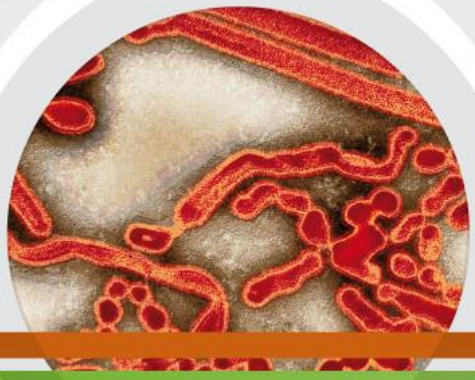

SURVEILLANCE REPORT

Influenza virus characterisation

Summary Europe, November 2014

Summary

In light of the emergence of antigenically distinct groups of influenza A(H3N2) and the altered prevalence of influenza B viruses, the WHO-recommended composition of influenza vaccines for use in the 2015 southern hemisphere influenza season differs from that recommended for use in the 2014–15 northern hemisphere influenza season.

Based on reports submitted to TESSy, the 2014–15 influenza season has not yet started in the WHO European Region. Although an increasing number of countries are reporting sporadic influenza detections, overall numbers are low (less than 1400 over weeks 40–50/2014). Influenza type A is predominating over type B by a ratio of 3:1, with A(H3N2) viruses predominating over A(H1N1)pdm09 by a ratio of 5:1 and B/Yamagata lineage viruses predominating over B/Victoria by a ratio of 16:1.

To date, only eight specimens with collection dates after week 39/2014 have been received at the London WHO Collaboration Centre (CC): three A(H1N1)pdm09, four A(H3N2), and one B/Yamagata lineage. Only one H1N1pdm09 and two H3N2 viruses were recovered. National Influenza Centres (NICs) are encouraged to send influenza-positive specimens as soon as possible, and no later than week 6/2015, to allow the London WHO CC to conduct detailed characterisation in time for the February (week 9/2015) WHO Vaccine Consultation Meeting.

The A(H1N1)pdm09 virus, as those circulating worldwide, belonged to genetic subgroup 6B and was antigenically similar to the vaccine virus A/California/07/2009.

The A(H3N2) viruses fell in genetic subgroups 3C.3 and 3C.3a and were poorly recognised by antisera raised against the A/Texas/50/2012 vaccine virus but well recognised by antisera raised against cell-propagated 3C.3a viruses.

The unrecovered B/Yamagata lineage virus fell in genetic clade 3 as for the majority of B/Yamagata viruses circulating worldwide.

The previously recognised difficulties with A(H3N2) viruses in relation to obtaining virus isolates for antigenic characterisation is exacerbated with genetic subgroup 3C.2a viruses. In culture, cytopathic effects are commonly seen, but HA titres are either very low or non-existent. This can have implications for virus detection and surveillance.

This report was prepared by Rod Daniels, Vicki Gregory and John McCauley on behalf of the European Reference Laboratory Network for Human Influenza (ERLI-Net), under contract to the European Centre for Disease Prevention and Control (ECDC).

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Influenza-positive samples, 22 viruses or clinical specimens, with collection dates after 31 May 2014 have been received at the MRC National Institute for Medical Research, WHO Collaborating Centre for Reference and Research on Influenza (WHO CC), from six countries in the EU/EEA. The majority (77.3%) were type A viruses, and A(H3N2) outnumbered A(H1N1)pdm09 at a ratio of 3:1 (Table 1). Of the five type B specimens received (~22.7% of the specimens), only three were recovered; all were of the B/Yamagata-lineage. Only eight of the viruses/clinical specimens received have been within the period since the start of monitoring (week 40/2014) for the 2014–2015 influenza season: three A(H1N1)pdm09, four A(H3N2) and one B/Yamagata-lineage viruses. Only one A(H1N1)pdm09 and two A(H3N2) viruses were recovered at sufficient HA titre to allow antigenic characterisation.

