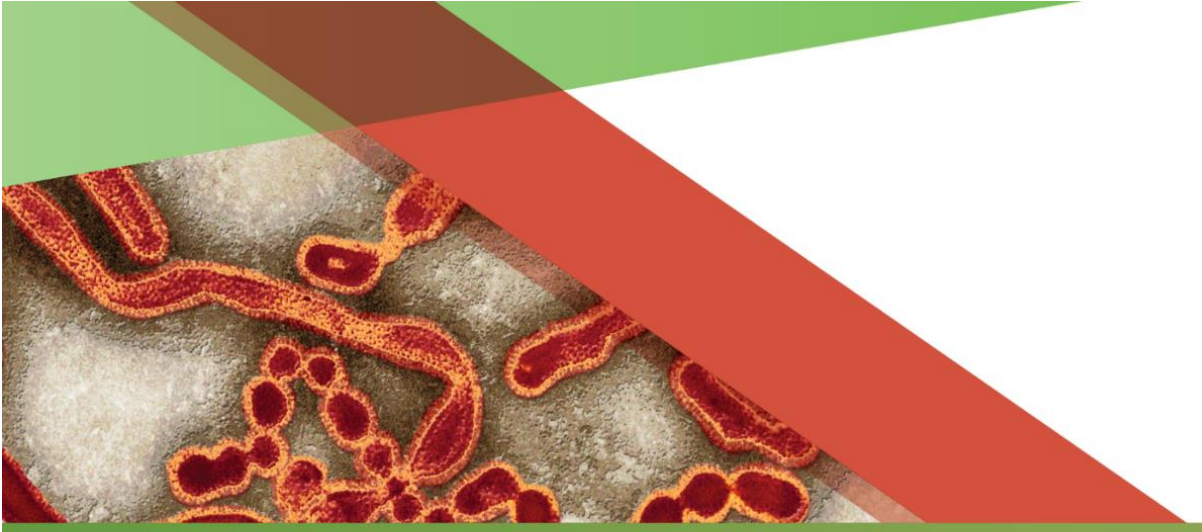

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, November 2021

Summary

This is the second influenza virus characterisation report for the 2021-2022 influenza season. As of week 47/2021, 2 707 influenza detections across the World Health Organization (WHO) European Region were reported to the European Surveillance System (TESSy); 93% type A viruses, with A(H3N2) (96%) dominating over A(H1N1)pdm09 (4%), and 7% type B, with only two having been ascribed to a lineage (B/Victoria). This represents a large increase (2 627, 3384%) in detections compared to the 2020-2021 season, following a large increase (354 256, 519%) in the number of samples tested, and is closer to the more usual number of detections seen at this time in earlier seasons. The increased testing is probably related to the emergence of SARS-CoV-2 and measures introduced to combat it.

Since the October 2021 characterisation report[1], no shipments from EU/EEA countries were received at the London WHO Collaborating Centre, the Francis Crick Worldwide Influenza Centre (WIC). This report therefore focuses on genetic characterisation of HA genes of representative seasonal influenza viruses submitted and/or released in GISAID during November 2021, with a focus on those viruses with collection dates after 31 August 2021, together with sequences recently determined at the WIC. The genetic clade nomenclature system adopted during the September 2021 vaccine composition meeting (VCM) is used throughout the document. The data continued to show low levels of influenza detections globally but with a predominance of A(H3N2) viruses.

Very low numbers of A(H1N1)pdm09 detections have been reported. The three with the most recent collection dates fell in the 6B.1A.5a.1 subgroup, represented by the vaccine virus for the northern hemisphere 2020-2021 season, A/Guangdong-Maonan/SWL1536/201960. A batch of viruses from Bangladesh with collection dates in September 2021 all fell in the 6B.1A.5a.2 subgroup, represented by A/Victoria/2570-like and A/Wisconsin/588/2019-like viruses which have been recommended respectively for egg- and cell-based vaccines in the 2021-2022 northern and 2022 southern hemisphere influenza seasons, but with additional HA amino acid substitutions seen previously in viruses from India.

All recently detected A(H3N2) viruses have fallen in subgroup 3C.2a1b.2a and been 'Bangladesh-like' (3C.2a1b.2a.2), with a number of EU/EEA countries reporting detections. A/Cambodia/e0826360/2020-like (3C.2a1b.2a.1) viruses were recommended for use in the 2021-2022 northern hemisphere season, while A/Darwin/9/2021-like and

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

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A/Darwin/6/2021-like (3C.2a1b.2a.2) viruses were recommended for egg- and cell-based vaccines in the 2022 southern hemisphere season. Recently detected viruses in Croatia were Bangladesh-like genetically and antigenically.

While the vast majority of B/Victoria-lineage HA sequences derived from viruses collected after 31 January 2021 were subclade V.1A.3, represented by B /Washington/02/2019, the vaccine virus recommended for inclusion in influenza vaccines for the 2021-2022 northern hemisphere season, such viruses have only been detected in the US recently. The vast majority of sequences from recently detected viruses, in geographically dispersed countries, have fallen in the V.1A.3.2 subgroup with defining HA1 A127T, P144L and K203R amino acid substitutions. B/Austria/1359417/2021-like (V.1A.3.2) viruses have been recommended for use in the southern hemisphere 2022 influenza season. Antigenically, viruses in subgroups of the V.1A.3a group differ and show some loss of reactivity with post-infection ferret antisera raised against B/Washington/02/2019.

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 Y3 and carry 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 2021-2022 northern hemisphere and 2022 southern hemisphere seasons. 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 during the 2021-2022 season (weeks 40-47/2021), compared to the 2019-2020 season. There has been a vast increase in the number of samples from patients fulfilling Influenza-Like Illness (ILI) and/or Acute Respiratory Infection (ARI) criteria being tested (354 256, 519%), even when compared with a more 'normal' season, 2019-2020 (326 453, 391%: results not shown), which preceded the COVID-19 pandemic. With this increased testing there has been a rise in the number of influenza-positive samples (2 627, 3384%), although there was a reduction compared to the same period in 2019-2020 (1 649, 37.9%: results not shown). These data probably relate 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 during of the 2020-2021 season, the ratio of type A to type B detections has increased compared to the 2020-2021 season (1.2:1 to 12.3:1), with a greater dominance of A(H3N2) over A(H1N1)pdm09 viruses. While the number of influenza B virus detections has increased from 37 to 203 (549%), only two viruses in each period were ascribed to a lineage (Table 1), though B/Yamagata lineage viruses were not detected after March of 2020. Currently, it appears that measures introduced relating to the COVID-19 pandemic are still having an effect but that A(H3N2) viruses will dominate in 2021-2022.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2021-22 season (weeks 40-47/2021)[a]

