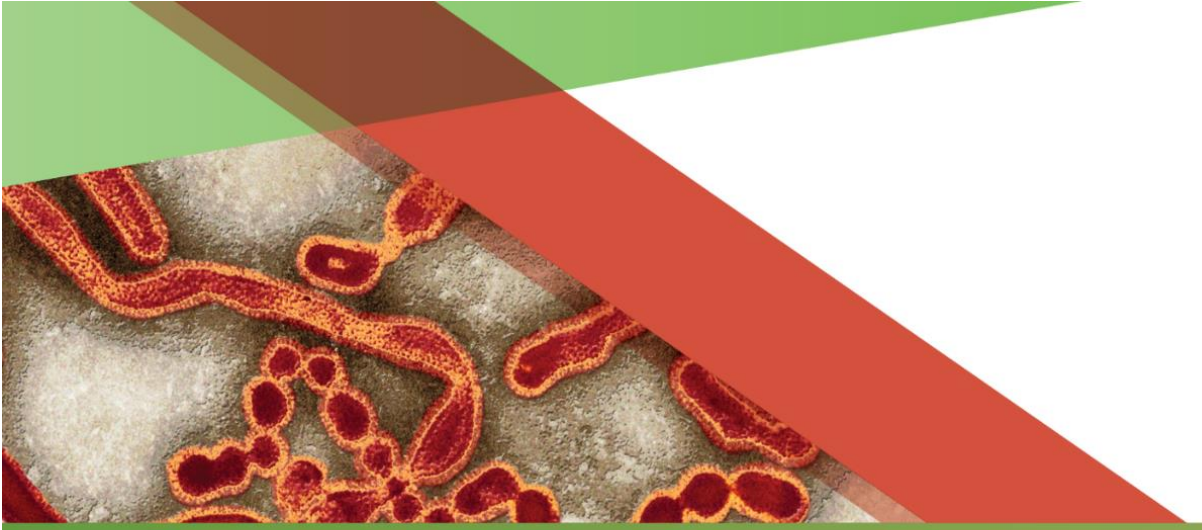

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, May 2020

Summary

This is the seventh report for the 2019–20 influenza season. As of week 20/2020, 164 868 influenza detections across the WHO European Region have been reported; 73% type A viruses, with A(H1N1)pdm09 prevailing over A(H3N2), and 27% type B viruses, with 4 479 (98%) of 4 568 ascribed to a lineage being B/Victoria.

Since the April 2020 characterisation report[1], eight shipments of influenza-positive specimens from EU/EEA countries have been received at the London WHO CC, the Francis Crick Worldwide Influenza Centre (WIC). In total, 1 362 virus specimens, with collection dates after 31 August 2019, have been received. No influenza antigenic characterisation has been conducted during this reporting period.

Previous analyses have shown the great majority of test viruses to be well recognised by antisera raised against the 2019–20 vaccine virus, A/Brisbane/02/2018. Those viruses showing poor reactivity generally carried amino acid substitutions (notably N156K) in the HA1 150-loop region. The 296 EU/EEA test viruses with collection dates from week 40/2019 genetically characterised at the WIC have fallen within subclades of clade 6B.1A: 263 6B.1A5A, 23 6B.1A5B, 1 6B.1A6 and 9 6B.1A7.

Previous analyses have shown clade 3C.3a-specific recognition by antisera raised against egg-propagated A/Kansas/14/2017, the current vaccine virus. Globally, approximately equal proportions of clade 3C.3a and subgroups 3C.2a1b+T131K and 3C.2a1b+T135K viruses have been detected, but for viruses detected since 1 February 2020, subgroups 3c.2a1b+T135KA/B have prevaled in the USA while those of clade 3C.3a and subgroup 3C.2a1b+T131K have dominated in Europe. In total, 355 viruses from EU/EEA countries have been characterised genetically at the WIC: 185 clade 3C.3a, 112 3C.2a1b+T131K, 43 3C.2a1b+T135K-A and 15 3C.2a1b+T135K-B.

Viruses detected in EU/EEA countries during February and March 2020, based on sequences available in GISAID, have all fallen in the 1A(Δ3)B subgroup. Viruses in this subgroup have been antigenically similar to B/Washington/02/2019, the vaccine virus for the 2020–2021 northern hemisphere influenza season. In total, 221 EU/EEA viruses have been characterised genetically at the WIC: 208 subgroup 1A(Δ3)B and 13 subclade 1A(Δ2).

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

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All eight EU/EEA viruses characterised genetically at the WIC since week 40/2019, as for all recently circulating B/Yamagata-lineage viruses, belong to genetic clade 3 and contain at least two HA amino acid substitutions (HA1 L172Q and M251V) compared to B/Phuket/3073/2013, the antigenic effects of which have been minimal as assessed in earlier reports.

Table 1 shows a summary of influenza virus detections in the WHO European Region reported to ECDC's TESSy database since the start of the 2019–20 season (weeks 40/2019–20/2020), with a total of 164 868 detections over this period. Since week 18/2020, reported in the April 2020 characterisation report, the proportion of type A viruses has continued to decrease (from 73.2% to 72.9%), with a concomitant rise in the proportion of type B viruses (from 26.8% to 27.1%). Of the type B viruses ascribed to a lineage (n = 4 568), B/Victoria-lineage viruses (n = 4 479) have continued to predominate over B/Yamagata-lineage viruses (n = 89) by a large margin, while for type A viruses subtyped (n = 47 193) there has been a continued increase in the proportion of A(H1N1)pdm09 viruses (55.9% to 56.0%) and a reduction in the proportion of A(H3N2) viruses (44.1% to 44.0%). Overall, there have been 41 079 (19.9%) less influenza detections reported than in 2018–19, but this is probably due to the increasing number of countries that either stopped influenza surveillance or stopped reporting (or reported sporadically) to TESSy from week 5/2020 due to responses to COVID-19 which WHO declared a pandemic on 11 March 2020 (week 11/2020). With this caveat, the ratio of type A to type B detections is dramatically reduced compared with the 2018–19 season (86:1 to 2.7:1), and while proportions of influenza A subtypes are similar, B/Victoria-lineage viruses have predominated among the type B viruses compared to near equivalence with B/Yamagata-lineage viruses in the 2018–19 season.