Table 1. Summary of clinical samples and virus isolates received from EU/EEA Member States, with collection dates after 31 May 2014

MONTH	TOTAL RECEIVED	A	H1N1pdm09		H3N2		B	B Victoria lineage		B Yamagata lineage	
Country			Number received	Number propagated[1]	Number received	Number propagated[2]		Number received	Number propagated[1]	Number received	Number propagated[1]
2014											
JUNE											
France	1		1	1							
Iceland	1				1	1					
Norway	4				3	2				1	1
Spain	1				1	1					
United Kingdom	1				1	1					
JULY											
Spain	4				1	1	1			2	2
AUGUST											
France	1				1	1					
Norway	1				1	0					
OCTOBER											
Norway	6		3	1	3	1					
Slovenia	2				1	1				1	0
	22	0	4	2	13	9	1	0	0	4	3
			18.2%		59.1%			0.0%		18.2%	
6 Countries			77.3%				22.7%				

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 presence of 20nM oseltamivir (the totalled number does not include any from batches that are in process)

Influenza A(H1N1)pdm09 virus analyses

The results of haemagglutination inhibition (HI) analyses of viruses performed since the September 2014 report[1] are shown in Table 2. The test virus, a genetic subgroup 6B virus, was antigenically similar to the vaccine virus, A/California/7/2009, indicating that no significant antigenic drift has occurred, in agreement with the September 2014 report.

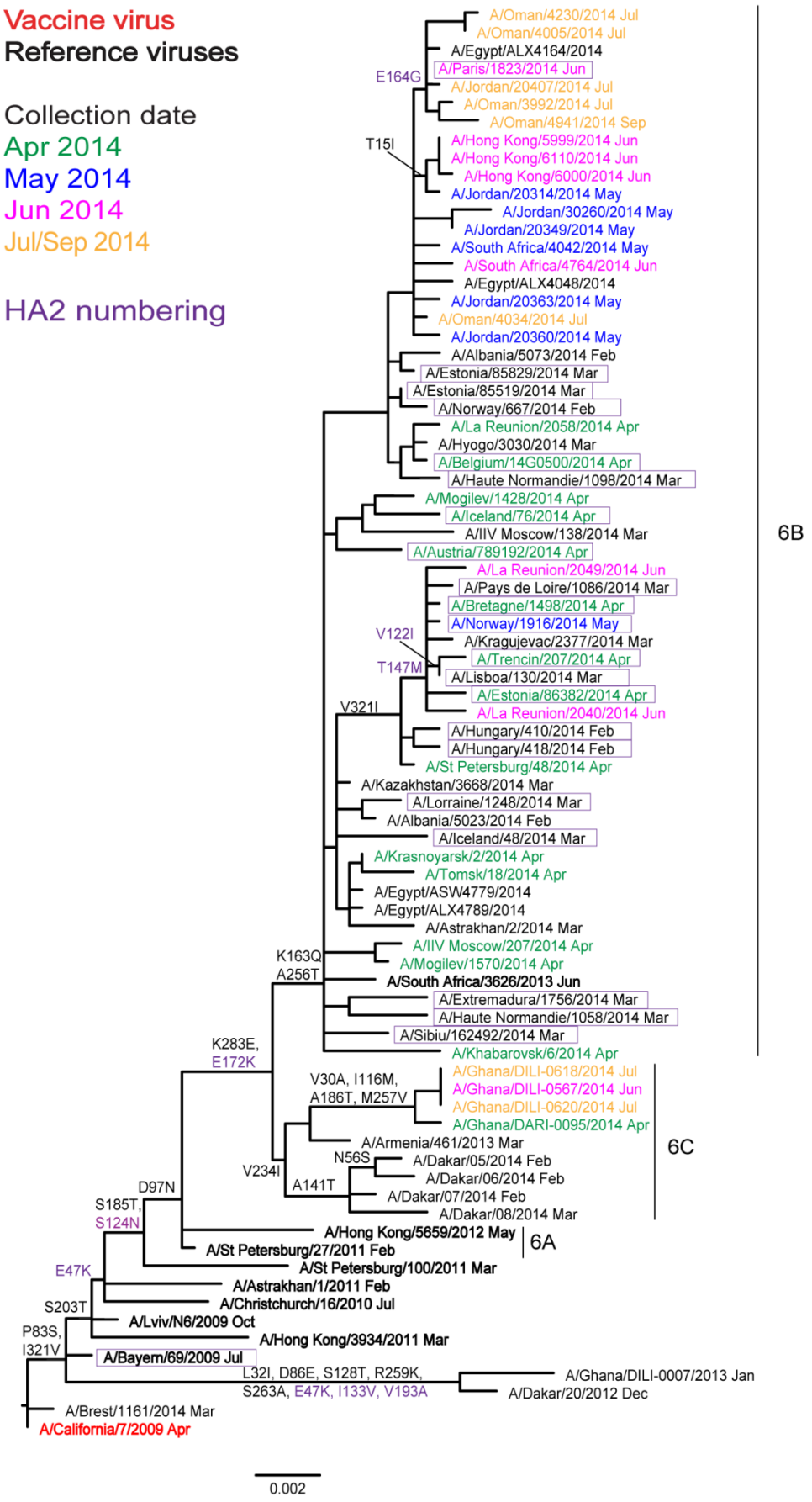
Figure 1 shows a phylogenetic tree for the HA genes of representative A(H1N1)pdm09 viruses. Since 2009, the HA genes have evolved, and eight genetic groups have been designated, with A/California/7/2009 representing group 1. However, over the last season, viruses in genetic group 6 have predominated, and all EU/EEA viruses characterised since the September 2014 report carried HA genes in genetic subgroup 6B. This subgroup carries the amino acid substitutions **D97N**, **K163Q, S185T, S203T, A256T** and **K283E** in **HA1** and **E47K, S124N and E172K** in **HA2** compared with A/California/7/2009.

