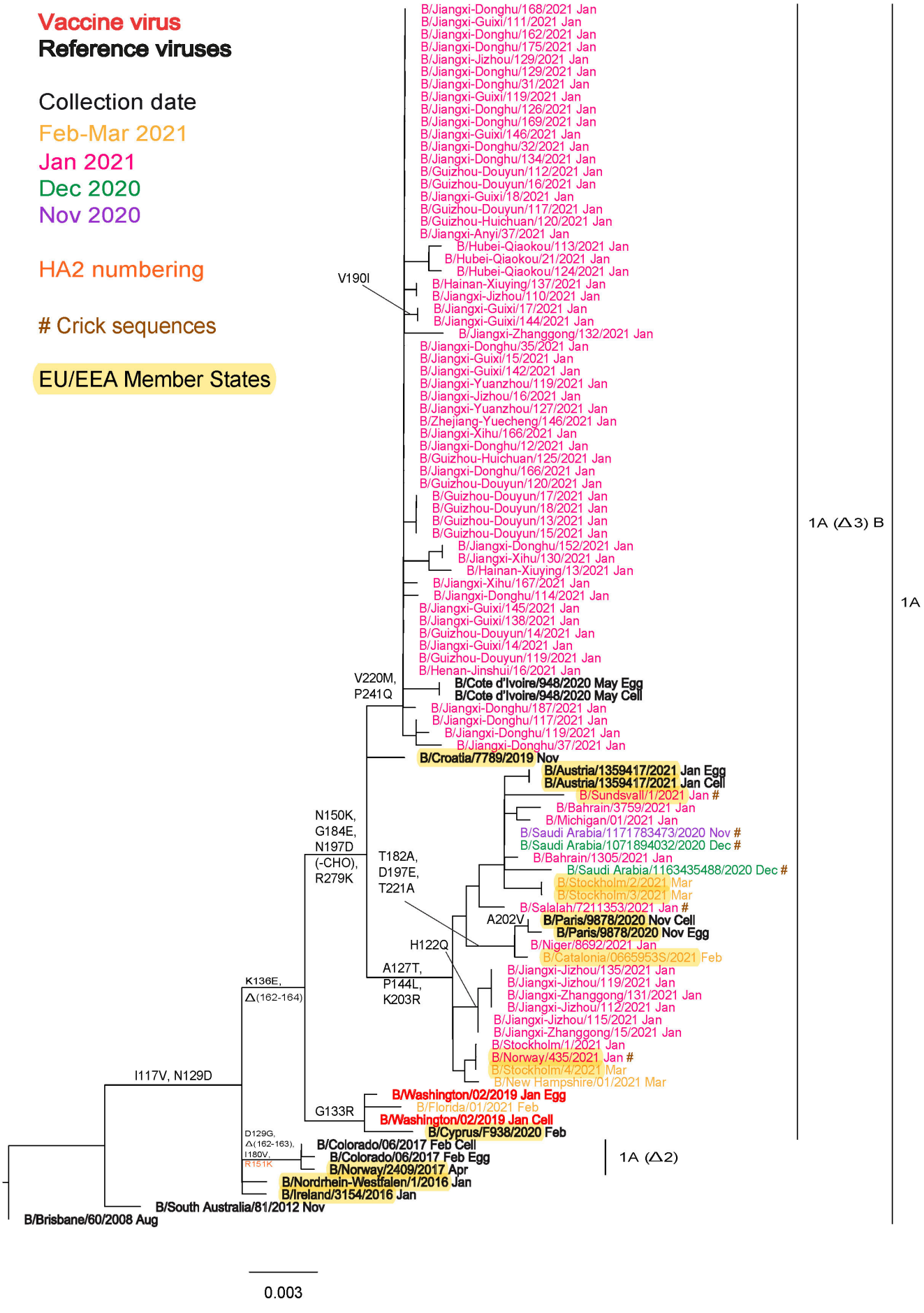
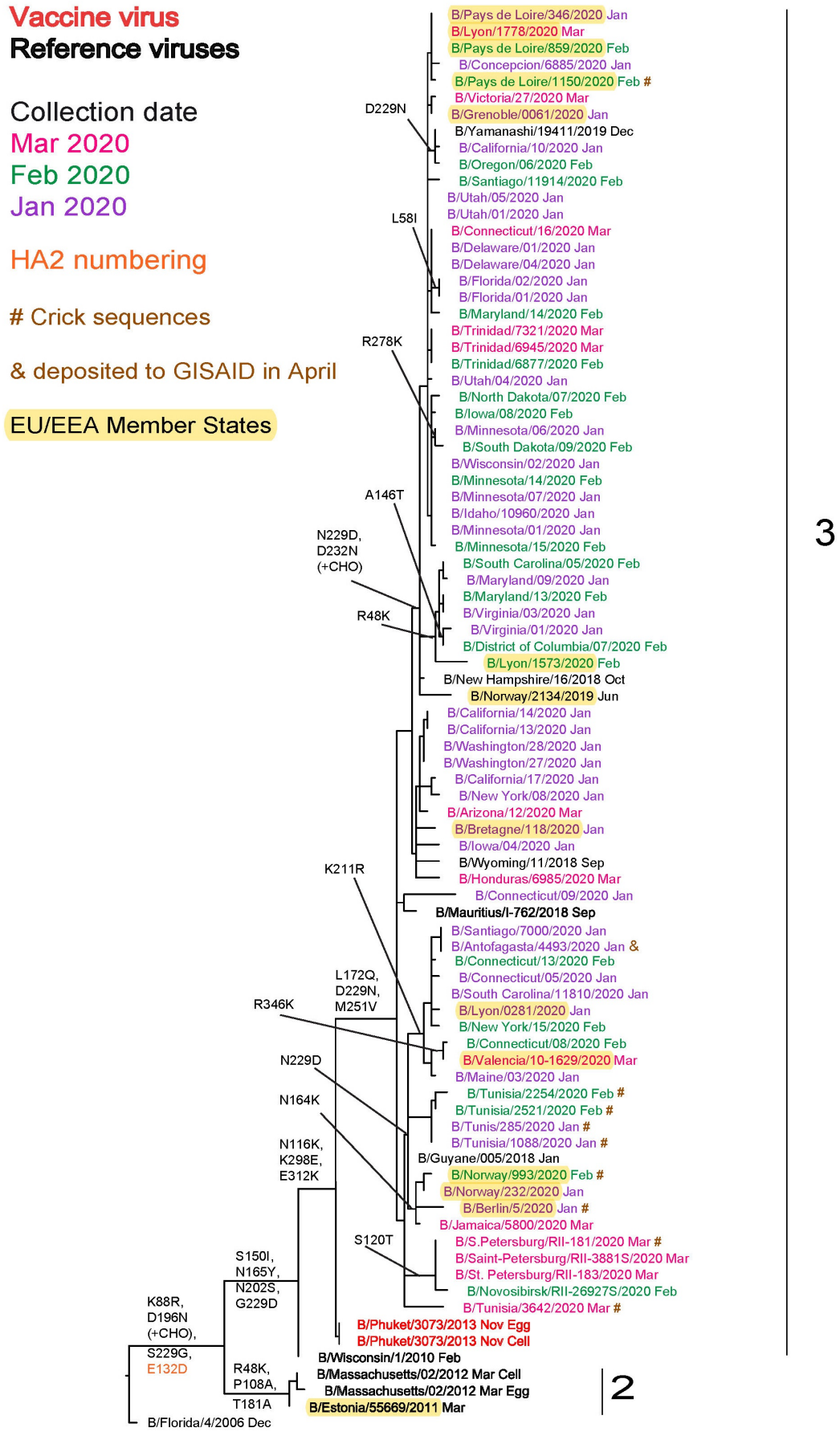