Virus type/subtype/lineage	Cumulative number of detections for weeks 40-47/2021			Totals*		Cumulative number of detections for weeks 40-47/2020			Totals*	
	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Sentinel sources	Non-sentinel sources	Totals	%	Ratios
Influenza A	**133**	**2371**	**2504**	**92.5**	**12.3:1**	**1**	**42**	**43**	**53.8**	**1.2:1**
A(H1N1)pdm09	1	63	64	3.8		1	6	7	28.0	
A(H3N2)	98	1501	1599	96.2	25.0:1	0	18	18	72.0	2.6:1
A not subtyped	34	807	841			0	18	18		
Influenza B	**6**	**197**	**203**	**7.5**		**0**	**37**	**37**	**46.2**	
Victoria lineage	0	2	2	100.0		0	2	2	100.0	
Yamagata lineage	0	0	0	0.0		0	0	0	0	
Lineage not ascribed	6	195	201			0	35	35		
Total detections (total tested)	**139 (10 461)**	**2 568 (>428 255)**	**2 707 (>438 716)**			**1 (4 433)**	**79 (>80 027)**	**80 (>84 460)**		

[a] **Numbers taken from Flu News Europe to week 47/2021 and week 47/2020 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 40/2021, two shipments of specimens (virus isolates and/or clinical specimens) were received at the Crick Worldwide Influenza Centre (WIC) from Croatia and the Netherlands containing five and 14 samples, respectively (Table 2). Of the 18 samples, 17 were type A viruses and one was type B/Victoria-lineage. No shipments were received in November 2021.

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. Data generated on viruses with collection dates after 31 January 2021 informed the recent VCM where recommendations were made for the 2022 southern hemisphere season. Recommendations for the 2020-2021 northern hemisphere, the 2021 southern hemisphere and upcoming 2021-2022 northern hemisphere and 2022 southern hemisphere seasons have been published [1, 2, 3, 4].

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. Four A(H3N2) viruses from Croatia were characterised antigenically since the October 2021 report (Table 3).

Table 2. Summary of seasonal influenza clinical samples and virus isolates* contained in packages received from EU/EEA Member States since week 40/2021

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]
2021														
August														
Croatia	2					2	2							
Netherlands	1					1	1							
September														
Croatia	3					3	2	0						
Netherlands	11					10	10				1	1		
October														
Netherlands	1					1	1							
2 Countries	18	0	0	0	0	17	16	0	0	0	1	1	0	0
		0.00%		0.0%		94.4%			0.0%		5.6%		0.0%	
		94.4%							5.6%					

*** 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)
Characterisation of Specimens with collection dates from 1 September 2021 (below red line) will be considered for the northern hemisphere VCM in February 2022

As of 2021-12-01

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, updated for the September 2020 WHO VCM and with a new nomenclature introduced at the time of the September 2021 WHO VCM (**6B.1A.1 to 6B.1A.7**). 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, 2022 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, 4]. The seven subclades are defined by the following HA amino acid substitutions:

1. Subclade **6B.1A.1** 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.1A.2** 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.1A.3** viruses, represented by **A/Norway/3737/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions.
4. Subclade **6B.1A.4** represented by **A/Hungary/20/2018** carries HA gene mutations encoding **HA1 N129D, A144E** and **S183P** amino acid substitutions.
5. Subclade **6B.1A.5** viruses carry HA gene mutations encoding **HA1 S183P** and **N260D** amino acid substitutions and splits into two groups designated **6B.1A.5a** represented by **A/Norway/3433/2018** with additional **HA1** amino acid substitutions of **N129D** and **T185A**, and **6B.1A.5b** represented by **A/Switzerland/3330/2017** with additional amino acid substitutions of **HA1 E235D** and **HA2 V193A**. Two subgroups within the **6B.1A.5a** group have been defined based on **HA1** amino acid substitutions of **D187V/A** and **Q189E** (**6B.1A.5a.1**) or **K130N**, **N156K**, **L161I** and **V250A** (**6B.1A.5a.2**).
6. Subclade **6B.1A.6** viruses, represented by **A/Ireland/84630/2018**, carry HA gene mutations encoding **HA1 T120A** and **S183P** amino acid substitutions, like subclade **6B.1A.3** viruses, but fall within a separate phylogenetic branch which is closer to subclade **6B.1A.5** viruses.
7. Subclade **6B.1A.7** 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 October 2021 report and included 35 recently submitted/released sequences available in GISAID, derived from 16 **6B.1A.5a.1** and 19 **6B.1A.5a.2** viruses (Figure 1a). The **6B.1A.5a.1** viruses were largely detected in West Africa but with one each detected in France (in March), Qatar (in August) and USA (in August), while the 6B.1A.5a.2 viruses were largely detected in India with one each in Bangladesh (in August) and USA (in September). The recently emerged **6B.1A.5a.2** viruses were mostly detected in India and carried additional **HA1** amino acid substitutions of **K54Q**, **K130N**, **A186T**, **Q189E** and **E224A**, often with **R259K** and **K308R**. A zoonotic A(H1N1)v H1N1pdm09-like virus, A/North Dakota/12226/2021, belonging to the swine 1A.3.3.2 clade was detected in September (Figure 1a). The second phylogeny is based on sequences submitted/released in November (n = 39), only 23 of which had collection dates after 31 August (Figure 1b).

Three of the latter viruses, one each from England, Spain and the US, fell in subgroup **6B.1A.5a.1**, with the remainder falling in subgroup **6B.1A.5a.2**, 19 from Bangladesh and one from the US. The 20 **6B.1A.5a.2** viruses were like those detected in India, carrying additional **HA1** amino acid substitutions of **K54Q**, **K130N**, **A186T**, **Q189E**, **E224A**, **R259K** and **K308R**.

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.1A.5a.2**, were antigenically similar to A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like viruses (**6B.1A.5a.1** 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 1 December 2021].