[1] European Centre for Disease Prevention and Control. Influenza virus characterisation, summary Europe, July 2014. Stockholm: ECDC; 2014. Available from: http://www.ecdc.europa.eu/en/publications/Publications/Influenza-ERLI-Net-report-Sept-2014.pdf

Table 2. Antigenic analysis of A(H1N1)pdm09 viruses by HI

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre: Post infection ferret antisera A/Cal 7/09	A/Bayern 69/09	A/Lviv N6/09	A/Chch 16/10	A/HK 3934/11	A/Astrak 1/11	A/St. P 27/11	A/St. P 100/11	A/HK 5659/12	A/Sth Afr 3626/13	
				F30/11	F11/11	F14/13	F30/10	F21/11	F22/13	F26/14	F24/11	F30/12	F3/14	
	Genetic group						4	3	5	6	7	6A	6B	
REFERENCE VIRUSES														
A/California/7/2009		2009-04-09	E1/E2	640	640	1280	160	160	160	160	320	160	160	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK2	160	320	320	40	40	40	40	80	40	80	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	320	1280	1280	160	80	80	80	160	160	160	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	1280	2560	5120	2560	2560	640	5120	2560	2560	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	320	160	160	160	640	640	320	1280	640	640	
A/Astrakhan/1/2011	5	2011-02-28	MDCK4/MDCK1	640	640	640	320	1280	1280	640	2560	2560	1280	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	640	1280	640	320	1280	1280	640	2560	1280	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	640	640	640	320	1280	1280	640	2560	2560	1280	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	320	160	160	160	640	640	320	1280	1280	640	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	320	640	640	320	640	640	640	2560	640	1280	
TEST VIRUSES														
A/Norway/2432/2014	6B	2014-10-02	MDCK2	1280	320	320	640	1280	1280	640	5120	2560	1280	
				Vaccine										

Figure 1. Phylogenetic comparison of influenza A(H1N1)pdm09 HA genes



Influenza A(H3N2) virus analyses

As described in many previous reports[2], influenza A(H3N2) viruses continue to be difficult to characterise antigenically by HI assay due to variable agglutination of red blood cells (RBCs) from guinea pigs, turkeys and humans. Of the four H3N2 samples received with collection dates since the start of monitoring (week 40/2014) for the 2014–2015 influenza season, two were not recovered (no cytopathic effect seen in culture, no HA titre on the RBCs indicated above and no NA activity [based on MUNANA assay]).

Results of HI tests performed with guinea pig RBCs in the presence of 20nM oseltamivir are shown in Table 3. A/Norway/3004/2014 falls in genetic subgroup 3C.3 and A/Slovenia/1944/2014 in genetic subgroup 3C.3a. Both test viruses reacted poorly in HI assays (≥ sixteenfold decrease) with post-infection ferret antiserum raised against the egg-propagated vaccine virus, A/Texas/50/2012, compared to the titre of the antiserum with the homologous virus. Similar results were seen with antisera raised against the egg-propagated reference viruses A/Hong Kong/146/2013 and A/South Africa/4655/2013. Somewhat better reactivity was observed with antisera raised against egg-propagated A/Stockholm/6/2014 and A/Switzerland/9715293/2013, with both test viruses reacting within fourfold and eightfold, respectively, of the titres of the antisera with their homologous viruses (Table 3).