Table 1. Influenza virus detections in the WHO European Region from the start of reporting for the 2019–20 season (weeks 40/2019–20/2020)[a]

	Cumulative number of detections			Totals*		Totals for 2018-19 season*		
Virus type/subtype/lineage	Sentinel sources	Non-sentinel sources	Totals	%	Ratios	Number	%	Ratios
Influenza A	**11302**	**108940**	**120242**	**72.9**	**2.7:1**	**203564**	**98.8**	**86:1**
A(H1N1)pdm09	6126	20302	26428	56.0		44179	57.2	
A(H3N2)	4174	16591	20765	44.0	0.79:1	33117	42.8	0.7:1
A not subtyped	1002	72047	73049			126271		
Influenza B	**6324**	**38302**	**44626**	**27.1**		**2380**	**1.2**	
Victoria lineage	2449	2030	4479	98.1		79	47.9	
Yamagata lineage	23	66	89	1.9	0.02:1	86	52.1	1.1:1
Lineage not ascribed	3852	36206	40058			2215		
Total detections (total tested)	**17626 (51764)**	**147242 (>858633)**	**164868 (>910397)**			**205947 (>849439)**		

[a] **Numbers taken from Flu News Europe week 20/2020**

*** 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 Yamagata:Victoria lineages.**

Since week 40/2019, 52 shipments of specimens (virus isolates and/or clinical specimens) have been received at the Crick Worldwide Influenza Centre (WIC), from 27 EU/EEA countries, eight of which were received in May 2020 (from Belgium, France, Iceland, Poland, Portugal, Slovakia, Sweden and the UK). The packages contained 1 362 virus-related samples with collection dates after 31 August 2019 and were made up of 997 type A viruses, with 468 and 520 subtyped as A(H1N1)pdm09 and A(H3N2), respectively, and 365 type B viruses, with 294 and 16 ascribed to B/Victoria and B/Yamagata lineages, respectively (Table 2). Genetic and antigenic characterisation data generated at the WIC for viruses with collection dates after 31 August 2019 until 31 January 2020, up to 21 February 2020, formed the basis of the February 2020 virus characterisation report and were presented at the WHO influenza vaccine composition meeting in February 2020 when recommendations were made for the northern hemisphere 2020–21 season. Recommendations for the current 2019–20 northern hemisphere and the subsequent 2020 southern hemisphere and 2020–21 northern hemisphere seasons have been published [1, 2, 3].

We (WIC) thank those eight WHO-recognised national influenza centres (NICs) that have responded to our e-mail message, sent after the Easter break and repeated in the April characterisation report, requesting sharing of influenza-positive samples with recent collection dates. We encourage other NICs and laboratories that share influenza-positive samples with the WIC to do so in coming months to allow virus characterisation in time for the September 2020 WHO vaccine consultation meeting (VCM). Please note that to inform the September 2020 southern hemisphere influenza VCM, we focus on samples with collection dates from 1 February 2020 on, and more than one shipment from a country is encouraged to ensure that we capture any 'end-of-season' samples from countries in the northern hemisphere.

During the lockdown imposed by the UK government, due to the COVID-19 pandemic, WIC has been operating with reduced staff numbers. Consequently, only gene sequencing has been performed to assess the emergence of any new genetic groups. Therefore, this report contains no antigenic data and is based on phylogenetic analyses of complete HA gene sequences submitted to the GISAID EpiFlu database during the month of May, together with sequences generated at the WIC, with those from EU/EEA countries highlighted. With lockdown restrictions now being relaxed in the UK, virus propagation for antigenic characterisation and antiviral susceptibility studies are being resumed in June 2020.

Table 2. Summary of clinical samples and virus isolates*, with collection dates from 1 September 2019, contained in packages received from EU/EEA Member States since week 40/2019

MONTH / Country	TOTAL RECEIVED Seasonal viruses	A Number received	A Number propagated[1]	H1N1pdm09 Number received	H1N1pdm09 Number propagated[1]	H3N2 Number received	H3N2 Number propagated[2]		B Number received	B Number propagated[1]	B Victoria lineage Number received	B Victoria lineage Number propagated[1]	B Yamagata lineage Number received	B Yamagata lineage Number propagated[1]
SEPTEMBER														
Czech Republic	1					1	1							
Finland	1					1	1							
France	6			1	1	3	2	1			2	2		
Norway	7			1	1	4	1	3			2	2		
Romania	1					1	0	1						
Sweden	3			2	2	1	1							
United Kingdom	4			2	2	2	0							
OCTOBER														
Denmark	3			2	2	1	1							
Finland	2			1	1	1	1							
France	5			3	3						2	2		
Germany	6			2	2	4	2	1						
Greece	1					1	1							
Iceland	9					8	4	4			1	1		
Ireland	11			1	1	9	1	1					1	0
Latvia	3			1	1						2	2		
Lithuania	1										1	1		
Netherlands	3			1	1	2	0	2						
Norway	28			5	4	19	3	15			3	2	1	0
Poland	1	1	0											
Portugal	7			2	2	2	1		3	0				
Spain	5			3	3						2	2		
Sweden	3					2	2				1	1		
United Kingdom	29			5	2	21	11	6			3	0		
NOVEMBER														
Austria	4			2	2	1	0	1					1	1
Belgium	3			2	1	1	1							
Croatia	3			2	2						1	1		
Czech Republic	2					2	2							
Denmark	16			7	7	6	3	3			3	3		
Finland	1			1	1									
France	16			8	8	4	3	1			2	2	2	2
Germany	8			5	5	3	0	3						
Greece	1					1	0							
Iceland	3					2	0	2			1	1		
Ireland	49			18	12	22	7	5	2	0	7	6		
Italy	7			2	2	3	1	2			2	2		
Latvia	10			2	2	3	3				5	5		
Lithuania	2			2	2									
Netherlands	3			2	2	1	1							
Norway	22			6	5	9	3	4			4	4	3	1
Poland	1	1	0											
Portugal	102	1	0	13	11	3	0		26	0	59	20		
Slovenia	1			1	1									
Spain	6			2	2	2	2		1	0	1	1		
Sweden	8			5	5	1	0	1			2	2		
United Kingdom	62			9	4	52	14	2			1	0		
DECEMBER														
Austria	18			5	5	9	7	2			4	4		
Belgium	21			5	3	11	in process				5	in process		
Bulgaria	2			1	0	1	1							
Croatia	6			4	1	1	0	1			1	0		
Cyprus	2					1	in process		1	in process				
Czech Republic	2					2	1	1						
Estonia	1			1	1									
France	36			14	14	7	3	4			15	14		
Germany	13			6	6	6	4	2			1	1		
Greece	6			4	0	2	0							
Iceland	5			2	2	2	0	1			1	1		
Italy	12			2	2	6	2	4			4	4		
Latvia	1					1	0	1						
Lithuania	20	1	0	6	6	12	9	3			1	1		
Netherlands	10			1	1	9	7	2						
Norway	15			8	5	1	in process				1	in process	5	2
Poland	5	2	0	1	0	2	1	0						
Portugal	20			2	2	3	2	1			15	15		
Romania	8										8	8		
Slovenia	9			5	5	3	3				1	1		
Spain	30			12	12	6	0	6			12	12		
United Kingdom	18			4	in process	11	in process				3	in process		
2020														
JANUARY														
Austria	2	1	0						1	0				
Belgium	52			29	in process	18	in process				5	in process		
Bulgaria	28			14	in process	12	in process				2	0		
Cyprus	26			4	in process	21	in process				1	in process		
Czech Republic	5			2	2	3	3							
Estonia	14			7	4	3	1	1			4	4		
France	1										1	1		
Germany	24			6	6	9	8	1			8	8	1	1
Greece	45			22	8	20	5	3	2	0	1	1		
Italy	3			1	1	1	1				1	1		
Lithuania	2					2	1	1						
Norway	17			1	0	11	in process				5	in process		
Poland	9	1	0	4	in process	2	in process		2	in process				
Portugal	7			1	in process	1	in process				5	in process		
Romania	15			4	3	7	in process		1	0	3	3		
Slovakia	1					1	in process							
Slovenia	7			4	in process	2	in process				1	1		
Spain	18			13	12				1	0	4	4		
United Kingdom	38			18	in process	11	in process		3	0	6	in process		
FEBRUARY														
Austria	2	1	0						1	0				
Belgium	50			28	in process	17	in process				5	in process		
Bulgaria	26			5	in process	12	in process				9	in process		
Cyprus	21			3	in process	13	in process				5	in process		
France	21			7	in process	5	in process				7	in process	2	in process
Germany	25			13	in process	7	in process				5	in process		
Iceland	10			4	in process	2	in process		4	in process				
Poland	22			10	in process	9	in process		3	in process				
Portugal	32			29	in process	2	in process				1	in process		
Slovakia	8			2	in process	4	in process				2	in process		
Slovenia	16			4	in process	9	in process				3	in process		
Sweden	18			8	in process	5	in process				5	in process		
United Kingdom	7			1	in process	2	in process				4	in process		
MARCH														
Belgium	6			4	in process						2	in process		
Bulgaria	9			1	in process	1	in process				7	in process		
France	9			2	in process	2	in process				5	in process		
Germany	5			2	in process	2	in process				1	in process		
Iceland	13			4	in process	6	in process		3	in process				
Poland	4			4	in process									
Portugal	4			3	in process	1	in process							
Slovenia	8					2	in process				6	in process		
United Kingdom	1										1	in process		
APRIL														
Iceland	1								1	in process				
	1362	9	0	468	203	520	132	92	55	0	294	146	16	7
27 Countries		0.66%		34.4%		38.2%			4.0%		21.6%		1.2%	
		73.2%							26.8%					

