Vaccine 38 (2020) 5707–5717

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Predominance of influenza virus A(H3N2) 3C.2a1b and A(H1N1)pdm09 6B.1A5A genetic subclades in the WHO European Region, 2018–2019

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ARTICLE INFO

Article history:
Received 4 December 2019
Received in revised form 5 June 2020
Accepted 9 June 2020
Available online 3 July 2020

Keywords:
Influenza
Surveillance
Europe
Genetic
Antigenic
Vaccine

ABSTRACT

Background: The 2018/2019 influenza season in the WHO European Region was dominated by influenza A (H1N1)pdm09 and (H3N2) viruses, with very few influenza B viruses detected.
Methods: Countries in the European Region reported virus characterization data to The European Surveillance System for weeks 40/2018 to 20/2019. These virus antigenic and genetic characterization and haemagglutinin (HA) sequence data were analysed to describe and assess circulating viruses relative to the 2018/2019 vaccine virus components for the northern hemisphere.
Results: Thirty countries reported 4776 viruses characterized genetically and 3311 viruses antigenically. All genetically characterized A(H1N1)pdm09 viruses fell in subclade 6B.1A, of which 90% carried the amino acid substitution S183P in the HA gene. Antigenic data indicated that circulating A(H1N1)pdm09 viruses were similar to the 2018/2019 vaccine virus. Genetic data showed that A(H3N2) viruses mostly fell in clade 3C.2a (75%) and 90% of which were subclade 3C.2a1b. A lower proportion fell in clade 3C.3a (23%) and were antigenically distinct from the vaccine virus. All B/Victoria viruses belonged to clade 1A; 30% carried a double amino acid deletion in HA and were genetically and antigenically similar to the vaccine virus component, while 55% carried a triple amino acid deletion or no deletion in HA; these were antigenically distinct from each other and from the vaccine component. All B/Yamagata viruses belonged to clade 3 and were antigenically similar to the virus component in the quadrivalent vaccine for 2018/2019.
Conclusions: A simultaneous circulation of genetically and antigenically diverse A(H3N2) and B/Victoria viruses was observed and represented a challenge to vaccine strain selection.
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1. Introduction

Influenza viruses are known for their rapid evolution and genetic heterogeneity. Recent years have seen extensive genetic diversification of the haemagglutinin (HA) gene of circulating A (H3N2) viruses with emergence of several subclades [1–3]. A (H1N1)pdm09 viruses have also evolved since 2009, although more slowly than A(H3N2) viruses, and there are now new subclade designations based on the HA gene sequences [4–6]. In addition, new B/Victoria deletion variants were detected during the 2017/2018 season [4,5]. Those newly emergent strains have spread in Europe and worldwide at varying proportions during recent influenza seasons [5,7,8].

Monitoring influenza virus diversification is necessary as it may affect vaccine effectiveness, population immunity, antiviral drug resistance and pandemic preparedness. Annual vaccine recommendations for the northern and southern hemispheres are based on global epidemiological and virological influenza surveillance data, genetic and antigenic virus characterization data, and the availability of candidate vaccine viruses (CVVs) at the time of the Vaccine Composition Meeting (VCM) in February or September. Global data are provided by the Global Influenza Surveillance and Response network (GISRS) and the WHO Collaborating Centres (WHO CC) [9]. Real-time tracking platforms, like Nextstrain (https://nextstrain.org/), provide important tools to monitor the evolution of influenza viruses and facilitate the vaccine decision making.

In February 2018, WHO recommended that the influenza trivalent vaccine for the northern hemisphere 2018/2019 season

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https://doi.org/10.1016/j.vaccine.2020.06.031
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contain an A/Michigan/45/2015 (H1N1)pdm09-like virus (clade 6B.1), an A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus (clade 3C.2a1) and a B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage) (clade 1A_Δ2). For the quadrivalent vaccines, the recommendation was to also include a B/Yamagata lineage B/Phuket/3073/2013-like virus (clade 3) [10].

Throughout the 2018/2019 influenza season, both influenza A subtypes circulated widely in the World Health Organization (WHO) European Region, with very few influenza B viruses of either lineage reported [3,11]. We used the virological data reported weekly by the national reference laboratories for influenza and the National Influenza Centres (NICs) to describe the virological, genetic and antigenic characteristics of the viruses circulating in the WHO European Region during the 2018/2019 season and compare them with the vaccine virus components for the northern hemisphere 2018/2019 influenza season.

2. Methods

In the WHO European Region, countries reported weekly influenza surveillance data to The European Surveillance System (TESSy) during the 2018/2019 influenza season. We used data from week 40/2018 to week 20/2019. The data were retrieved on 30 May 2019. Viruses were characterised according to pre-defined criteria and category definitions described below.

2.1. Genetic characterization

Influenza viruses were genetically characterised by the national influenza reference laboratories/NICs using Sanger and/or next generation sequencing techniques directly on clinical specimens or virus isolates. To report a virus to TESSy as belonging to a specific genetic category, the phylogenetic and amino acid sequence analysis had to fulfil the following criteria: (a) based on the HA gene sequence phylogeny, the virus clustered within the clade represented by the indicated vaccine/reference virus, and (b) the virus contained neither more non-synonymous nor critical amino acid substitutions that may alter the antigenicity compared to reference viruses from the given clade. In October 2018, the WHO CC at the Francis Crick Institute, London, United Kingdom provided the list of reference viruses for genetic analysis and the TESSy reporting categories for influenza virus characterization related to the HA gene. Fourteen different representative influenza virus categories proposed by WHO CC were available for reporting genetic characterization data to TESSy. In addition, 'not attributed to any clade' and 'subgroup not listed' were available for each subtype and lineage to accommodate viruses that either did not match any of the predefined genetic groups or were assigned to a previously designated category that was no longer included in the reporting scheme. Characterisation results in aggregated and strain-based manner and, often, the Global Initiative for Sharing All Influenza Data (GISAID) database EpiFlu reference number were reported to TESSy.