Ferret antisera raised against reference viruses exclusively propagated in tissue culture cells – A/Samara/73/2013, A/Stockholm/6/2014, A/Norway/466/2014, A/Switzerland/9715293/2013 (Table 3), and the exclusively cell-propagated cultivar of A/Victoria/361/2011 – recognised the test viruses more effectively. The reference viruses A/Stockholm/6/2014, A/Norway/466/2014 and A/Switzerland/9715293/2013 are representative of genetic subgroup 3C.3a viruses. The A/Slovenia/1944/2014 virus (3C.3a) was recognised less well by antisera raised against the cell-propagated cultivar of A/Victoria/361/2011 and A/Samara/73/2013 than A/Norway/3004/2014 (3C.3) as observed previously for viruses in these two genetic groups (see September 2014 report). Antisera raised against cell-propagated reference viruses A/Stockholm/6/2014, A/Norway/466/2014 and A/Switzerland/9715293/2013 recognised both test viruses at titres within fourfold of the titres of the antisera with their homologous viruses.

Since 2009, seven genetic groups based on the HA gene have been defined for H3N2 viruses. Phylogenetic analysis of the HA genes of representative, recently circulating H3N2 viruses is shown in Figure 2. The vaccine virus A/Texas/50/2012 belongs to genetic subgroup 3C.1. Viruses characterised genetically since the September 2014 report fall into subgroups 3C.2, 3C.2a, 3C.3 and 3C.3a, with viruses in 3C.2a and 3C.3a increasing in frequency over recent months (Figure 2). Amino acid substitutions that define subgroups 3C.2, 3C.2a, 3C.3 and 3C.3a are:

- 3C.2 **N145S** in **HA1**, and **D160N** in **HA2**, e.g. A/Hong Kong/146/2013;
- 3C.2a: Those in 3C.2 plus **L3I, N144S** (resulting in the loss of a potential glycosylation site)**, F159Y, K160T** (resulting in the gain of a potential glycosylation site)**, N225D** and **Q311H** in **HA1,** e.g. A/Hong Kong/5738/2014;
- 3C.3 **T128A** (resulting in the loss of a potential glycosylation site), **R142G,** and **N145S** in **HA1**, e.g. A/Samara/73/2013;
- 3C.3a: Those in 3C.3 plus **A138S**, **F159S** and **N225D** in **HA1**, many with **K326R**, e.g. A/Switzerland/9715293/2013.

Most recently, based on genetic characterisation, the 3C.2a viruses have risen to prominence. The previously recognised issues with H3N2 viruses in relation to obtaining virus isolates for antigenic characterisation is exacerbated with genetic subgroup 3C.2a viruses. In culture, cytopathic effects are commonly seen, but HA titres are either very low or non-existent on turkey, guinea pig and human red blood cells, and those viruses with HA titres in the range 4–8 often lose titre in the presence of 20nM oseltamivir (added to overcome agglutination mediated by NA) or lose titre on short-term storage at 4 °C. Consequently, there could be a systematic underreporting of H3N2 virus isolation, and NICs are encouraged to use measurement of NA activity as a surrogate biological assay to indicate virus isolation, allowing subsequent genetic characterisation.

The relatively few 3C.2a viruses that have been subjected to HI analysis show poor reactivity with post-infection ferret antiserum raised against the egg-propagated vaccine virus, A/Texas/50/2012, compared to the titre of the antiserum with the homologous virus. Somewhat better reactivity has been observed with antisera raised against 3C.3a viruses in both HI and MN assays.

In light of the emergence of antigenically distinct groups of influenza A(H3N2) viruses, notably 3C.3a viruses at the time of the September WHO Vaccine Consulation Meeting, the recommendation for the H3N2 component of influenza vaccines for use in the 2015 southern hemisphere influenza season differed from that recommended for use in the 2014–15 northern hemisphere influenza season. The recommendation made on 25 September 2014 was that the vaccine should contain an

[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 http://www.ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-sep-2013.pdf

A/Switzerland/9715293/2013 (H3N2)-like virus, and advised that A/Norway/466/2014, A/Stockholm/6/2014 and A/South Australia/55/2014 are A/Switzerland/9715293/2013 (H3N2)-like viruses. (Available from: http://www.who.int/influenza/vaccines/virus/recommendations/201409_recommendation.pdf). Egg-propagated cultivars of these four viruses are available for development for use in vaccines.