*** 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 clinical samples in lysis-mix from Northern Ireland and Scotland and RNA extracts from Greece and Portugal for which genetic characterisation only can be performed. In addition, some clinical samples from Bulgaria, Estonia, Greece, Ireland, Poland and Portugal were not cultured as either sequencing from the clinical sample failed or sequences generated were identical to those from other clinical samples.

As of 2020-06-01

Influenza A(H1N1)pdm09 virus analyses

The first A(H1N1)pdm09 HA phylogeny is repeated from the April 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 in April 2020 (Figure 1a). The second is again based on viruses with collection dates from 1 February 2020, but with sequences deposited in GISAID during May 2020 (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 Vaccine Consultation Meeting (6B.1A/183P-1 to -7, abbreviated to 6B.1A1 to 6B.1A7); the recommended vaccine viruses for the northern hemisphere 2019–2020 and 2020–2021 influenza seasons are shown in red [1, 3]. The seven subclades are defined by the following HA amino acid substitutions:

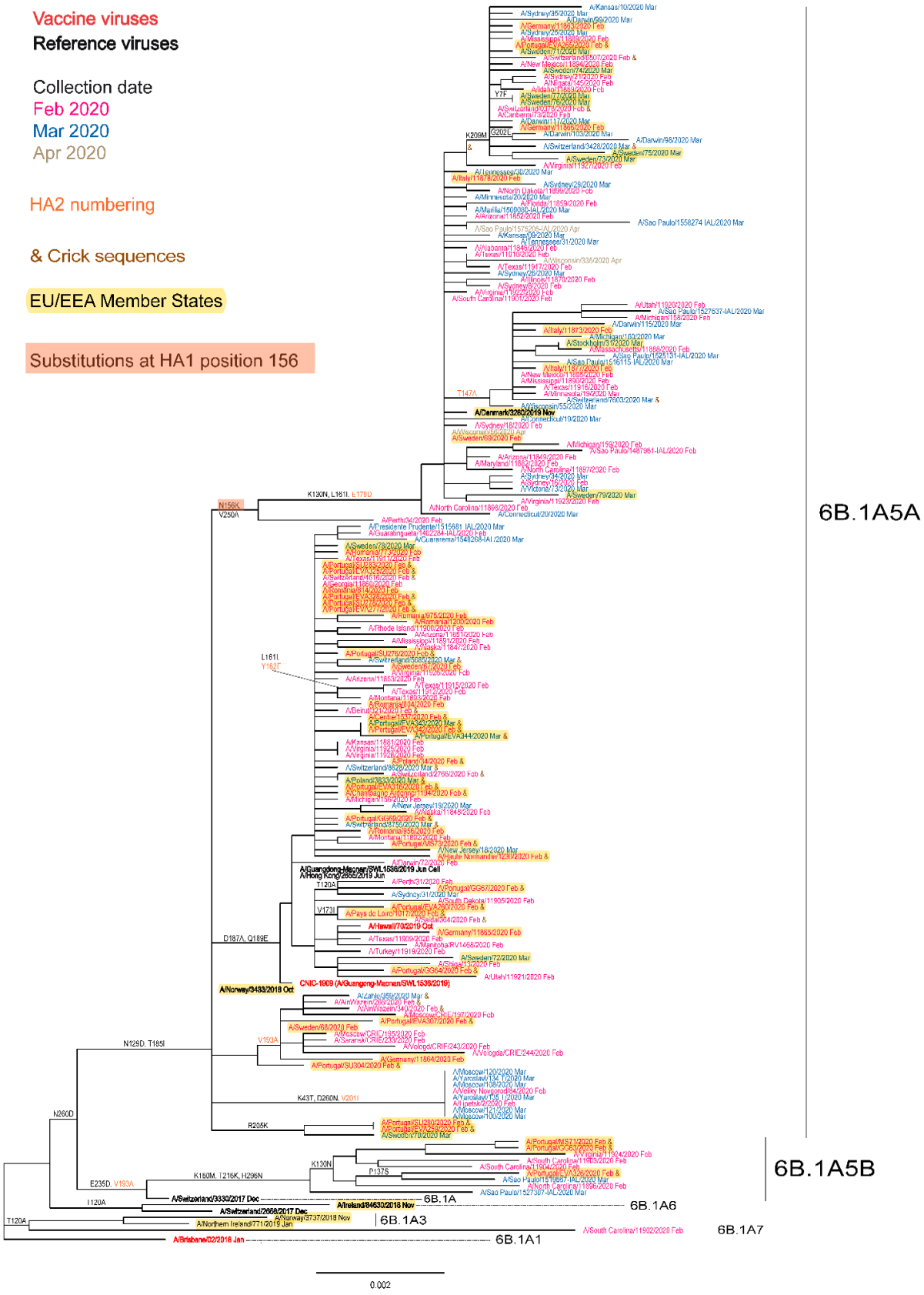
1. Subclade **6B.1A1** viruses, represented by the current vaccine virus **A/Brisbane/02/2018**, carry an HA gene mutation encoding **HA1 S183P** amino acid substitution;
2. Subclade **6B.1A2** viruses, represented by **A/Denmark/2728/2019**, carry HA gene mutations encoding **HA1 S183P** and **L233I** with **HA2 V193A** amino acid substitutions - a subgroup 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 subgroups 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**;
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 subgroup of this subclade has emerged with **P183S** (reversion)**, T185I, I240V** and **I286L** substitutions in **HA1** (e.g. **A/Estonia/120012/2019**).