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

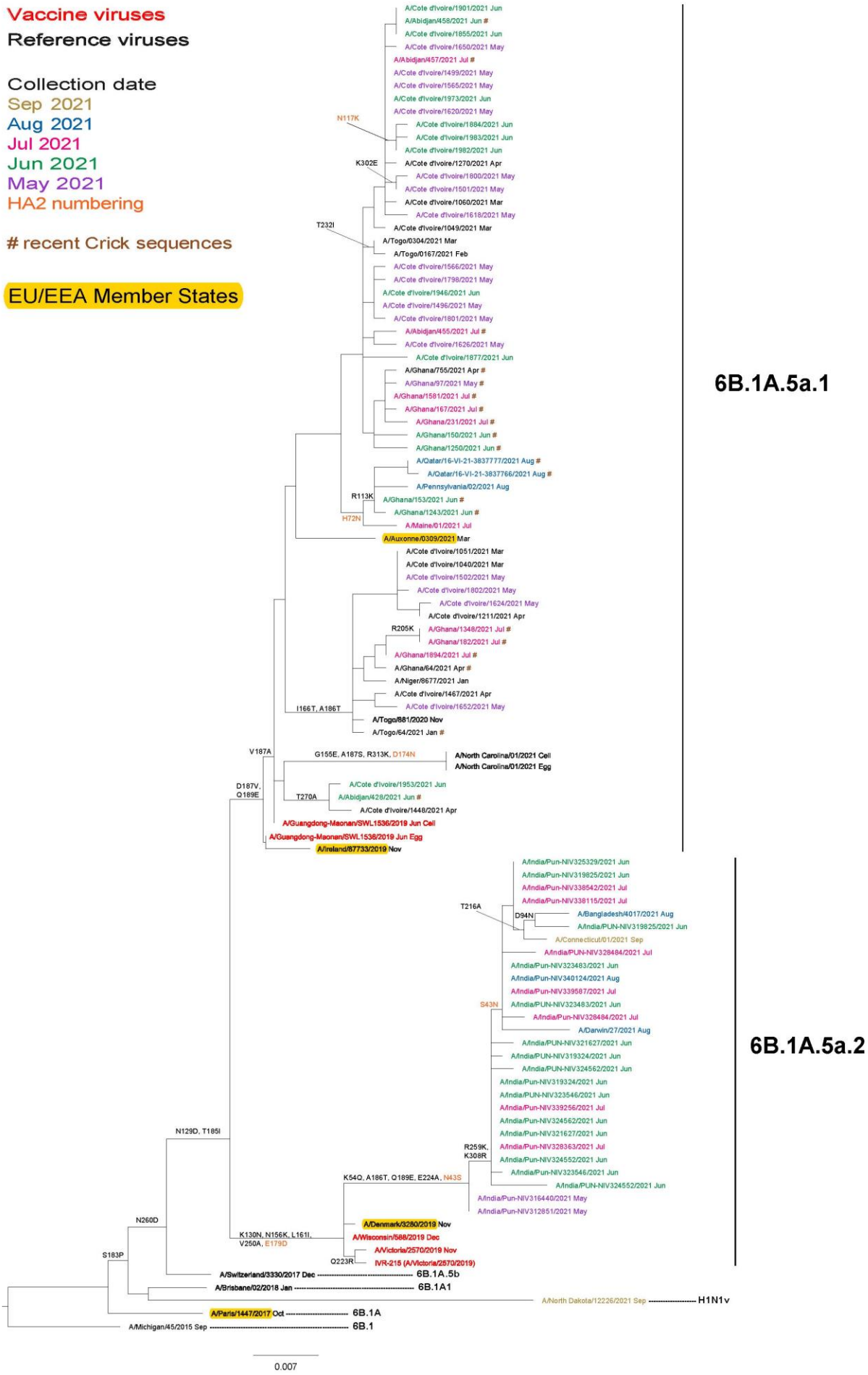
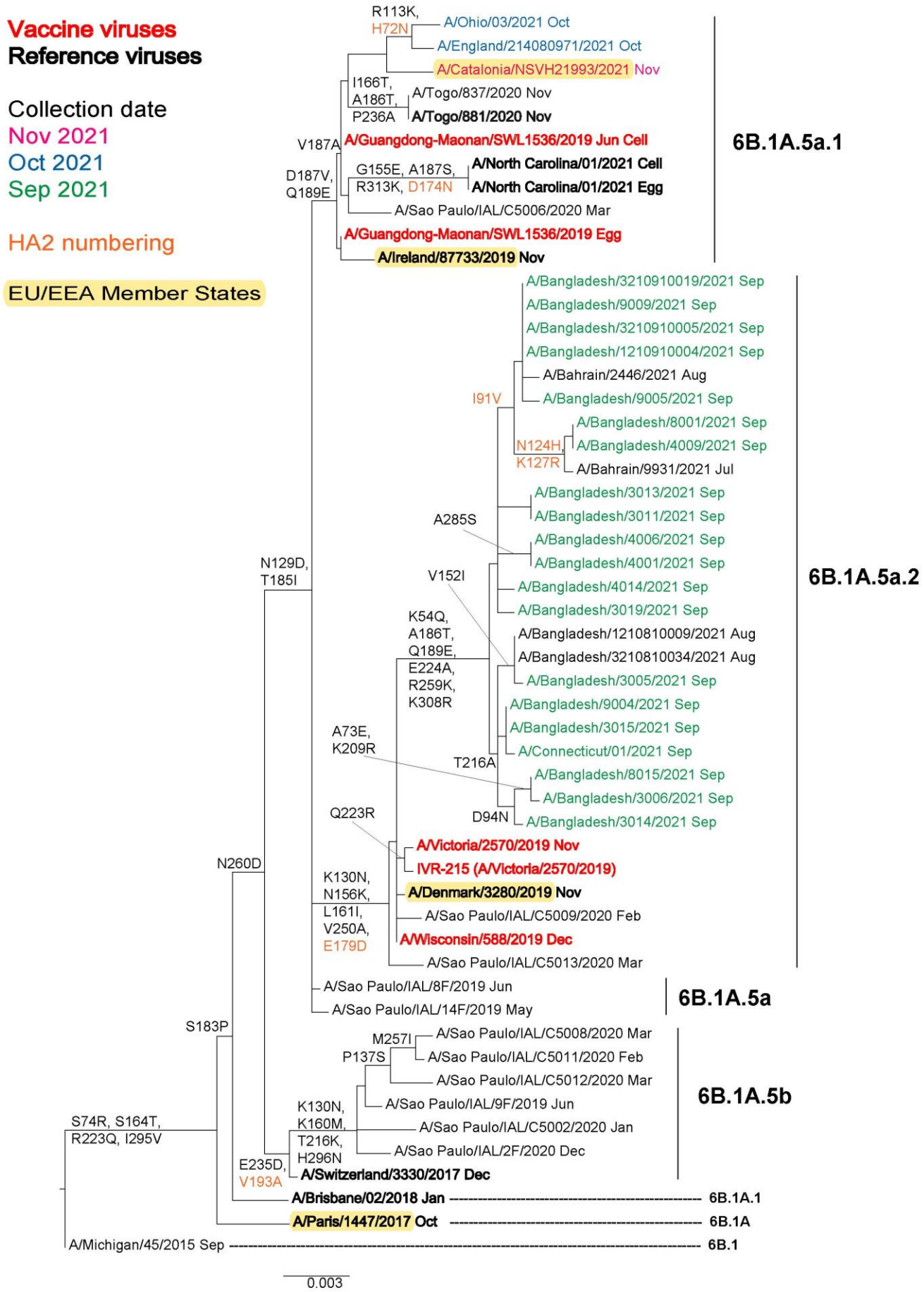


Figure 1b. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes (GISAID, November 2021)