2.2. Antigenic characterization

National influenza reference laboratories/ NICs cultured influenza viruses, from a subset of influenza-positive clinical specimens, in MDCK, MDCK-SIAT or other variants of MDCK cell lines and/or embryonated chicken eggs [12]. A haemagglutination inhibition (HI) assay was used for antigenic characterization of recovered influenza viruses using post-infection ferret antisera raised against vaccine/reference influenza viruses (supplied by WHO CC London or Atlanta or produced in the laboratory) to inhibit virus agglutination of red blood cells [12]. A virus isolate was considered antigenically similar to a reference virus if the HI titre with the respective post-infection ferret antiserum differed by no more than 4-fold down in a 2-fold dilution series, from the homologous HI titre of the antiserum against the reference virus itself. To consider an isolate antigenically different from a reference virus, the HI titre had to show a decrease of more than 4-fold or more compared to the homologous titre. For antigenic characterization of A(H3N2) viruses, some laboratories conducted HI assays in the presence of oseltamivir, to prevent haemagglutination by the neuraminidase, and/or performed virus neutralization assays. Ten different representative influenza virus categories proposed by WHO CC were available for reporting antigenic characterization data to TESSy. In addition, 'not attributed to any category' was available for each subtype and lineage to accommodate viruses that either did not match one of the predefined major antigenic groups, did not yield a conclusive HI assay result (showed >4-fold reduced HI titres against all the reference virus antisera that the laboratory had used) or were not tested against the appropriate reference antisera. 'Subgroup not listed' was available for each subtype and lineage and was used when a virus was assigned to a designated category that is no longer in the reporting scheme. Interpretations of HI assays from the laboratory were reported to TESSy. Raw data for antigenic characterization are not submitted to TESSy.

2.3. Analysis

We performed a descriptive analysis of virological data that were reported by the laboratories to TESSy between week 40/2018 and 20/2019. Viral genetic and antigenic characterization data were reported weekly in aggregated and/or virus-based format by date of sampling. If any laboratory reported both aggregated and virus-based data for the same week, the more detailed virus-based data were used. All data originated from ambulatory and inpatient populations from sentinel primary care and non-sentinel (e.g., diverse populations, including outpatients, hospitals, outbreak investigations, long-term care facilities) sources. For Supplemental Fig. 1, October/November as well as April/May were merged due to the very low number of A(H3N2) virus genetic characterizations that were reported during October 2018 and May 2019.

2.4. Phylogenetic analysis

We conducted phylogenetic analysis on reported influenza HA sequences for A(H1N1)pdm09, A(H3N2), B/Victoria, and B/Yamagata viruses. Sequences were downloaded from the EpiFlu database of GISAID. An ECDC in-house programme was used to process the sequence data for each subtype separately. All entries for HA sequences in TESSy were matched with the respective GISAID data. HA sequences were excluded if the sequence was not released for public access, or if the entry had errors in the accession number or the name of the virus in the TESSy report did not match GISAID. Alignment was performed using mafft v7, first aligning the reference sequences and then adding the available test sequences, and the alignment was trimmed to include only the HA1-coding region in order to include as many TESSy reported sequences as possible. RAxML v8.2.7 was used to construct a phylogenetic tree and a maximum likelihood search [13]. We used the maximum likelihood best tree and branch likelihood for the output that are not affected by the number of bootstraps. The tree was rooted on the oldest reference sequence using treesub (https://github.com/tamuri/treesub) and PAML baseml v4.9f was used to reconstruct the ancestors of the HA1 sequences for all internal nodes of the tree. Treesub was used to annotate the tree branches with amino acid substitutions based on the root sequence. The nodes were coloured according to month and the

Table 1
Distribution of antigenically and genetically characterized influenza viruses as reported to TESSy by country, WHO European Region, weeks 40/2018–20/2019.

Countries	Genetic clades																Antigenic categories										
	AH1/ Michigan/ 45/ 2015_6B.1 (1)	AH1Subgroup NotListed	AH3/Alsace/ 1746/ 2018_3C.2a1b	AH3/Cote d'Ivoire/544/ 2016_3C.2a3	AH3/Greece/ 4/ 2017_3C.2a1a	AH3/ England/ 538/ 2018_3C.3a	AH3/ Switzerland/ 8060/ 2017_3C.2a2	AH3/Hong Kong/ 4801/ 2014_3C.2a	AH3/ Singapore/ INFIMH-16–0019/ 2016_3C.2a1 (1)	AH3NOClade	AH3Subgroup NotListed	BVic/Hong Kong/269/ 2017_1A_Δ3	BVic/ Colorado/06/ 2017_1A_Δ2 (1)	BVic/ Brisbane/ 60/ 2008_clade 1A	BYam/ Phuket/ 3073/ 2013_3 (2)	Total number of viruses	AH1/ Michigan/ 45/2015-like (1)	AH1NOCAT	AH3/ Switzerland/ 8060/2017-like	AH3/ Hong Kong/ 4801/ 2014-like	AH3/ Singapore/ INFIMH-16–0019/ 2016-like (1)	AH3NOCAT	BVic/ Colorado/ 06/2017-like (1)	BVic/ Brisbane/ 60/2008-like	BVicNOCAT	BYam/ Phuket/ 3073/ 2013-like (2)	Total number of viruses
Austria	104	0	71	2	0	8	1	0	0	0	2	0	0	0	3	**191**	43	0	0	0	0	0	0	0	0	0	**43**
Belgium	31	0	60	0	0	12	7	0	22	0	0	0	0	2	0	**134**	0	0	0	0	0	0	0	0	0	0	**0**
Bulgaria	16	0	5	0	0	0	4	0	0	0	0	0	0	0	0	**25**	0	0	0	0	1	0	0	0	0	0	**1**
Czech Republic	5	0	25	0	0	1	0	0	0	0	0	0	0	0	0	**31**	23	0	0	0	0	0	0	0	0	0	**23**
Denmark	161	0	89	1	0	1	1	0	0	0	0	0	0	0	0	**253**	23	0	0	0	0	0	0	0	0	0	**23**
Finland	74	0	60	0	0	9	1	0	0	0	0	0	0	0	0	**144**	0	0	0	0	0	0	0	0	0	0	**0**
France	99	0	96	7	0	83	16	0	1	0	0	0	0	2	0	**304**	69	1	2	24	0	35	0	0	2	0	**133**
Germany	90	0	158	3	0	19	7	0	0	0	2	1	0	0	1	**281**	764	0	0	0	327	0	0	0	0	1	**1092**
Greece	24	3	6	0	0	0	2	1	0	0	1	0	0	0	0	**37**	16	0	0	1	1	0	0	0	0	0	**18**
Hungary	4	0	4	0	0	0	0	0	0	0	0	0	0	0	0	**8**	4	0	0	0	4	0	0	0	0	0	**8**
Ireland	139	0	38	0	0	13	0	0	0	0	0	0	1	0	1	**192**	73	3	0	0	1	0	0	0	0	1	**78**
Italy	38	0	51	0	0	21	9	0	0	0	0	0	0	0	1	**120**	12	0	0	0	0	0	0	0	0	0	**12**
Kazakhstan	24	0	19	0	0	0	0	0	1	0	0	0	6	0	0	**50**	19	0	0	0	11	0	6	0	0	0	**36**
Latvia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	61	0	7	0	3	0	0	0	0	0	**71**
Lithuania	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	16	0	0	0	0	0	0	0	0	0	**16**
Luxembourg	26	0	69	4	0	17	0	0	0	0	0	0	0	0	0	**116**	0	0	0	0	0	0	0	0	0	0	**0**
Republic of Moldova	13	0	0	0	7	0	1	0	0	0	0	0	0	0	0	**21**	13	0	0	8	0	0	0	0	0	0	**21**
Netherlands	309	0	305	3	0	65	4	0	2	0	0	2	0	0	1	**691**	0	0	0	0	0	0	0	0	0	0	**0**
Norway	126	0	121	0	0	6	7	4	0	0	0	12	2	1	18	**297**	0	0	0	0	0	0	0	0	0	0	**0**
Portugal	30	0	55	7	0	21	1	0	0	0	0	0	0	0	3	**117**	97	0	0	0	2	21	0	0	0	2	**122**
Romania	39	0	37	0	0	0	3	0	0	0	0	0	0	0	0	**79**	94	0	0	0	1	0	0	0	0	0	**95**
Russian Federation	19	0	10	0	0	0	0	0	0	0	0	0	1	0	0	**30**	439	0	0	92	100	0	1	8	0	14	**654**
Slovakia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	174	0	0	0	64	0	0	0	0	0	**238**
Slovenia	28	0	33	0	0	2	1	0	0	0	0	0	0	0	0	**64**	33	0	7	0	0	0	3	0	0	0	**43**
Spain	201	0	266	6	0	322	3	0	0	0	0	0	0	0	1	**799**	38	0	0	0	11	0	0	0	0	0	**49**
Sweden	153	0	83	0	0	3	10	0	0	0	0	4	0	1	2	**256**	0	0	0	0	0	0	0	0	0	0	**0**
Switzerland	49	0	13	0	0	1	3	0	0	0	33	0	0	0	0	**99**	26	0	0	0	27	0	0	0	0	0	**53**
Ukraine	13	0	0	0	0	0	0	0	45	1	0	0	0	0	0	**59**	13	0	0	0	45	1	0	0	0	0	**59**
United Kingdom	238	0	112	0	0	9	5	0	0	0	0	3	2	0	9	**378**	418	0	0	3	0	0	0	0	0	2	**423**
Total	**2053**	**3**	**1786**	**33**	**7**	**613**	**86**	**5**	**71**	**1**	**38**	**22**	**12**	**6**	**40**	**4776**	**2468**	**4**	**16**	**128**	**598**	**57**	**10**	**8**	**2**	**20**	**3311**