The majority of recently circulating viruses have fallen in subgroup **6B.1A5A**, which contains a number of virus clusters, three of which have been detected in significant numbers defined by: (i) **HA1 D187A** and **Q189E** substitutions, (ii) by **HA2 V193A** substitution, and (iii) by **HA1 N156K** substitution, with the great majority in this cluster also having **HA1 K130N**, **L161V**, **V250A** and **HA2 E179D** substitutions. Relatively few viruses in subgroup **6B.1A5B** (with **HA1 K130N**, **K160M**, **T216K**, **E235D**, **H296N** and **HA2 V193A** substitutions) have also been detected. However, as indicated in the April report, based on sequences deposited in GISAID for viruses detected in February and March 2020, the vast majority fell in subgroup 6B.1A5A, with an approximately equal split between two of the genetic clusters defined above, (i) and (iii), with a minority falling in a cluster defined by **R205K** substitution, and small numbers falling in subclade **6B.1A7** and subgroup **6B.1A5B** (Figures 1a and 1b). This pattern was seen for viruses detected in the USA and EU/EEA countries. Both phylogenies are made up largely with sequences from viruses detected in February and March, and have very similar profiles. Significant differences are an apparent reduction in the number of viruses falling in genetic cluster (ii), though this is largely due to a large number in this cluster being deposited in GISAID in April by Russia (Figure 1a), and detection of a new genetic cluster in Russia defined by **HA1 K43T**, **D260N** and **HA2 V201I** substitutions (Figure 1b). The three viruses with collection dates in April all fall in genetic cluster (iii) (Figure 1b) and carry **HA1 N156K** substitution, which is known to have significant antigenic effect based on HI assays conducted with post-infection ferret antisera raised against A(H1N1)pdm09 vaccine viruses

The great majority of viruses in the various subgroups characterised to date, with the exception of those in genetic cluster (i), have remained antigenically similar to the northern hemisphere 2019–2020 vaccine virus, A/Brisbane/02/2018, as assessed with post-infection ferret antisera and shown in earlier characterisation reports; this is also the case for the relatively small number of viruses tested with antisera raised against A/Guangdong-Maonan/SWL1536/2019 (H1N1)pdm09-like viruses (with **HA1 D187A** and **Q189E** amino acid substitutions) that were recommended for use in the northern hemisphere 2020–2021 influenza season [3].

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

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



Influenza A(H3N2) virus analyses

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 is a particular problem for many viruses that fall in genetic clade 3C.2a. Previous reports have shown: (i) poor cross-reactivity of antisera raised against subclade 3C.2a2 viruses, (ii) significant clade specificity of antisera raised against cell culture-propagated clade 3C.3a viruses, A/England/538/2018 and A/Kansas/14/2017, and (iii), of antisera raised against cell culture-propagated viruses from different genetic clades/subclades, the one raised against A/Hong Kong/5738/2014 (clade 3C.2a) to give the broadest cross-clade/subclade reactivity.

The first A(H3N2) HA phylogeny is repeated from the April 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 in April 2020 (Figure 2a). The second is again based on viruses with collection dates from 1 February 2020, but with sequences deposited in GISAID during May 2020 (Figure 2b).

Viruses in clade 3C.2a have been dominant since the 2014–15 influenza season, and subgroup 3C.2a1b viruses predominated over the course of the 2018–19 season, but 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, and levels of detection since January 2019 had increased in a number of WHO European Region countries and North America. Greater variation has been observed among clade 3C.2a viruses, resulting in the designation of new subclades/subgroups. Amino acid substitutions that define these subclades/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)
- Subgroup **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**
- Subgroup **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** cluster (e.g. **A/South Australia/34/2019**) 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-A** cluster (e.g. **A/La Rioja/2202/2018**) or a recently emerged, antigenically distinct group with **HA1 T135K, T128A, S137F, A138S** and **F193S**, the **3C.2a1b+T135K-B** cluster (e.g. **A/Hong Kong/2675/2019**)
- Clade **3C.3a**: represented by **A/Switzerland/9715293/2013** (see above), but recently a resurgence of 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 2019–20 influenza season.

The HA phylogeny generated for the April report, based on sequences recently deposited in GISAID, showed viruses in the **3C.2a1b** subgroup to have circulated recently in the greatest numbers, with approximately equal distribution between the **3C.2a1b+T131K**, **3C.2a1b+T135K-A** and **3C.2a1b+T135K-B** clusters (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 influenza season [3]. The geographic distribution of clade 3C.3a viruses was more restricted with the great majority of recently detected viruses being reported from the European Region (Figure 2a). The updated phylogeny, for sequences deposited in May is again made up largely with sequences from viruses detected in North America and Europe during February and March, with only two from April both in cluster **3C.2a1b+T131K** (Figure 2b). The two phylogenies are very similar but for there being no sequences deposited in May for viruses from EU/EEA countries in the **3C.2a1b+T135K-B** cluster.