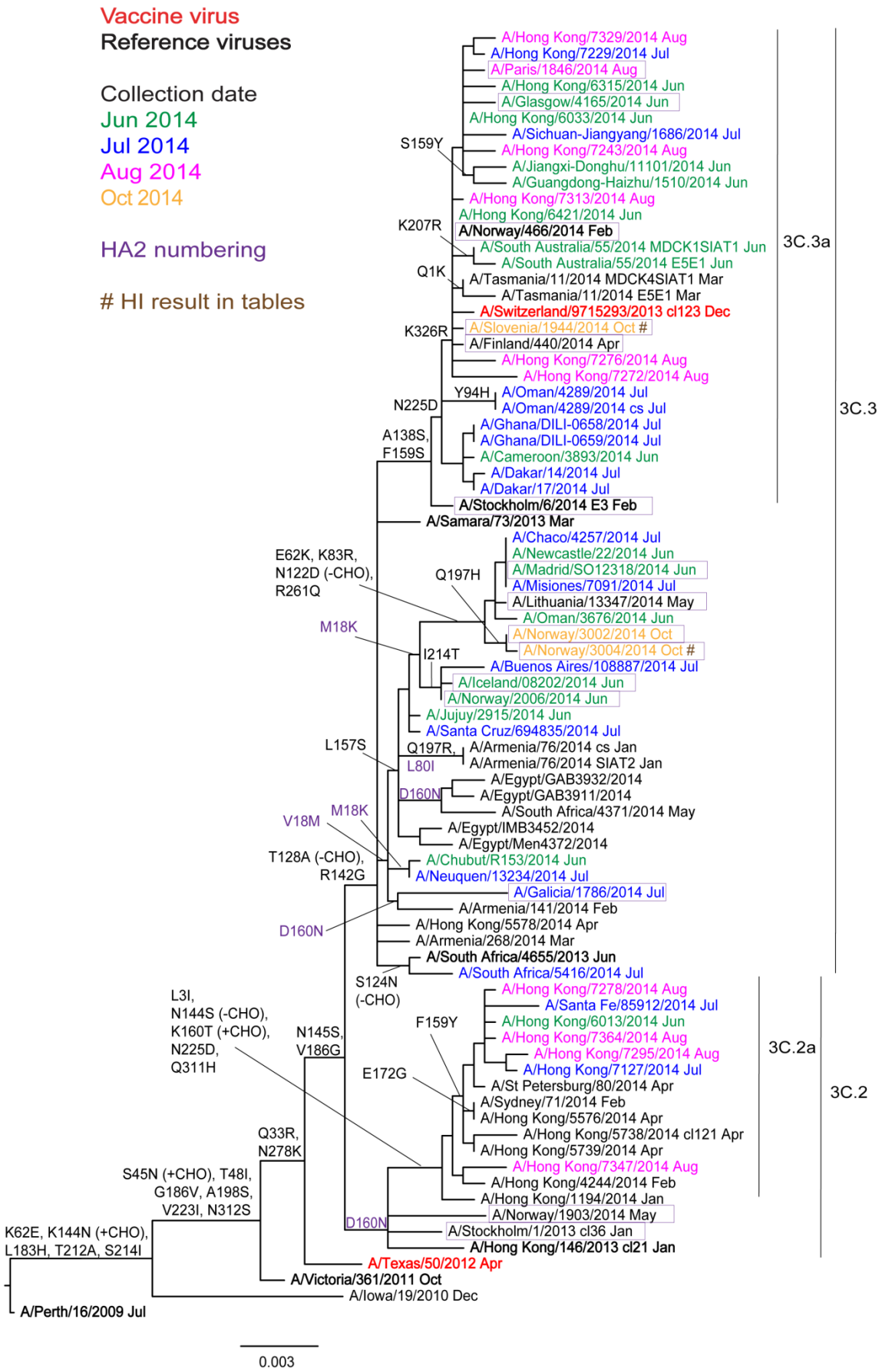
Table 3. Antigenic analysis of A(H3N2) viruses by HI (guinea pig RBC with 20nM oseltamivir)