(1) Vaccine component for the trivalent vaccines used in the northern hemisphere 2018/2019 season.
(2) Additional vaccine component for the quadrivalent vaccines for use in northern hemisphere 2018/2019 season.

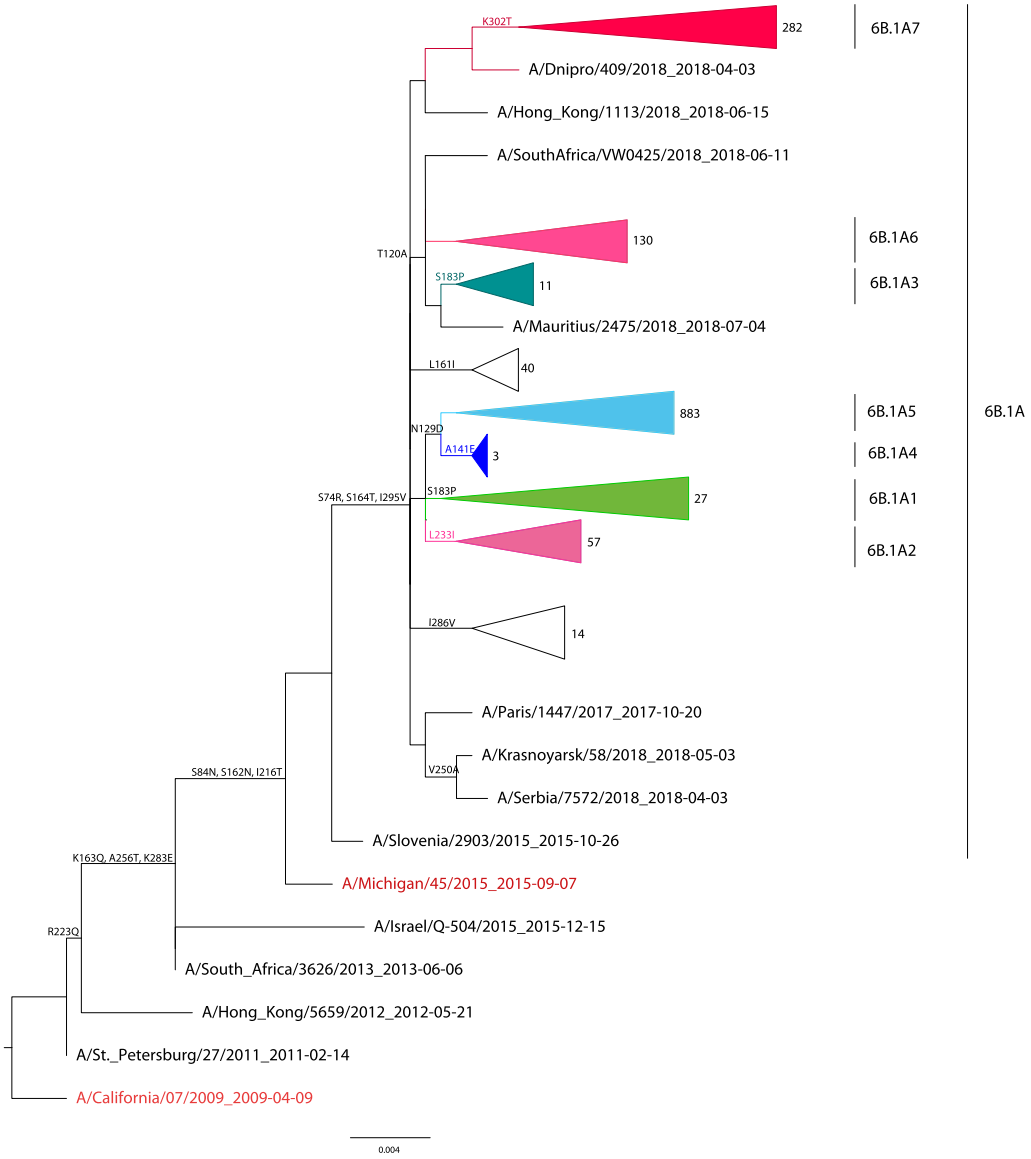

Fig. 1. Phylogenetic comparison of influenza A(H1N1)pdm09 HA gene sequences. Colour coding indicates the northern hemisphere 2018/2019 vaccine virus in red and reference strains in black. The number of collapsed sequences (including reference sequences) are mentioned next to the branches. Supplemental Fig. 3 shows all TESSy reported sequences in color according to the virus collection month. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tree was exported in nexus format. PDF trees were edited and annotated using FigTree and PDF Illustrator. HA amino acid sequence alignments were used to inspect amino acid substitutions in Bioedit and compare them with the respective vaccine viruses for 2018/2019.