The locations of A/Kansas/14/2017 (3C.3a), the A(H3N2) virus recommended for inclusion in vaccines for the northern hemisphere 2019–20 influenza season [1], and A/South Australia/34/2019 (3C.2a1b+T131K), the A(H3N2) virus recommended for inclusion in vaccines for the southern hemisphere 2020 influenza season [2], are indicated in Figures 2a and 2b in red. The location on the A/Hong Kong/2671/2019 (**3C.2a1b+T135K-B**) virus 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 season [3], are also indicated.

[2] For example, the September 2013 report: European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, September 2013. Stockholm: ECDC; 2014. 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, April 2020)

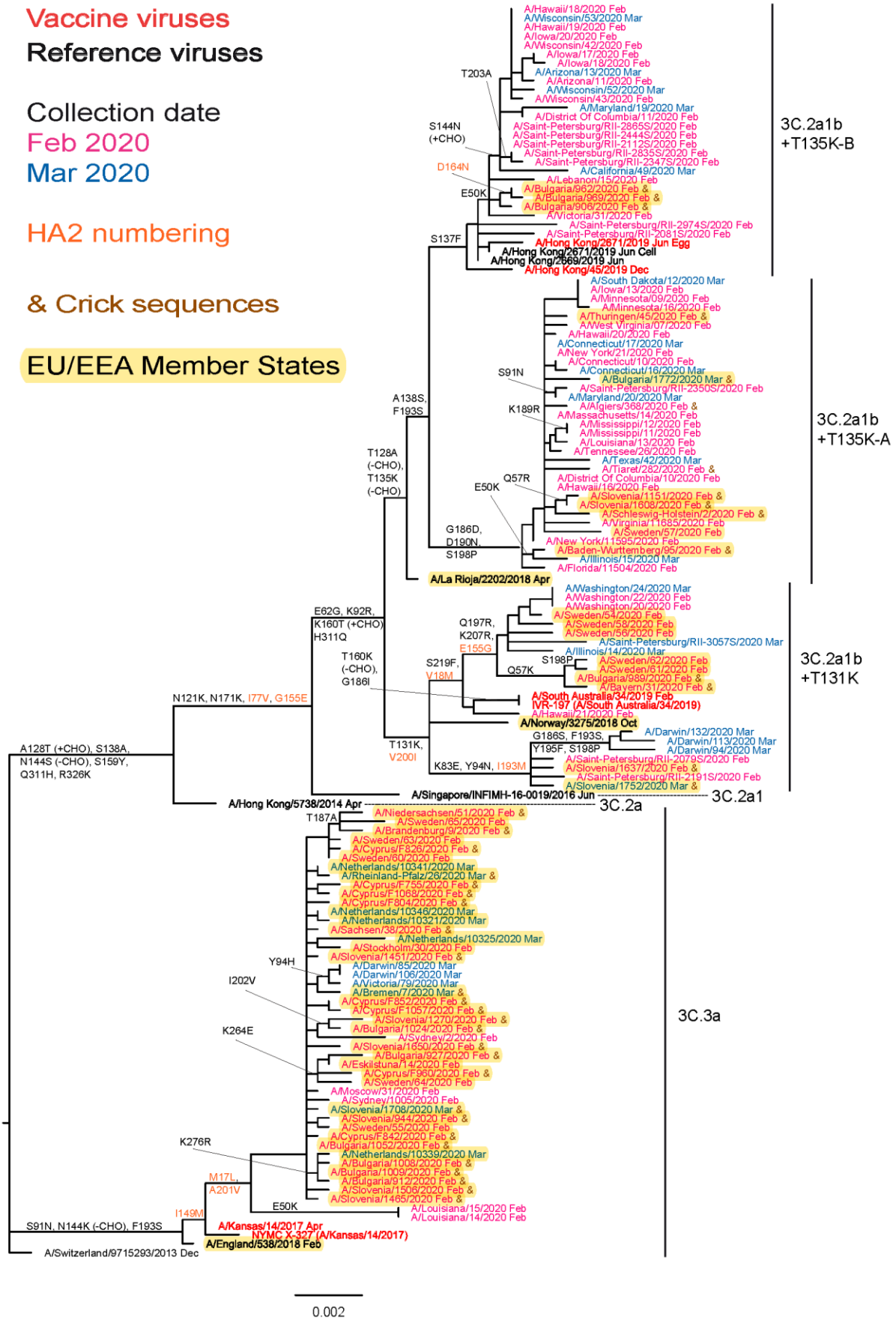
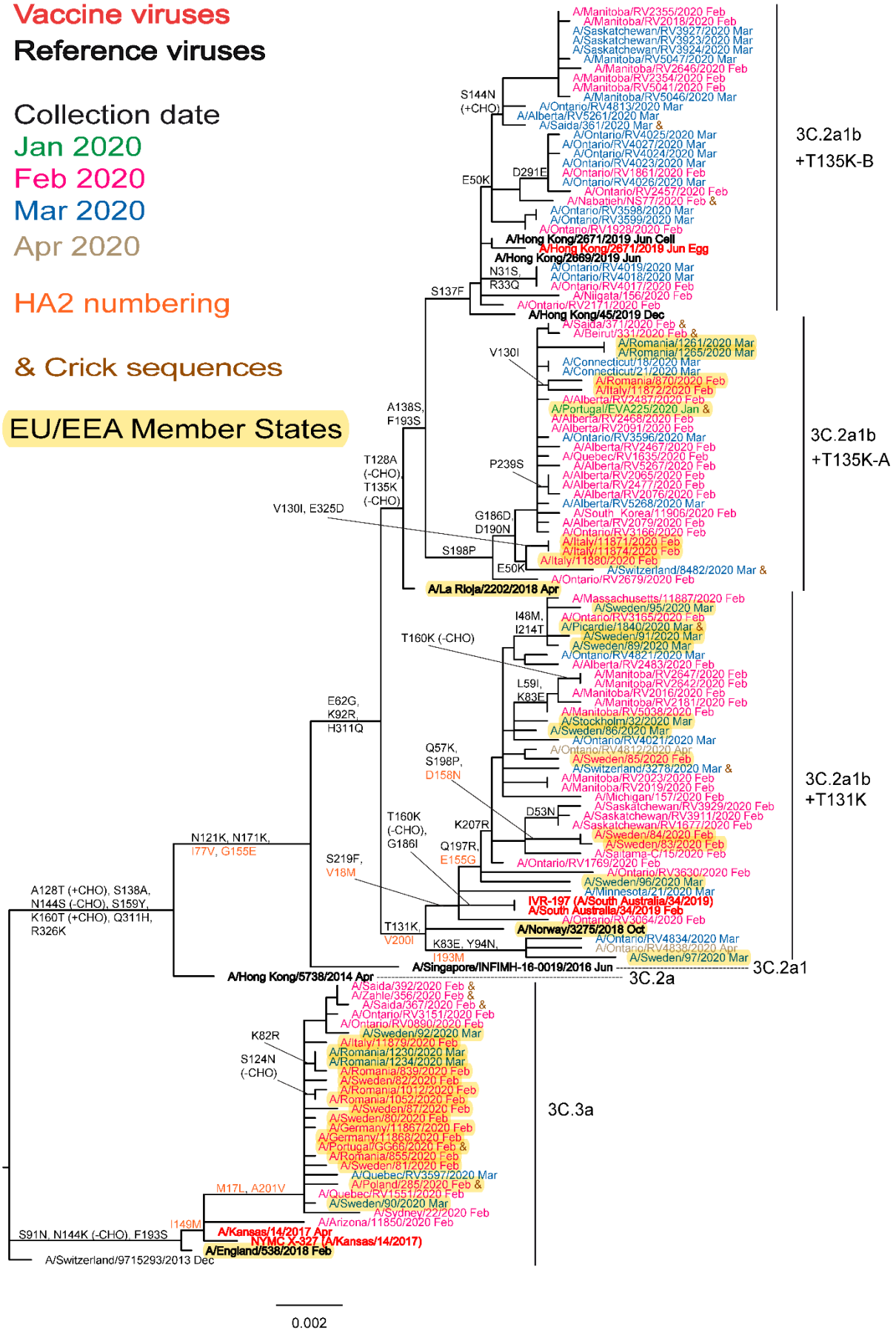