Influenza A(H3N2) virus analyses

The first A(H3N2) HA phylogeny is repeated from the October 2021 report and was based on H3 HA sequences deposited/released in GISAID in October (n = 453), including only those with collection dates in October included together with sequences that had been generated recently at the WIC (Figure 2a).The second phylogeny is based on H3 HA sequences deposited/released in GISAID in November (n = 511), including only those with collection dates from 21 October included (n = 147) together with sequences generated recently 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 but for the European Region, where there was equivalence of clade **3C.3a** viruses. The HA gene sequences of viruses in both clades **3C.2a** and **3C.3a** continue to diverge. Notably, clade **3C.3a**1 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 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.1** subgroup (e.g. **A/La Rioja/2202/2018**) or **HA1 T131K** and **HA2 V200I**, the **3C.2a1b.2** subgroup (e.g. **A/South Australia/34/2019**). Distinct clusters of viruses within both these subgroups have emerged defined by specific **HA1** and/or **HA2** amino acid substitutions: **3C.2a1b.1a** with additional amino acid substitutions of **HA1 A138S**, **F193S** and **S198P**, many also with **G186D** and **D190N** (e.g. **A/Denmark/3284/2019**); **3C.2a1b.1b** with additional amino acid substitutions of **HA1 S137F, A138S** and **F193S** (e.g. **A/Hong Kong/2671/2019**); **3C.2a1b.2a** with additional amino acid substitutions of **HA1 K83E** and **Y94N** with **HA2 I193M** (e.g. **A/Slovenia/1637/2020**); **3C.2a1b.2b** with **HA2 V18M** substitution, often with additional **HA1** substitutions (e.g. **A/Bretagne/1323/2020**).
- Clade **3C.3a**: represented by a former vaccine virus, **A/Switzerland/9715293/2013**, with recently circulating clade **3C.3a1** 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.1b** cluster influenced the selection of an A/Hong Kong/2671/2019-like or an A/Hong Kong/45/2019-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 October report showed that all viruses detected in October were 'Bangladesh-like' (**3C.2a1b.2a.2 with HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N** and **Y195F**) (Figure 2a). Within this group, subgroups showing limited geographic spread had emerged defined by specific **HA1** amino acid substitutions. For example, s subgroup defined by **S205F** and **A212T** substitutions detected in Qatar in August and recently detected in the Russian Federation and Spain, and a more geographically dispersed subgroup defined by **H156S** substitution. The latter subgroup showed more diversification with viruses carrying **D53N**, **N96S** and **I192F** substitutions detected in Qatar, Spain and the Netherlands; viruses with **D53G**, **R201K** and **S219Y** substitutions detected in the Russian Federation; viruses with **D53G**, **D104G** and **K276R** substitutions detected in Lebanon with a branch of this subgroup carrying additional **L157I** and **S262N** substitutions as identified in an outbreak in Maryland USA.

The second phylogeny incorporated 147 sequences submitted/released in GISAID in November with collection dates from 21 October 2021, together with sequences recently determined at the WIC (Figure 2b). All viruses with collection dates after 31 August 2021 (n = 176) were 'Bangladesh-like' (**3C.2a1b.2a.2 with HA1** substitutions of **Y159N**, **T160I** (loss of a glycosylation site), **L164Q**, **G186D**, **D190N** and **Y195F**). The latter viruses were split into three subgroups defined by specific **HA1** amino acid substitutions: (i) **S205F** and **A212T** (n = 5) with viruses from the Netherlands, Spain and the Russian Federation; (ii) **D53G** (n = 58), often with additional amino acid substitutions, in viruses from Lebanon, Spain, the Russian Federation and the US; and (iii) **D53N**, **N96S** and **I192F** (n = 113) with viruses from Croatia, Italy, the Netherlands (n = 86), Spain (n = 23) and the US.

'Bangladesh-like' **3C.2a1b.2a.2** viruses, A/Darwin/9/2021 and A/Darwin/6/2021 for egg- and cell-based vaccines respectively, were recently recommended for use in the southern hemisphere 2022 influenza season [4].

The locations of HA sequences for egg- and cell culture-propagated cultivars of A/Cambodia/e0826360/2020 (**3C.2a1b.2a.1**) recommended for use in northern hemisphere 2021-2022 vaccines [3], are indicated on the phylogenies, as are egg- and cell-culture based vaccines to be used in the 2022 southern hemisphere season, A/Darwin/9/2021 and A/Darwin/6/2021 (**3C.2a1b.2a.2**) respectively [4] (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. This issue is now much alleviated for 'Bangladesh-like' **3C.2a1b.2a.2** viruses which agglutinate guinea pig RBCs well, allowing HI assays to be performed.

While the number of detections of seasonal influenza viruses was low from April 2020 to July 2021, compared to previous years, the WHO Collaborating Centres for Influenza have shown viruses in these emerged virus clusters to be antigenically distinguishable from one another and other A(H3N2) virus subgroups.

Antigenic characterisation of four A(H3N2) viruses from Croatia, all of which are **3C.2a1b.2a.2** viruses with **HA1 H156S** substitution, is presented here (Table 3). All test viruses were inhibited well, by antisera raised against **3C.2a1b.2a.2** viruses, notably so for antisera raised against cell culture-propagated A/Stockholm/5/2021 and slightly less well by antisera raised against cell culture-propagated A/Bangladesh/4005/2020 and egg-propagated A/Darwin/9/2021. Antisera raised against single 3C.2a1b.1a, 3C.2a1b.1b and 3C.3a1 viruses, and two 3C.2a1b.2a.1 did not recognise any of the test viruses.

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, October 2021)