3. Results

3.1. Genetic and antigenic characteristics of circulating influenza viruses, 2018/2019

Genetic characterization results were reported for a total of 4776 viruses (2056 A(H1N1)pdm09, 2640 A(H3N2), 40B/Victoria and 40B/Yamagata) from 26 countries (Table 1). Of the genetically characterized viruses, accession numbers for HA sequences in GISAID EpiFlu were available for 1467 (74%) A(H1N1)pdm09, 2083 (79%) A(H3N2), 20 (50%) B/Yamagata and 16 (40%) B/Victoria viruses. Antigenic characterization results were reported for a total of 3311 viruses from 23 countries.

3.2. A(H1N1)pdm09

3.2.1. Genetic characterization

Of 2056 genetically characterized A(H1N1)pdm09 viruses reported to TESSy, 2053 (99.9%) were assigned to the A/Michigan/45/2015 subgroup (6B.1) (Table 1). The phylogenetic analysis included 1432 HA gene sequences from A(H1N1)pdm09 viruses (Fig. 1). Similarly to the reported characterization data, they all fell in phylogenetic clade 6B.1 which is defined by amino acid substitutions at positions S84N, S162N and I216T in HA1 and includes the 2018/2019 vaccine virus A/Michigan/45/2015. All of the viruses further clustered into a genetic subgroup designated 6B.1A with additional amino acid substitutions S74R, S164T and I295V in HA1. Most of the viruses (1290, 90%) also carried amino acid substitution S183P in HA1. Subgroup 6B.1A diversified in subclade

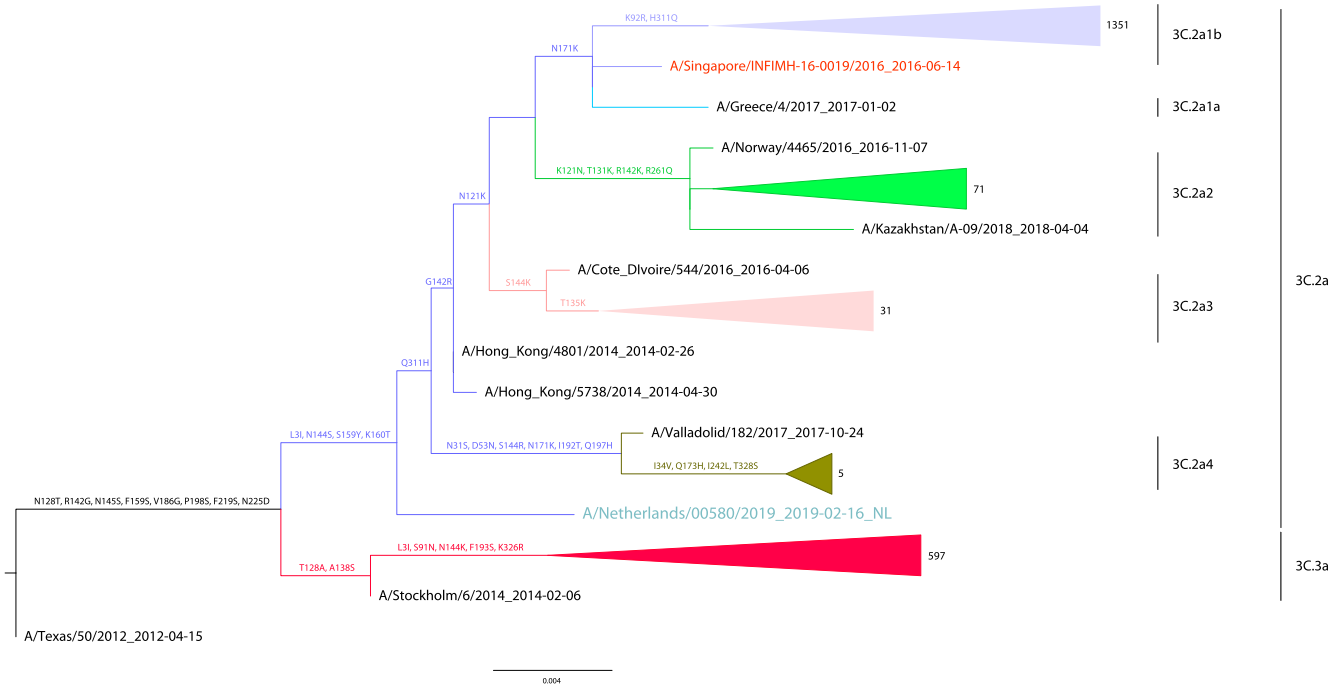

Fig. 2. Phylogenetic comparison of influenza A(H3N2) HA gene sequences. Colour coding indicates the northern hemisphere 2018/2019 vaccine strain in red and reference strains in black. The number of collapsed sequences (including reference sequences) are mentioned next to the branches. Branch colouring indicates the different clades and subclades. Supplemental Fig. 2 shows all TESSy reported sequences in color according to the virus collection month. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

6B.1A1 (n = 23) that carried only S182P and further into subgroups defined by specific amino acid substitutions in addition to S183P including L223I (n = 56) (subclade 6B.1A2), or T120A (n = 9) (6B.1A3), or N129D together with A144E (n = 3) (subclade 6B.1A4), or N260D together with N129D (n = 830) (subclade 6B.1A5A) or E235D (n = 50) (subclade 6B.1A5B), or T120A (n = 122) (subclade 6B.1A6), or K302T (n = 282) (subclade 6B.1A7), often in combination with other substitutions.

Two of the HA sequences were derived from seasonal A(H1N2) reassortant viruses that were detected in Sweden and Denmark, respectively. These viruses carried an HA sequence similar to currently circulating A(H1N1)pdm09 viruses [14,15].

3.2.2. Antigenic characterization

Of 2472 antigenically characterized A(H1N1)pdm09 viruses, 2468 (99.8%) were reported as similar to the 2018/2019 vaccine virus component A/Michigan/45/2015. Only four were not attributed to a predefined antigenic category (Table 1).

3.3. A(H3N2)aaa

3.3.1. Genetic characterization

Of 2640 A(H3N2) viruses that were genetically characterized and reported to TESSy, 1988 (75%) belonged to clade 3C.2a, including 71 (4%) in 3C.2a1, 1786 (90%) in subclade 3C.2a1b, 86 (4%) in 3C.2a2, 33 (2%) in 3C.2a3 and 7 (<1%) in 3C.2a1a. Clade 3C.3a accounted for 613 viruses (23%) from 15 countries (Table 1). The proportion of clade 3C.3a viruses among all A(H3N2) viruses increased from 0 to 27% through mid-season and then decreased to 11% by the end of the season (Supplemental Fig. 1). Thirty-eight A(H3N2) viruses (1%) were reported as 'subgroup not listed' and one virus was reported as 'not attributable to any predefined group'.