Figure 2b. Phylogenetic comparison of influenza A(H3N2) HA genes (GISAID, May 2020)



Influenza B virus analyses

A total of 365 influenza type B viruses with collection dates after 31 August 2019 have been received at the WIC (Table 2). Of these, 310 were sent with pre-assignment to a lineage: 294 B/Victoria and 16 B/Yamagata.

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 antigenically similar 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 the current 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 spread worldwide and is 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 in recent months and is represented by the recently recommended vaccine virus **B/Washington/02/2019**.

The HA phylogeny generated for the April report showed continued dominance of **subclade 1A(△3)B** viruses, with the great majority having **HA1 K136E,** often with **G133R** substitution, and a number of virus clusters had emerged defined by specific amino acid substitutions, e.g. **HA1 N126K** or **E128K** or **D129N** or **N150K** with **G184E** or **N233K** (loss of a glycosylation site) and **R279K**, and relatively few **subclade 1A(△2)** viruses had been detected (Figure 3a). The updated phylogeny for sequences deposited in GISAID during May is again largely made up of viruses detected in the USA and Europe during February and March, with just two from April for viruses detected in Sweden (Figure 3b); the phylogeny profile is very similar to that of Figure 3a. Viruses representative of some of these genetic clusters have not yet been characterised antigenically at the WIC.

Following the spread of **1A(Δ2)** viruses a representative, B/Colorado/06/2017, was recommended for use in trivalent influenza vaccines for the 2019–20 northern hemisphere season [1]. Recent predominance of **1A(Δ3)B** viruses led to recommendation of a representative (B/Washington/02/2019) for use in trivalent influenza vaccines for the 2020 southern hemisphere and northern hemisphere 2020–2021 seasons [2, 3].

Influenza B/Yamagata-lineage

A single B/Yamagata-lineage virus from France was characterised genetically at the WIC since the April report (Figure 4). The HA phylogeny, for viruses with collection dates from 1 January 2020, was updated from the April report to contain two sequences submitted to GISAID in April, one each from France and the USA detected in February and March, respectively. 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 has occurred, defined by specific amino acid substitutions (e.g. **HA1 N164K, K211R, D229N** or **D232N** [introducing a potential N-linked glycosylation site] sometimes with **R48K**). The two sequences recently deposited in GISAID both encode the **D232N** substitution. As noted in previous characterisation reports for 2018, 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 has been recommended for inclusion in quadrivalent vaccines for the 2019–2020 and 2020–2021 [1, 3] northern hemisphere and the 2020 [2] southern hemisphere seasons.

[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, April 2020)