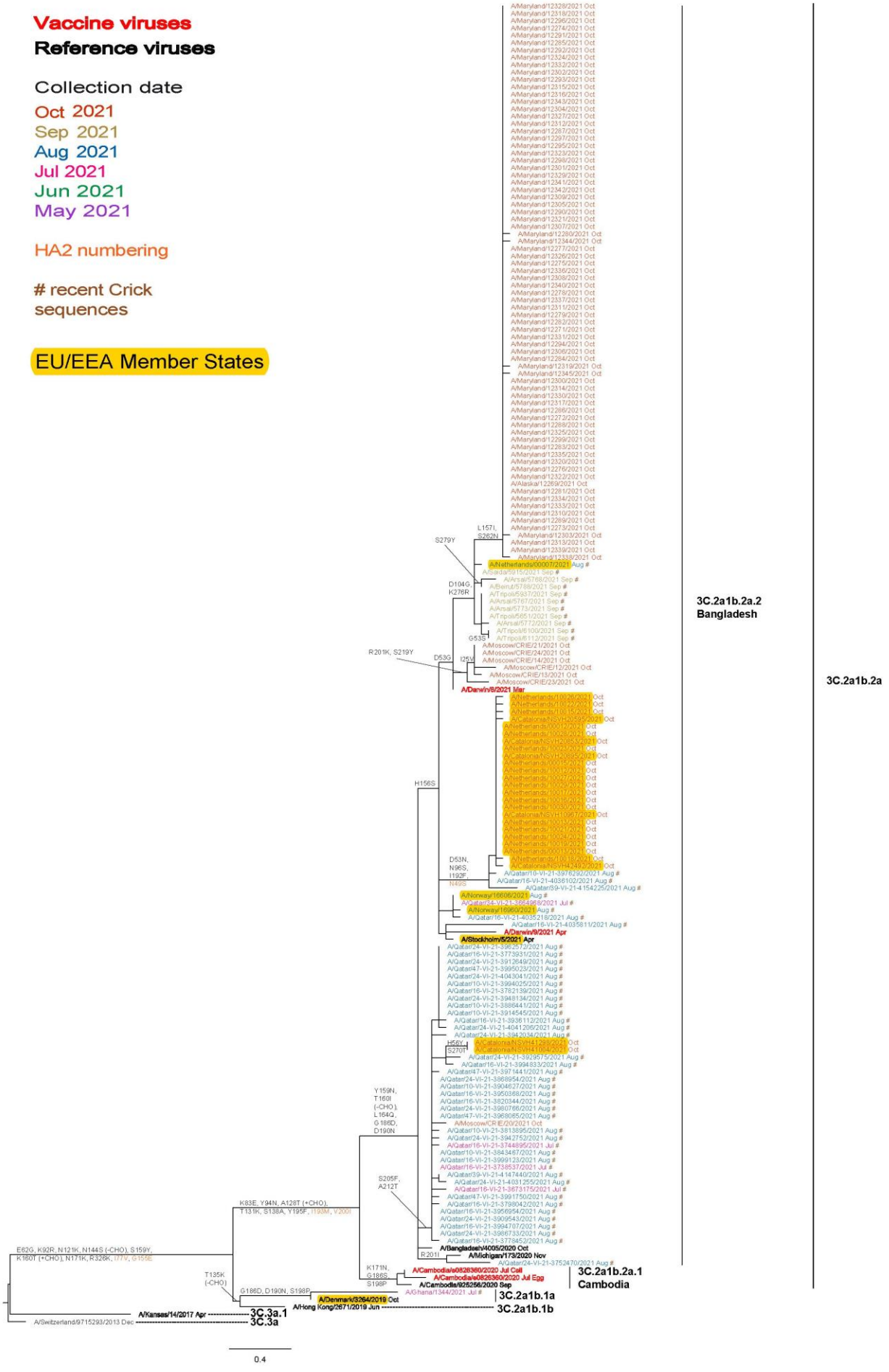


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, November 2021)