The phylogenetic analysis of A(H3N2) viruses was performed on 2034 HA gene sequences (Fig. 2). Similarly to the reported characterization data, 71% of viruses carried HA genes that fell into genetic groups within clade 3C.2a (n = 1439) and 29% in clade 3C.3a (n = 595). Among the 3C.2a clade viruses, 93% fell in the 3C.2a1b subgroup (n = 1343), 4% in the 3C.2a2 subclade (n = 61), 2% in the 3C.2a3 subclade (n = 30) and <1% in 3C.2a4 (n = 5). The major clades and subclades with the characteristic amino acid substitutions in HA1 are presented in Table 2.

3.3.2. Antigenic characterization

Seven hundred and forty-two (93%) of 799 A(H3N2) viruses were attributed to a predefined antigenic category and were reported as antigenically similar to the 2018/2019 vaccine component A/Singapore/INFIMH-16-0019/2016 or to reference viruses that are considered antigenically similar to the vaccine strain (A/Hong Kong/4801/2014-like, A/Switzerland/8060/2017-like) (Table 1).

Fifty-seven A(H3N2) viruses (7%) were not assigned to any antigenic reporting category. Of the unassigned viruses, 56 had a reported genetic category; 37 (65%) were genetically characterized as A/England/538/2018 (3C.3a clade). The remainder of the unassigned viruses belonged to subclades 3C.2a1b (n = 13), 3C.2a3 (n = 3) or 3C.2a2 (n = 2) or were not attributable to any predefined group (n = 1).

3.4. B/Victoria lineage

3.4.1. Genetic characterization

Of the 80B viruses genetically characterized and reported to TESSy, 40 were B/Victoria lineage viruses. Of these, six (15%) were genetically assigned to the B/Brisbane/60/2008 group, 12 (30%) to the B/Colorado/06/2017 group that carries a HA1 double amino acid deletion (Δ162-163), and 22 (55%) to the B/Hong Kong/269/2017 group that carries a HA1 triple amino acid deletion (Δ162-164) (Table 1).

Table 2
Influenza A(H3N2) viruses by (sub)clade and amino acid substitutions (retrospective analysis based on GISAID accession numbers reported to TESSy), WHO European Region, weeks 40/2018–20/2019. Coloured circles indicate the respective branch in the phylogenetic tree (Fig. 2 and Supplemental Fig. 2).

(Sub)clade *(Sub)clade-specific amino acid substitutions in HA1*[*] + Additional frequent substitutions	**Number of viruses and percentage of total A(H3N2) viruses or of specific (sub)clade as indicated**
● **3C.2a** *L3I+N128T(+CHO)+N144S(-CHO)+N145S+F159Y+K160T(+CHO)+P198S+F219S+N225D+Q311H*	1439 (71%)
● **3C.2a1** *N121K+N171K*	1343 (93% of 3C.2a)
● **3C.2a1b** *K92R+H311Q* *+T135K(-CHO) +T128A(-CHO)* *+S198P* *+D53N* +T131K+K135T(+CHO) +S219F+/-Q197R+/-K83E	1343 (100% of 3C.2a1) 712 (53% of 3C.2a1b) 133 (9% of 3C.2a1b) 148 (10% of 3C.2a1b) 625 (43% of 3C.2a1b) 367 (26% of 3C.2a1b)
● **3C.2a2** *K121N+T131K+R142K+R261Q* *+A212T*	61 (4% of 3C.2a) 57 (93% of 3C.2a2)
● **3C.2a3** *N121K+T128A+T135K (-CHO)+S144K* +R142G+R261Q+/-T30A+T128A+/-R150K +T128A+I192V	30 (2% of 3C.2a) 27 (90% of 3C.2a3) 3 (1% of 3C.2a3)
● **3C.2a4** *N31S+D53N+S144R+N171K+I192T+Q197H* +I34V+ Q137H+ I242L+T328S	5 (<1% of 3C.2a) 5 (100% of 3C.2a4)
● **3C.3a** *T128A(-CHO)+ A138S +R142G+* L3I+S91N+N144K(-CHO)+F193S +R326K	595 (29%) 57 (10% of 3C.3a)
Total number of A(H3N2) virus HA sequences	2034

[*]Major (sub)clades in bold with characteristic amino acid substitutions in italics.

Nineteen HA gene sequences from B/Victoria lineage viruses were included in the phylogenetic analysis (Fig. 3). All but two were correctly assigned to the genetic categories. All of the viruses with reported GISAID accession numbers belonged to clade 1A and carried additional amino acid substitutions I117V and N129D or V146I in HA1 compared with B/Brisbane/60/2008. Two (11%) of the reported HA sequences belonged to the Δ162-163 subclade, represented by the trivalent and quadrivalent 2018/2019 vaccine virus B/Colorado/06/2017 and carried additional substitutions D129G and I180V in HA1. Two HA sequences (11%) did not have any amino acid deletion. Fifteen (79%) of the reported HA sequences fell into the Δ162-164 subgroup, similar to B/Hong Kong/269/2017 (see Fig. 3).

Of the Δ162-164 viruses, 13 (87%) carried K136E in HA1, placing them in the 1A(Δ3)B subgroup (West African); 92% (n = 12) also carried D164K, often with G74E and E198K or G133R in HA1; one also carried K52N in HA1. The remaining two (13%) B/Victoria viruses of the Δ162-164 group also carried amino acid substitutions I180T and K209N in HA1, thus belonging to the 1A (Δ3)A subgroup (Asian group) together with B/Hong Kong/269/2017.

3.4.2. Antigenic characterization

Of the 20 antigenically characterized B/Victoria lineage viruses, ten (50%) were characterized as B/Colorado/06/2017-like (Δ162-163) similar to the 2018/2019 vaccine virus component, eight (40%) were reported as B/Brisbane/60/2008-like that is antigenically distinct from the vaccine virus component and two (10%) were not attributed to any predefined category (Table 1). No viruses were assigned to the Δ162-164 antigenic group, possibly due to the lack of the corresponding reference antisera for the HI assay.

3.5. B/Yamagata lineage

3.5.1. Genetic characterization

Of the 80B viruses genetically characterized and reported to TESSy, 40 (50%) were reported as B/Yamagata lineage and they were all assigned to the B/Phuket/3073/2013 clade (clade 3) that was included only in the quadrivalent vaccine and was similar to the vaccine virus component (Table 1).