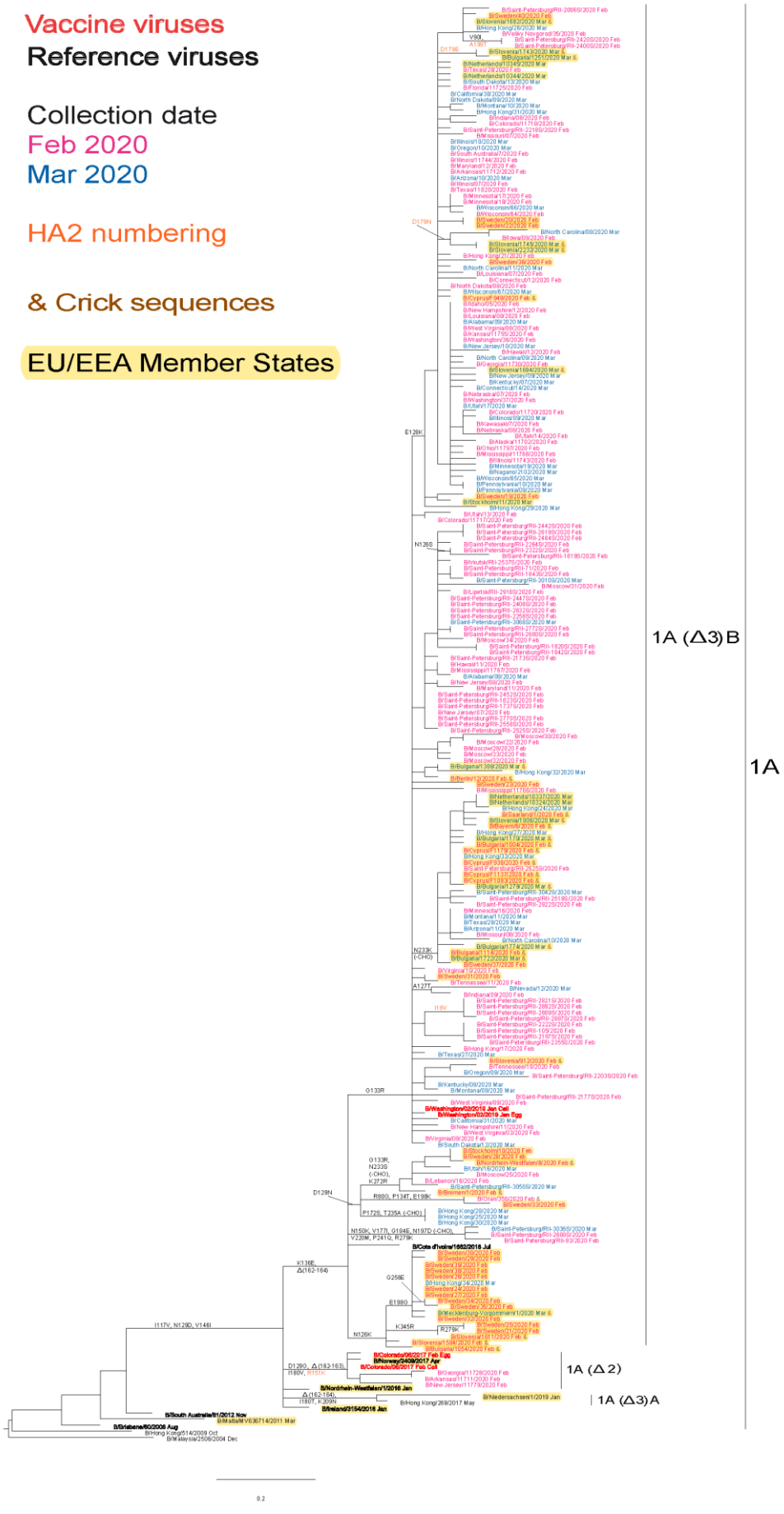


Figure 3b. Phylogenetic comparison of influenza B/Victoria-lineage HA genes (GISAID, May 2020)

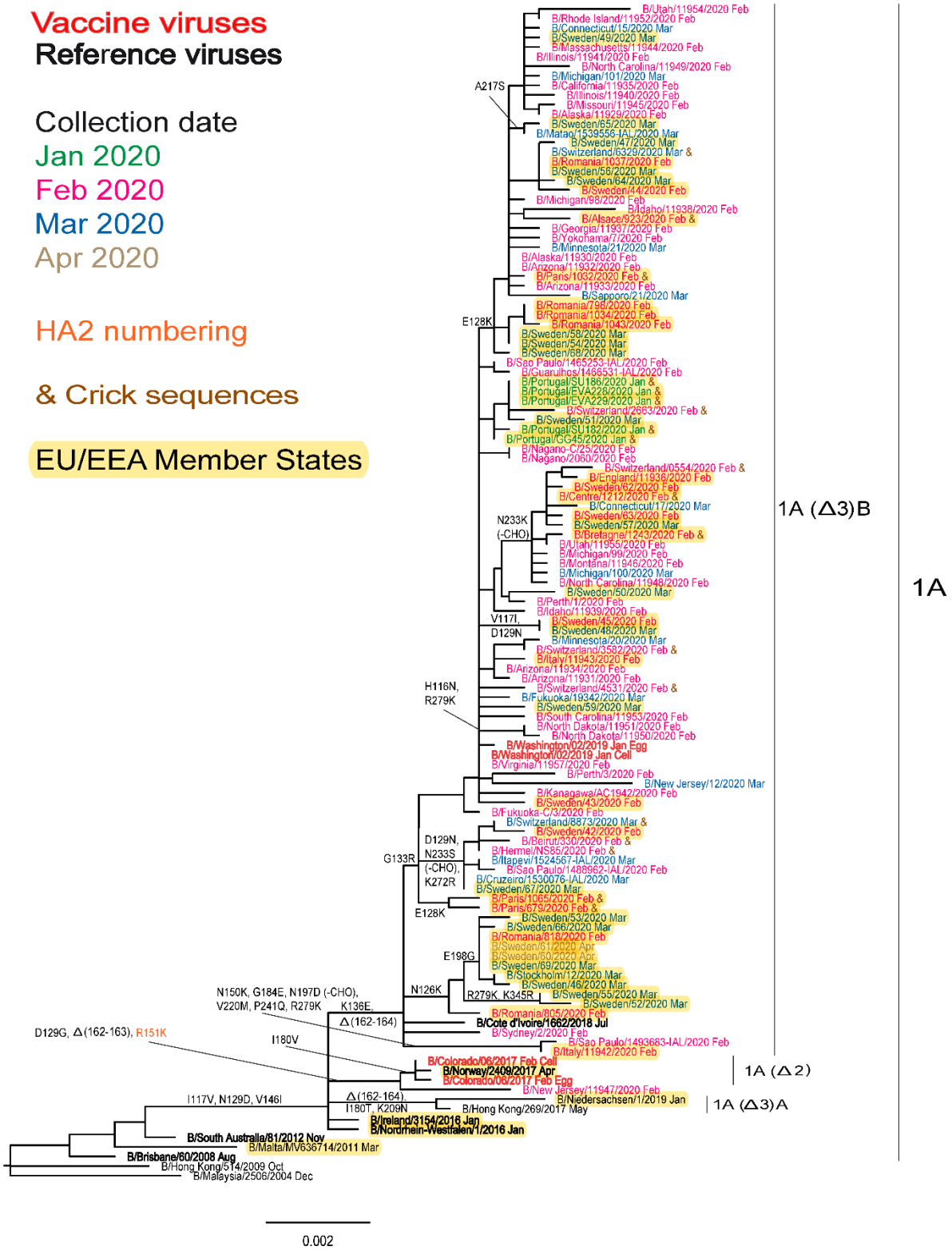
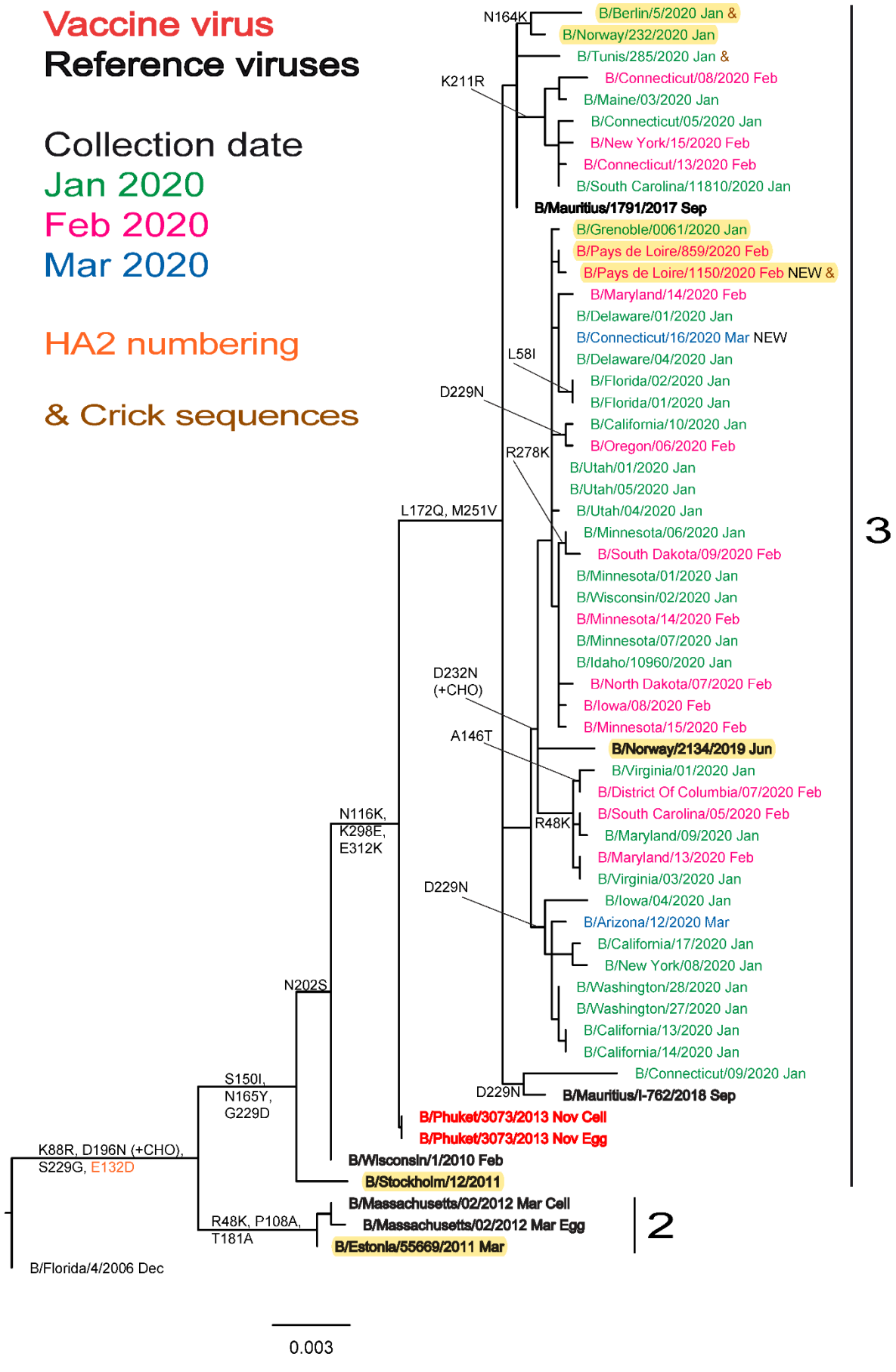