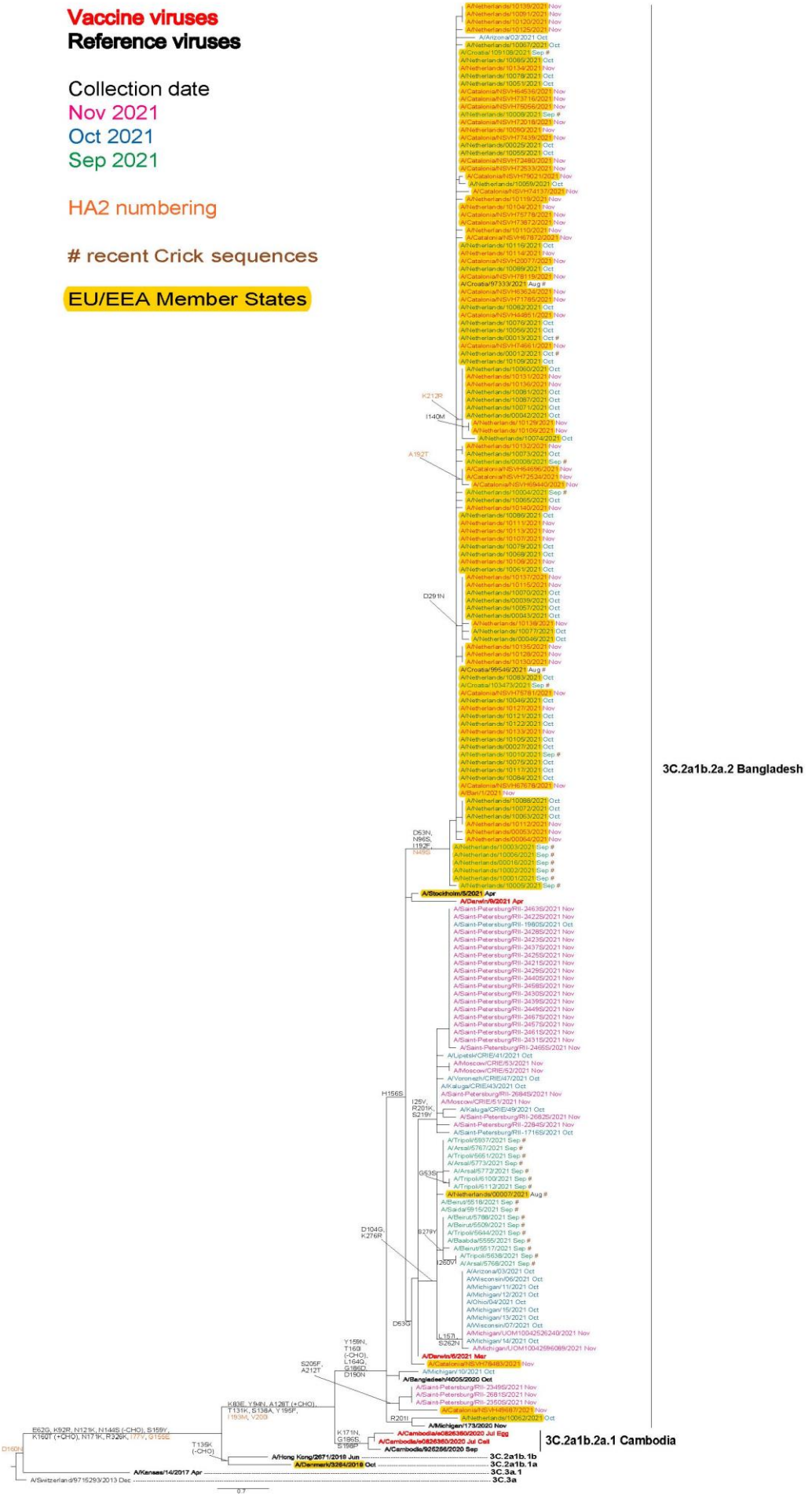


Table 3. Antigenic analysis of A(H3N2) viruses by HI

Viruses	Other information	Collection date	Passage history	Haemagglutination inhibition titre									
				Post-infection ferret antisera									
						NEW						NEW	
	Passage history			A/Denmark 3264/19 SIAT	A/HK 2671/19 Cell	A/Camb e0826360/20 Egg	A/Camb 925256/20 SIAT	A/Camb 925256/20 SIAT	A/Bang 4005/20 SIAT	A/Darwin 9/21 Egg	A/Stock 5/21 SIAT	A/Stock 5/21 SIAT	A/Kansas 14/17 SIAT
	Ferret number			F19/20[*1]	St Judes F21/20[*1]	F10/21[*1]	F03/21[*1]	F43/21[*1]	F07/21[*1]	F38/21[*1]	F35/21[*1]	F42/21[*1]	F17/19[*1]
	Genetic group			3C.2a1b.1a	3C.2a1b.1b	3C.2a1b.2a.1	3C.2a1b.2a.1	3C.2a1b.2a.1	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.2a1b.2a.2	3C.3a1
REFERENCE VIRUSES													
A/Denmark/3264/2019	3C.2a1b.1a	2019-10-25	SIAT5	320	320	160	640	320	320	320	160	160	80
A/Hong Kong/2671/2019	3C.2a1b.1b	2019-06-17	MDCK1/SIAT4	320	320	160	640	160	320	320	160	80	80
A/Cambodia/e0826360/2020	3C.2a1b.2a.1	2020-07-16	E5/E2	160	<	1280	160	40	320	320	160	40	80
A/Cambodia/925256/2020	3C.2a1b.2a.1	2020-09-25	SIAT4	160	160	160	640	320	320	320	160	160	80
A/Bangladesh/4005/2020	3C.2a1b.2a.2	2020-10-04	SIAT3	160	80	320	160	80	640	640	640	160	160
A/Darwin/9/2021	3C.2a1b.2a.2	2021-04-17	E3/E2	160	<	640	160	80	640	2560	640	320	40
A/Stockholm/5/2021	3C.2a1b.2a.2	2021-04-16	SIAT0/SIAT3	80	<	80	80	<	320	1280	640	320	40
A/Kansas/14/2017	3C.3a.1	2017-12-14	SIAT3/SIAT2	40	<	80	40	<	40	80	80	<	320
TEST VIRUSES													
A/Croatia/97333/2021	3C.2a1b.2a.2	2021-08-17	MDCKx/SIAT1	<	<	80	80	<	160	640	320	160	<
A/Croatia/99546/2021	3C.2a1b.2a.2	2021-08-25	MDCKx/SIAT1	<	<	80	40	<	160	640	320	160	<
A/Croatia/103473/2021	3C.2a1b.2a.2	2021-09-07	MDCKx/SIAT1	<	<	80	40	<	160	640	320	160	<
A/Croatia/109108/2021	3C.2a1b.2a.2	2021-09-20	MDCKx/SIAT2	<	<	80	40	<	160	320	320	160	<
						Vaccine NH 2021-22				Vaccine SH 2022			

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

Influenza B virus analyses

Influenza B/Victoria-lineage

All recently circulating B/Victoria-lineage viruses have fallen in genetic clade **V1A**, 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 **V1A.1**) 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 **V1A.2**) 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 **V1A.3**) 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 October report included 58 new full-length HA gene sequences from viruses with collection dates in 2021 available in GISAID, or generated by the WIC, in October (Figure 3a). One of these viruses, B/La Reunion/438 2021 collected in July, was B/Washington/02/2019-like (**V1A.3**). All other viruses fell in subgroup **V1A.3a.2** (with **HA1 A127T**, **P144L** and **K203R** substitutions).

The second phylogeny incorporated 52 sequences submitted/released in GISAID in November with collection dates from 1 July 2021 (Figure 3b). Of these, just 20 had collection dates after 31 August 2021, seven viruses from the US fell in subclade **V1A.3** being **B/Washington/02/2019**-like, and 13 (three from England, one from the Netherlands and nine from India) fell in subgroup **V1A.3a.2** being **B/Austria/1359417/2021**-like.

The WHO Collaborating Centres for Influenza have shown the **V.1A.3a** 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 in the subgroup. **B/Austria/1359417/2021**-like (**V.1A.3a.2**) viruses were recently recommended for southern hemisphere 2022 vaccines [4].

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 in GISAID as of 30 November 2021. Figure 4 is repeated from the September report with recently designated nomenclature indicted in bold/red type 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** (**Y3**), 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 and 2021-2022 northern hemisphere, 2021 and 2022 southern hemisphere seasons [1, 2, 3, 4]. 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, October 2021)