By week 20/2019, 15 HA gene sequences from B/Yamagata-lineage viruses were included in the phylogenetic analysis

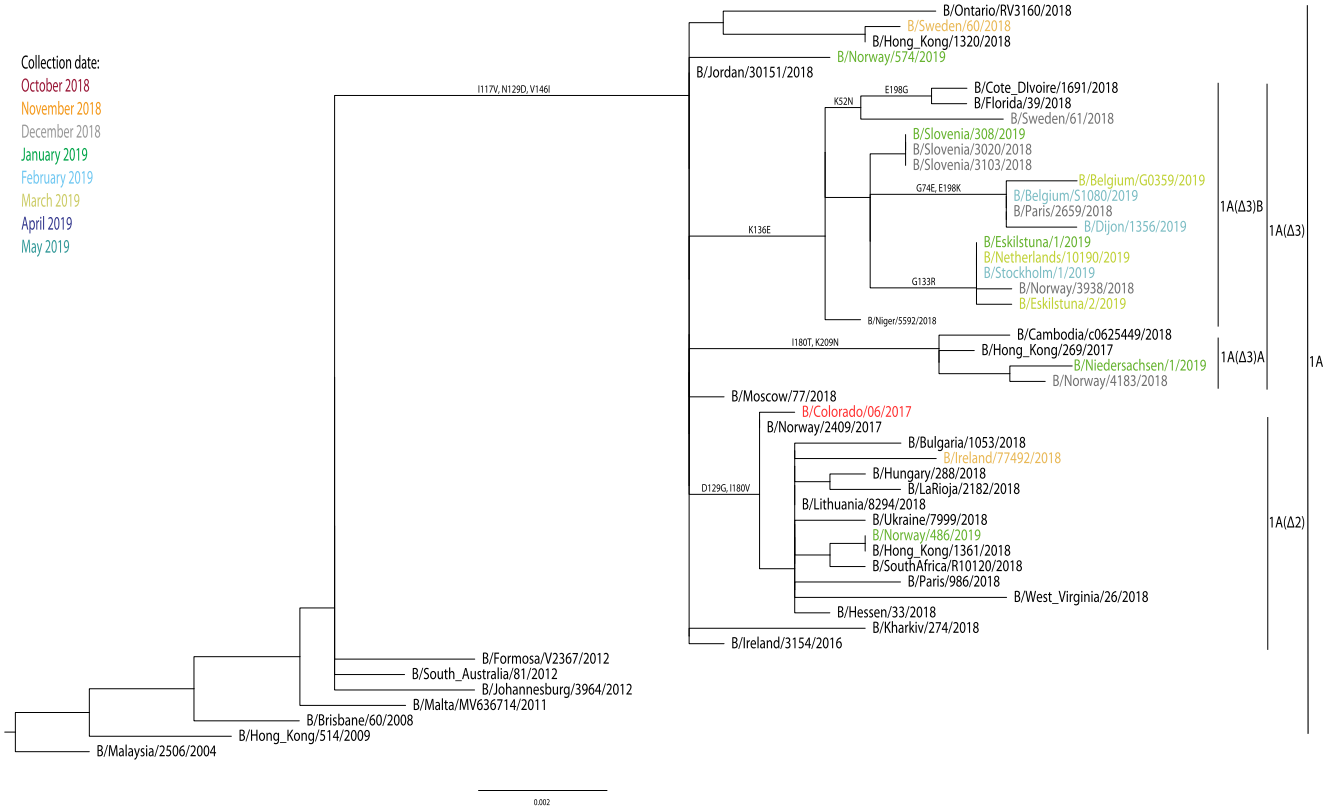

Fig. 3. Phylogenetic comparison of influenza B/Victoria-lineage HA gene sequences. Colour coding indicates the northern hemisphere 2018/2019 vaccine strain in red, reference strains in black and TESSy sequences according to the virus collection date by month. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4). Similarly to the reported characterization data, all sequences fell in clade 3 represented by B/Phuket/3073/2013, the additional vaccine virus recommended for inclusion in quadrivalent influenza vaccines for the 2018/2019 northern hemisphere season and in a subgroup defined by the amino acid substitutions L172Q and M251V in HA1 compared with B/Phuket/3073/2013 (Fig. 4). Few viruses carried additional amino acid substitutions in HA1, namely D232N introducing a potential N-linked glycosylation site in HA1 (n = 4), D229N (n = 2), S120T (n = 2), G141R with D232N (n = 2).

3.5.2. Antigenic characterization

All 20 antigenically characterized B/Yamagata lineage viruses were characterized as similar to the quadrivalent 2018/2019 vaccine virus component B/Phuket/3073/2013 (Table 1).

4. Conclusions

During the 2018/2019 season, influenza A(H1N1)pdm09 and A (H3N2) viruses co-dominated in the WHO European Region, while there were low levels of influenza B virus circulation. The genetic analysis of circulating viruses showed that both influenza A subtypes as well as influenza B lineage viruses are evolving. A (H1N1)pdm09 viruses have evolved from their 2009 ancestor and are becoming genetically more variable, but at a slower pace than A(H3N2) viruses [16]. In contrast, A(H3N2) viruses continued to exhibit high genetic heterogeneity, with a higher prevalence of clade 3C.3a viruses compared with 2017/2018, but with 3C.2a1b viruses being the most prevalent. B/Victoria viruses were also highly divergent, with four distinct antigenic variants co-circulating in the Region and worldwide. The evolution of B/Yamagata viruses did not have implications for the vaccine strain selection so far.

Based on data from the 2018/2019 season, WHO recommended for the 2019/2020 season to change the A(H1N1)pdm09 and A (H3N2) components to an A/Brisbane/02/2018 A(H1N1)pdm09-like virus (clade 6B.1A1) and an A/Kansas/14/2017 A(H3N2)-like virus (clade 3C.3a), respectively [17,18].

In large parts of the WHO European Region, during 2018/2019, influenza A(H1N1)pdm09 viruses predominated and consistently resembled the 2018/2019 vaccine virus component A/Michigan/45/2015 in both antigenic and genetic characterization data. However, phylogenetic analysis showed that 90% of circulating subgroup 6B.1A viruses carried amino acid substitution S183P, which is on an antigenic epitope of HA1. Together with the observation that post-vaccination human sera showed reduced titres against recent 6B.1A viruses compared with the titres against the 2018/2019 vaccine virus (6B.1), the fixation of the S183P substitution in the viral population supported the change in the A(H1N1) pdm09 vaccine virus component to a 6B.1A1 virus for the 2019/2020 influenza season [5,6].