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes (GISAID, May 2020)



Summaries of data submitted to TESSy

Genetic characterisation

For the 2019–20 season, as of week 20/2020, 2 752 viruses had been characterised genetically and ascribed to a genetic clade:

- 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.
- 1 048 were 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.
- 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.
- 694 were 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

Up to week 20/2020, a total of 1 789 viruses (758 A(H1N1)pdm09, 626 A(H3N2) and 405 type B) collected in the course of the 2019–20 season had been tested for susceptibility to neuraminidase inhibitors, oseltamivir and zanamivir. Three A(H1N1)pdm09 viruses carried amino acid substitution H275Y in NA, with one of them also having H295S substitution, both of which are indicative of highly reduced inhibition (HRI) by oseltamivir. An additional A(H1N1)pdm09 virus showed reduced inhibition (RI) by oseltamivir and zanamivir by phenotypic assay. One A(H3N2) virus showed HRI by oseltamivir with reduced inhibition (RI) by zanamivir and carried NA R292K amino acid substitution.

At the WIC this season, 535 viruses from EU/EEA countries have been assessed phenotypically against oseltamivir and zanamivir: 185 A(H1N1)pdm09, 198 A(H3N2), 145 B/Victoria-lineage and seven B/Yamagata-lineage. Two A(H1N1)pdm09 viruses (A/Denmark/3295/2019 and A/Denmark/3311/2019) showed HRI by zanamivir associated with NA Q136K amino acid substitution, one A(H3N2) virus (A/Limoges/2326/2019) showed RI by zanamivir associated with NA T148I substitution (resulting in the loss of a potential N-linked glycosylation motif) and one B/Victoria-lineage virus (B/Estonia/125782/2020) showed RI by zanamivir.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [4] reported that the China Health and Family Planning Commission notified the WHO of three cases of human infection with influenza A(H7N9). A description of the characteristics of H7N9 viruses can be found on the WHO 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, though 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 the WHO monthly summary and assessment of influenza at human-animal interface (accessed 4 June 2020). The assessment, published on 8 May 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 [9]. The most recent human case was detected in mid-March 2019 [10]. The latest overview of avian influenza by ECDC in collaboration with the European Food Safety Authority and the EU Reference Laboratory for Avian Influenza was published on 31 March 2020 and can be found on the ECDC website [11].

Influenza A(H5) virus

The most recent monthly risk assessment of influenza at the human–animal interface was published by WHO on 8 May 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 [9]. 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 been, however, reports of A(H5N1) infection in domestic birds since February 2019 [12]. On 18 November 2016, ECDC published a rapid risk assessment related to outbreaks of highly pathogenic avian influenza H5N8 viruses in Europe [13]. As described above, the EU Reference Laboratory for Avian Influenza, in collaboration with ECDC and the European Food Safety Authority, published the latest overview of avian influenza on 31 March 2020, which can be found on the ECDC website [11].

Influenza A(H9N2) virus

Since the last update on 28 February 2020, two new laboratory-confirmed human cases of influenza A(H9N2) virus infections in China, both in children with mild disease symptoms and exposure to poultry, were reported [9]: one in Guangdong province (30 March 2020, disease onset 22 March) and one in Hunan province (1 May 2020, disease onset 20 April). Avian influenza A(H9N2) viruses are enzootic in poultry in Asia and increasingly reported in poultry in Africa.

WHO CC reports

A description of results generated by the London WHO CC at the WIC and used at the most recent WHO vaccine composition meeting (held in Geneva, Switzerland 24–28 February 2020), and previous ones, can be found at https://www.crick.ac.uk/partnerships/worldwide-influenza-centre/annual-and-interim-reports (accessed 4 June 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 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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