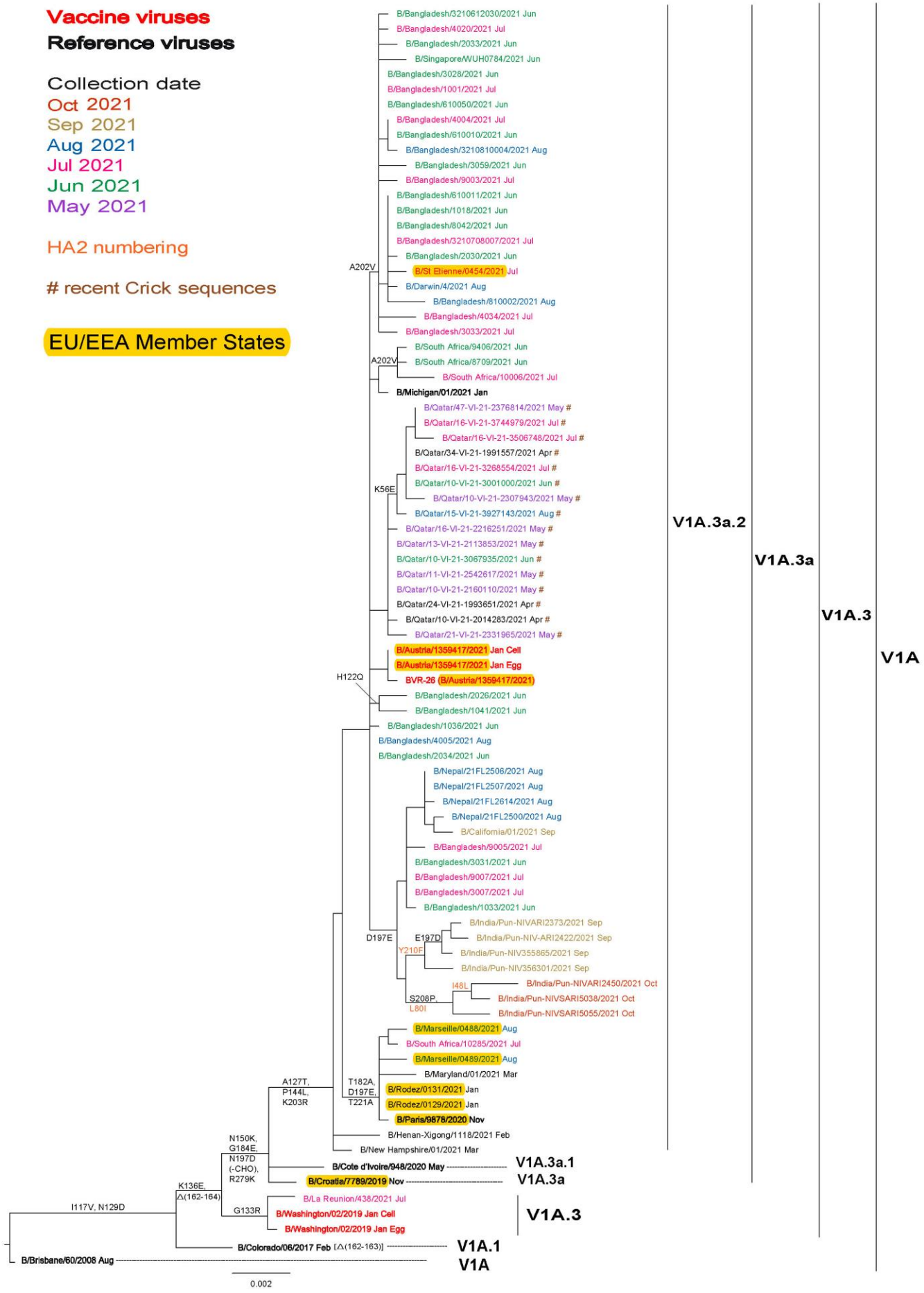


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, November 2021)

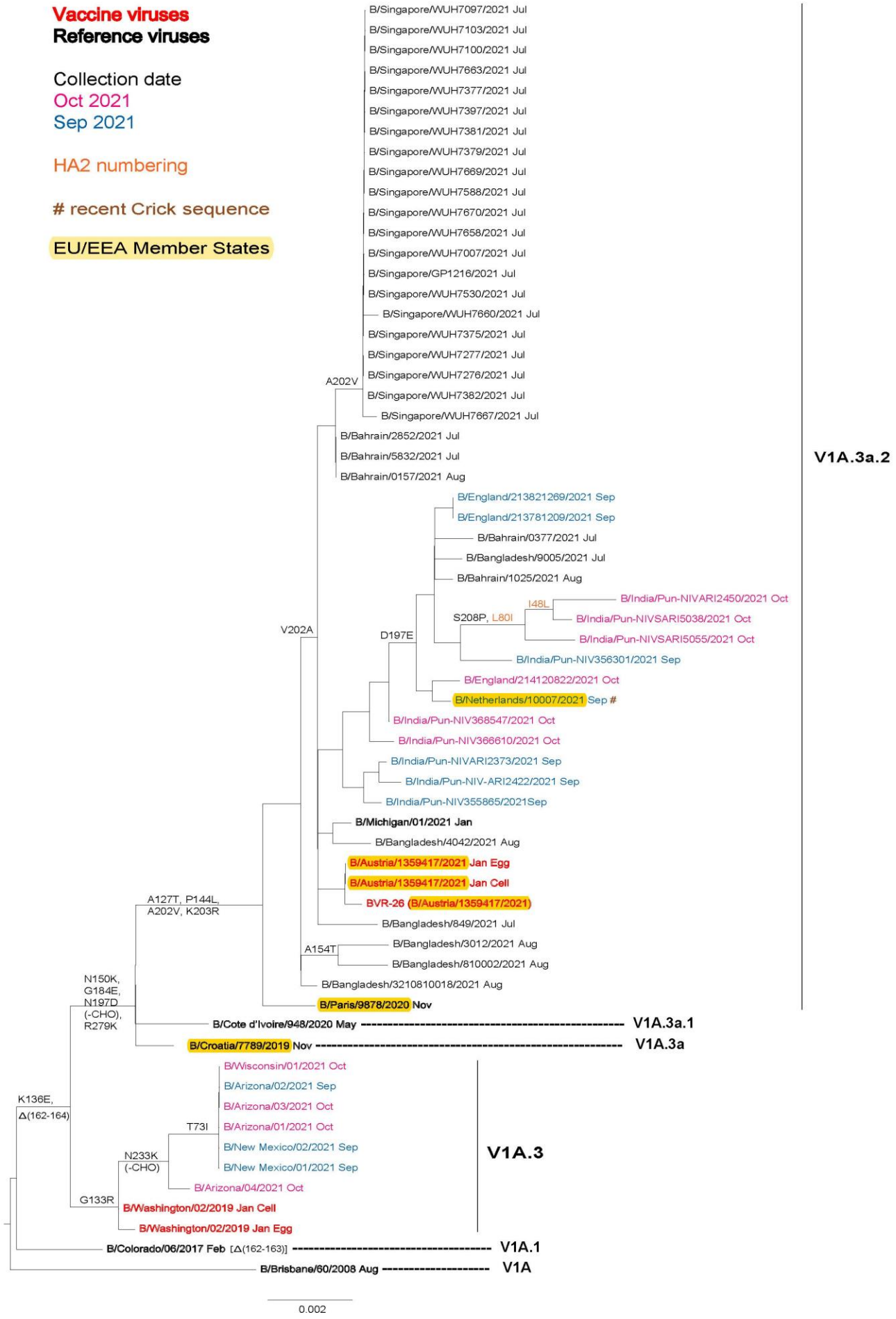
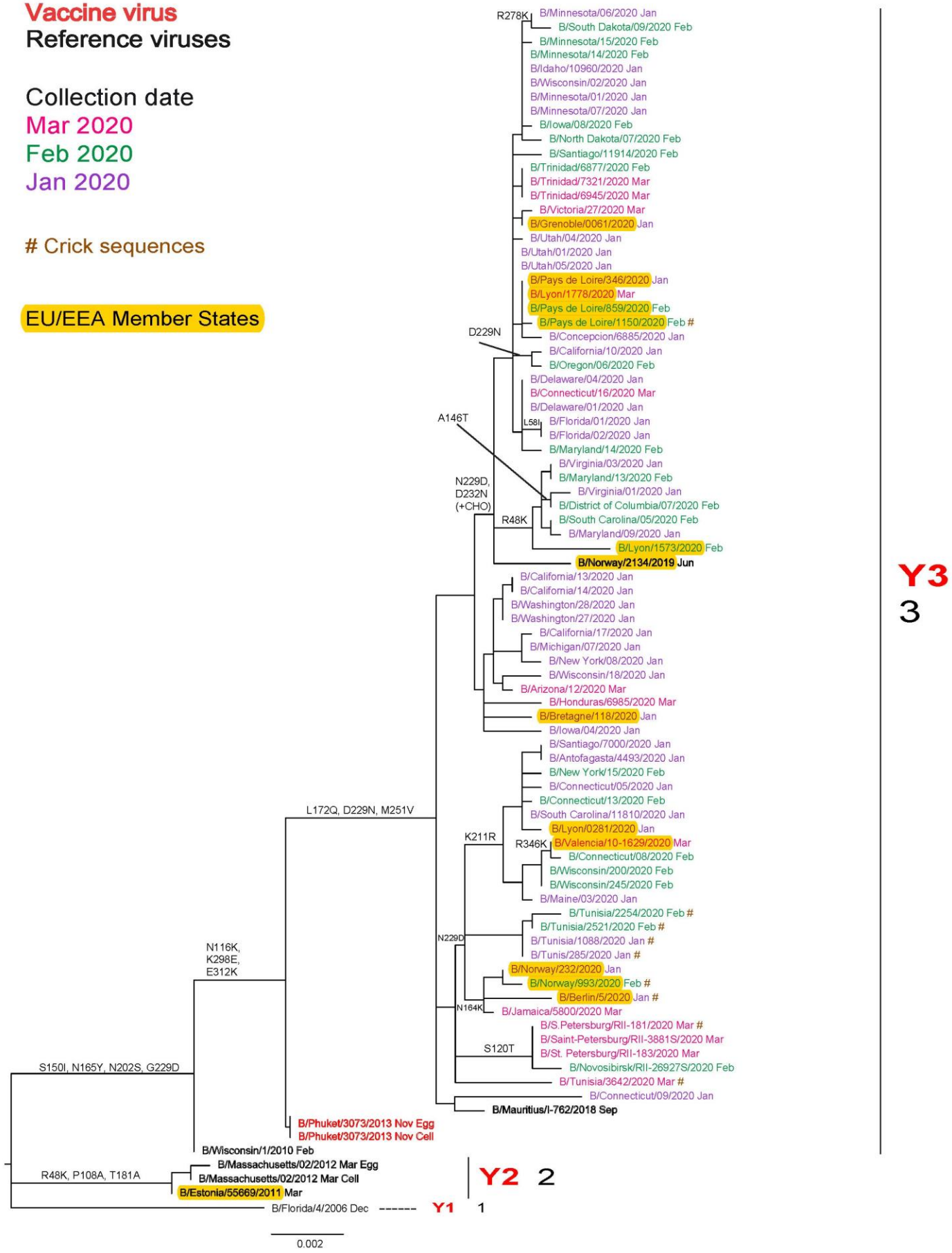