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, April 2021)



Summaries of data submitted to TESSy

Genetic characterisation

Twelve viruses detected over the course of the 2020-2021 season (weeks 40/2020-17/2021) have been genetically characterised:

- One A(H1N1)pdm09 virus attributed to the 6B.1A5A+187V/A group represented by A/Guangdong-Maonan/SWL1536/2019.
- Six A(H3N2) viruses with four 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 week15/2021 (two each A(H3N2) and B/Victoria-lineage viruses), have been tested for susceptibility to neuraminidase inhibitors (NAIs) and sequence analysis 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, six influenza viruses detected within EU/EEA countries during the 2020-2021 season have been assessed phenotypically against oseltamivir and zanamivir: two 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 10 May 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 5 May 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 were still hospitalised and two in children, one of whom had recovered while the other died [9]. According to reports compiled by the Food and Agricultural Organization of the United Nations (FAO), various influenza A(H5Nx) subtypes continue to be detected in wild and/or domestic birds in Africa, Europe and Asia and since 24 February 2021 a total of 166 outbreaks had been reported [13]. 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 [14]. In addition, Lao People's Democratic Republic reported a case of human H5N6 infection in a 5-year old male [13]. On 30 September 2020, ECDC published an alert related to outbreaks of avian influenza viruses in Europe [15]. 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]. The latest FAO Global AIV with zoonotic potential situation update reported three cases of human infection with A(H9N2) in China, all in children, since the previous update on 24 February 2021 [13].

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, had been 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 4-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 4 May 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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