The situation was more complex for A(H3N2) viruses as several genetic subclades continued to co-circulate and diversify. 3C.2a viruses exhibited high genetic heterogeneity and several subclades co-circulated, in some cases with additional amino acid substitutions. Subclade 3C.2a1b was the most divergent group and included the majority of emerging A(H3N2) subclusters, the most prevalent ones being those with additional amino acid substitutions in HA1, either T131K or T135K combined with T128A. Although these viruses carried substitutions at HA antigenic epitopes, antigenic data from the NICs and from the WHO CC indicated that viruses within the 3C.2a subclade remained

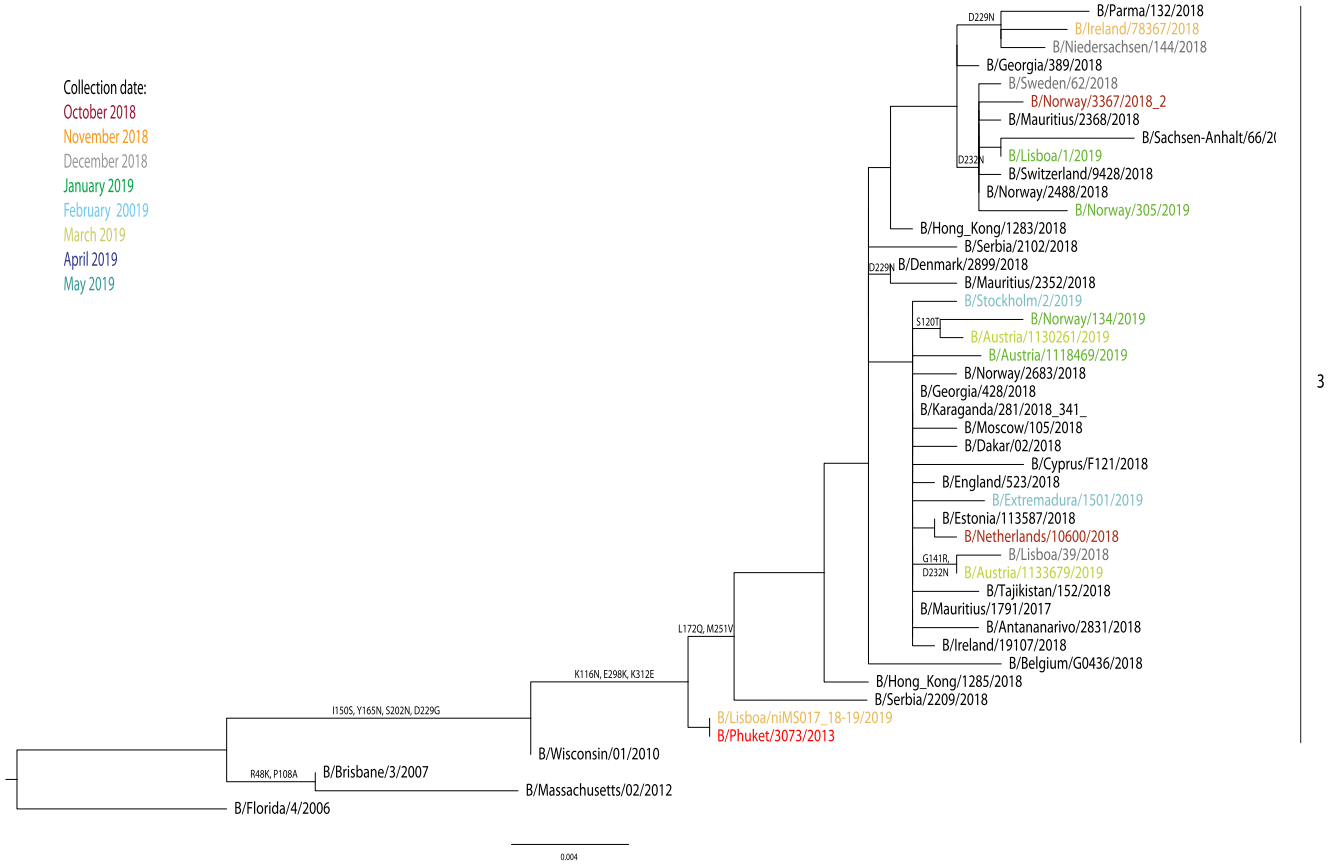

Fig. 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA gene sequences. Colour coding indicates the northern hemisphere 2018/2019 vaccine strain in red, reference strains in black and TESSy sequences according to the virus collection date by month. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

antigenically similar to the cell-based 2018/2019 vaccine virus component [2,5].

Antigenically distinct clade 3C.3a viruses were circulating in smaller numbers than 3C.2a; based on the genetic characterization data, their proportion among all characterized A(H3N2) viruses increased from 0% in the 2017/2018 season to 23%. The United States reported a more pronounced increase of 3C.3a viruses, where their relative proportion reached 60% in 2018/2019 [19,20]. WHO recommended including a 3C.3a virus in the 2019/2020 vaccine because of the wide circulation of viruses that belonged to this clade in some regions; the increasing trend observed until February in countries of the WHO European Region supported the recommendation at the time [5,17,19]. However, the proportion of this clade decreased to 11% of viruses tested in April, and 89% were subclade 3C.2a1b. This change was reflected in the subsequent recommendation for the 2020 southern hemisphere vaccine, in which a 3C.2a1b variant was included as the A(H3N2) component [21].

Interim VE estimates against all influenza A viruses for 2018/2019 from six studies in Europe ranged from 32 to 43% in persons of all ages seen in primary care. VE was higher (40–71%) against A(H1N1)pdm09 viruses, while the vaccine was not effective against A(H3N2) [22]. Estimates from studies in Canada, Hong Kong and the United States varied depending on the population studied and the proportions of circulating influenza A virus subtypes in each region [23–25]. Overall, a lower VE against A (H3N2) viruses was observed globally during 2018/2019, and was mainly driven by lack of effectiveness in the 15–64 year old people. A lower VE against A(H3N2) was also observed in previous seasons and has been partly attributed to the egg-adaptive mutations that the virus acquires during the preparation of candidate vaccine virus and which impact its antigenicity [26–29]. Our results show co-circulation of antigenically divergent influenza A (H3N2) viruses in the European Region, which could be another reason for the poor VE against these viruses during 2018/2019. The analyses of characterization data suggest that the proportions of circulating influenza subtypes/lineages and their subclades may differ across countries or regions and this may have differing implications for the VE in any given country.

New B/Victoria-lineage groups have also recently emerged and are circulating in the Region since 2017/2018 [8,30]. In the Δ2 group, the HA gene encodes a double deletion of amino acid residues 162–163 of HA1. The two additional Δ3 groups (Asian and West African) both encode a triple deletion of residues 162–164 of HA1. Although there were very few B/Victoria-lineage viruses during the 2018/2019 influenza season, the newly emerged antigenically distinct subgroups with triple deletions predominated over the ancestral B/Brisbane/60/2008 variant and the 2018/2019 vaccine virus B/Colorado/06/2017. Furthermore, the phylogenetic analysis revealed that the West African group was more frequent than the Asian group. As these groups are antigenically distinct from the virus component of 2018/2019 and 2019/2020 vaccines and from each other, it is crucial to continue to monitor them [2,31]. In contrast, the few characterisations of B/Yamagata lineage viruses in European national reference laboratories suggested that they remain uniformly close to the recommended B/Phuket/3073/2013 strain included in the quadrivalent vaccine for 2018/2019 and 2019/2020.