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, September 2021)



Summaries of data submitted to the European Surveillance System

Genetic characterisation

165 viruses detected over the course of the 2021-2022 season (weeks 40-47/2021) were genetically characterised:

- Two A(H1N1)pdm09 viruses, one of which was not ascribed to a genetic group, while the other belonged to clade 6B.1A.5a.1 (represented by A/Guangdong-Maonan/SWL1536/2019).
- 161 A(H3N2) viruses, all of which belonged to the 'Bangladesh-like' clade (3C.2a1b.2a.2) represented by A/Bangladesh/4005/2020.
- Two B/Victoria-lineage viruses, one each belonging to clades V1A.3 (represented by B/Washington/02/2019) and V1A.3a.2 (represented by B/Austria/1359417/2021).

Antiviral susceptibility

Up to week 47/2021, 122 and 69 A(H3) viruses, respectively, were assessed for susceptibility to neuraminidase inhibitors and the PA inhibitor baloxavir marboxil. No amino acid substitutions previously associated with reduced susceptibility to any of the drugs were identified.

At the WIC, fifteen A(H3N2) influenza viruses detected within EU/EEA countries during the 2021-2022 season have been assessed phenotypically against oseltamivir and zanamivir. All showed NI by both NAIs.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response System [5] 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 [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 can be found on WHO's website https://www.who.int/teams/global-influenza-programme/avian-influenza/monthly-risk-assessment-summary (accessed 1 December 2021). The assessment published on 1 October 2021 indicated that there had 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]. 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 1 December 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 29 September 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 1 October 2021. Since the previous risk assessment on 8 August 2021, 11 human cases of infection with avian influenza A(H5N6) viruses were reported by China with disease onset dates in July through September [9]. All cases reported exposure to poultry and, at the time of report publication, two cases were fatal, seven were severe/critical, one was mild and information was not available for the 11th case. The last human case of known A(H5N1) infection was reported by India [13].

On 30 September 2020, ECDC published an alert related to outbreaks of avian influenza viruses in Europe [14]. The latest collaborative report from ECDC and the European Food Safety Authority (EFSA), reported 162 highly pathogenic avian influenza (HPAI) A(H5) detections between 25 May and 15 September 2021, 51 in poultry, 91 in wild birds and 20 in domestic birds [12]. Detections occurred in 17 EU/EEA countries and the UK. Of the poultry detections, 20 were reported by Kosovo, 17 by Poland and six by Albania, and the wild bird detections were reported in resident populations mainly in northern Europe. Nineteen different virus genotypes have been detected in Europe and Central Asia since July 2020, confirming a high propensity for A(H5) viruses to undergo reassortment events, with most recent detections being subtype A(H5N8). According to reports compiled by the Food and Agricultural Organization of the United Nations (FAO) as of 24 November 2021, various influenza A(H5Nx) subtypes continued to be detected in wild and/or domestic birds in Africa, Asia and Europe, and since 27 October 2021 a total of 272 HPAI (14 H5Nx, 254 H5N1, one H5N5 and three H5N8) and 14 LPAI outbreaks had been reported, with one A(H5N6) human infection in China (symptom onset 29 August 2021) [15].

Influenza A(H9N2) virus

Since the previous WHO update on 8 August 2021, three laboratory-confirmed human cases of influenza A(H9N2) virus infection in children were reported by China with onset dates in June, August and September [9]. For two cases, poultry exposure was reported and disease symptoms were mild, this information was not available for the third case. The most recent FAO report mentions a further case in China with disease onset date of 29 October 2021 [15]. Public Health England recently published an updated risk assessment of avian influenza A(H9N2) [16]. 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 WHO update on 8 August 2021, five A(H1N1)v zoonotic events with swine-related variant influenza A viruses were reported by China with disease onset dates in late 2020/early 2021 [9]. The swine exposure history of these five cases is unknown and while four of the cases had mild disease, the fifth developed pneumonia. In addition, two adult cases were detected in Wisconsin USA, both reported exposure to swine, one was hospitalised but both recovered.

Four zoonotic cases of A(H1N2)v infection were also reported, one each by Austria and France, and two by the US (one each in Iowa and Ohio). The European cases were in adults and the US cases in children, with all reporting exposure to swine. All cases recovered and there was no evidence of human-to-human transmission.

One case of A(H3N2)v infection was reported from Iowa, USA. The infected child was not hospitalised, made a full recovery and no human-to- human transmission was identified.

WHO Collaborating Centre reports

A description of results generated by the London WHO Collaborating Centre at the WIC and used at the September 2021 WHO vaccine composition meeting (held online: 13-23 September 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 1 December 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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