Although regional analyses of national laboratory characterisation data are helpful, they also have limitations. The results from such analyses cannot be used to generate conclusions for the Region overall. Only 30 (60%) of 50 countries that reported influenza detection data contributed virus characterization data to varying extents and less than 5% of viruses detected by surveillance were characterized. In addition, there was no information on the selection criteria, so data may be biased. There may, however, also be biases in the selection of viruses shipped to the Collaborating Centres, and to the subset of data presented at real-time tracking platforms, so the national characterisation data can serve to substantiate these analyses. Furthermore, not all sequences had been submitted to GISAID at the time of analysis and therefore there are small discrepancies in the proportions of genetic clades between the TESSy reported genetic data and the data derived from the phylogenetic analyses. Finally, incomplete reference antiserum panels may have been used for antigenic characterisation; NICs are encouraged to request the most updated set of reference antisera for their antigenic assessment to be able to discriminate and accurately characterise the different circulating strains [12].

Simultaneous circulation of genetically and antigenically diverse A(H3N2) and B/Victoria viruses present a challenge to vaccine strain selection. While the genetic diversity observed among A (H1N1)pdm09 subclade 6B.1A viruses and A(H3N2) clade 3C.2a viruses appeared not to cause antigenic dissimilarity in HI assays compared to their egg/cell or cell-derived vaccine viruses respectively, antigenically distinct A(H3N2) clade 3C.3a and low levels of antigenically distinct B/Victoria viruses were detected in the WHO European Region. Influenza surveillance in the Region would be further strengthened by increasing the number of countries reporting genetic and antigenic data, increasing the number and frequency of antigenic and genetic reports per country, and improving the representativeness of viruses selected for characterization. As it was illustrated in this paper when comparing TESSy categories with the more detailed phylogenetic analysis, moving away from weekly reporting of genetic categories to TESSy to real-time analyses of weekly reported sequences to GISAID will increase accurate and timely reporting of emerging clades, subgroups and amino acid substitutions with antigenic implications, highlighting the important role of platforms for real-time tracking of pathogen evolution for public health decision making. Timely sharing and reporting of genetic data before the VCM is critical to the decision-making process of recommending influenza strains for inclusion in the vaccines.

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Marius Valentin Valcu from the ECDC TESSy management team for technical support. We would like to thank Erik Alm (ECDC) for the development of the influenza genetic analysis tool that was used in this study. We would also like to thank Phillip Zucs, Mike Catchpole (ECDC) and the reviewers of the 'Summary of influenza virus antigenic and genetic characterizations in Europe: data reported by National Influenza Centres to The European Surveillance System for weeks 40/2018 to week 20/2019′ for their valuable comments. We would like to thank John McCauley and Rodney Daniels (WHO Collaborating Centre in London) for their support and efforts during the influenza seasons. We acknowledge the providers of the HA sequences retrieved from the GISAID database EpiFlu for use in this study. We acknowledge the members of the European Region influenza surveillance network and all of the involved parties for their efforts for influenza surveillance data collection. The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions or policies of the institutions with which they are affiliated.

Country acknowledgements

Albania: We would like to acknowledge all the staff of the unit of laboratory surveillance of viral emergent diseases and the head of Epidemiology and control of communicable disease department, Dr Silvia Bino. Also the support in sequencing, antiviral resistance and further analyses of our samples from WHO CCs in London and CDC in Atlanta, Georgia. The support received from SECID, WHO and CDC.

Belgium: We would like to thank Ilham Fdillate for excellent technical assistance. Next Generation Sequencing was performed at the service Transversal activities in Applied Genomics at Sciensano.

England: We would like to acknowledge all staff of the Respiratory Virus Unit, National Infection Service, Public Health England.

Greece: We would like to acknowledge Professor Anna Papa and Maria Christoforidi for their contributions to the NIC work.

Finland: We would like to thank physicians and nurses of sentinel network sites and intensive care units as well as clinical laboratories for their contribution in providing respiratory specimens.

France: We would like to acknowledge the general practitioners and pediatricians of the Sentinelles network, the members of the RENAL network of hospital laboratories for contributing specimens and our colleagues from Hospices Civils at Lyon: Martine Valette and Bruno Lina.

Italy: Angela Di Martino, Marzia Facchini, Antonino Bella (Istituto Superiore di Sanità, Rome); Caterina Rizzo (Bambino Gesù Children's Hospital, Rome); the Regional Laboratory Network for influenza (InfluNet).

Netherlands: Mark Pronk and Ruud van Beek performing characterisation of non-sentinel influenza viruses, Viroscience, Erasmus Medical Centre, National Influenza Centre location Rotterdam. We thank all laboratories in The Netherlands that submitted influenza virus positive specimens for further

characterisation to the NIC location Rotterdam. Pieter Overduin and Sharon van den Brink performing characterisation of sentinel influenza viruses, and further technicians involved in influenza surveillance, Centre for Infectious Disease Research, Diagnostics and laboratory surveillance, National Institute for Public Health and the Environment, National Influenza Centre location Bilthoven; Gé Donker, coordinator of Nivel Primary Care Database sentinel GPs; Wim van der Hoek, Anne Teirlinck, Marit de Lange, Daphne Reukers, epidemiologists at Centre for Infectious Diseases, Epidemiology and Surveillance, National Institute for Public Health and the Environment, Bilthoven. Sequencing of sentinel influenza viruses was made possible through a grant from ECDC: OJ/2018/OCS/9105 "Monitoring influenza vaccine effectiveness (seasonal and pandemic) in EU/EEA" and the Specific Contracts n°1 — ECD.9118 implementing activities to the Framework Contract n° ECDC/2018/029.

Norway: We would like to acknowledge the contributing laboratories and sentinel practices in the national influenza virus surveillance, as well as all staff in the National Influenza Centre in the Norwegian Institute of Public Health, in particular Marie Paulsen Madsen who carried out most of the sequencing.

Romania: Odette Popovici and Rodica Popescu – coordinators from the National Institute of Public Health Romania-National Centre for Communicable Diseases Surveillance and Control.

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Wales: We would like to acknowledge the Wales specialist virology centre and public health Wales pathogen genomics centre. For virology: Laura Gifford, Joanne Watkins, Tom Connor, Matt Bull and Joel Southgate. For epidemiology: Ember Hilvers, Richard Lewis, Caroline Harris and Malory Perry.

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Nataša Berginc, National Laboratory for Health, environment and Food.

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Wales (United Kingdom): Catherine Moore, Simon Cottrell.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2020.06.031.

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