				Haemagglutination inhibition titre[1]										
				Post infection ferret antisera										
Viruses	Genetic group	Collection Date	Passage History	A/Perth 16/09	A/Vic 361/11	A/Texas 50/12	A/Samara 73/13	A/HK 146/13	A/Sth Afr 4655/13	A/Stock 6/14	A/Stock 6/14	A/Nor 466/14	A/Switz 9715293/13	A/Switz 9715293/13
				F18/11 T/C	F09/12	Egg F42/13	F24/13	F40/13	F10/14 T/C	F14/14	Egg F20/14	F13/14	NIB F13/14	F25/14
					3C.1	3C.1	3C.3	3C.2	3C.3 cl 101-60	3C.3a	3C.3a isolate 2	3C.3a	3C.3a	3C.3a cl123
REFERENCE VIRUSES														
A/Perth/16/2009		2009-07-04	E3/E3	640	160	160	160	320	40	80	80	80	40	80
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT5	160	320	160	640	320	80	320	160	160	80	160
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	640	1280	1280	1280	1280	160	320	640	80	80	1280
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT4	640	320	320	1280	640	160	640	320	320	160	640
A/Hong Kong/146/2013	3C.2	2013-01-11	E6	640	640	640	1280	2560	160	320	640	160	80	1280
A/South Africa/4655/2013	3C.3	2013-06-25	E7 clone 101-60	80	80	160	640	320	640	160	80	160	80	40
A/Stockholm/6/2014	3C.3	2014-02-06	SIAT1/SIAT4	<	80	80	320	160	80	640	320	320	320	160
A/Stockholm/6/2014	3C.3	2014-02-06	E4/E1 isolate 2	80	320	160	160	320	<	160	640	160	80	640
A/Norway/466/2014	3C.3a	2014-02-03	SIAT1/SIAT2	<	40	40	160	160	40	640	160	320	160	160
A/Switzerland/9715293/2013	3C.3a	2013-12-06	SIAT1/SIAT3	<	80	40	320	160	40	640	160	320	320	160
A/Switzerland/9715293/2013	3C.3a	2013-12-06	E4/E1 clone 123	40	160	160	160	320	<	320	320	160	80	1280
TEST VIRUSES														
A/Slovenia/1944/2014	3C.3a	2014-10-09	SIAT1	<	40	40	160	80	40	640	160	320	160	160
A/Norway/3004/2014	3C.3	2014-10-16	SIAT1	40	80	80	320	160	40	320	160	320	80	160
						Vaccine SH2014 NH 2014/15								Vaccine SH2015

1. < = <40

Sequences in phylogenetic tree

Figure 2. Phylogenetic comparison of influenza A(H3N2) HA genes



Influenza B virus analyses

Only one B/Yamagata-lineage sample with a collection date after 31 August 2014 has been received from EU/EEA countries since the September 2014 report[1]; the sample was not recovered as a virus isolate, but genetic sequencing from the clinical specimen was performed.

Influenza B – Victoria lineage

Figure 3 shows a phylogenetic analysis of the HA genes of representative B/Victoria-lineage viruses. The HA genes of viruses collected since 01 April 2014 fall into the B/Brisbane/60/2008 genetic clade (clade 1A) and have been antigenically similar to the recommended vaccine virus, B/Brisbane/60/2008, for use in quadrivalent vaccines.

Influenza B – Yamagata lineage

Figure 4 shows a phylogenetic analysis of the HA genes of representative B/Yamagata-lineage viruses. The HA genes of viruses collected since 1 April 2014 fall into the B/Massachusetts/02/2012 clade (clade 2) and the B/Wisconsin/1/2010 and B/Phuket/3073/2014 clade (clade 3), with those in clade 3 being in the majority in recent months. The A/Slovenia/1964/2014 specimen, which was not recovered in culture, fell in genetic clade 3. A small number of viruses were identified with HA genes of clade 3 of the B/Yamagata lineage combined with NA genes of the B/Victoria lineage. Similar reassortant viruses have been detected in many parts of the world.

As a result of the prevalence of influenza B viruses of the B/Yamagata lineage, with the majority having HA genes falling in clade 3, WHO recommended that the influenza vaccine for use in the 2015 southern hemisphere influenza season should contain a virus from that clade and recommended that the vaccine contain a virus antigenically similar to B/Phuket/3073/2013.

Figure 3. Phylogenetic comparison of influenza B/Victoria-lineage HA genes

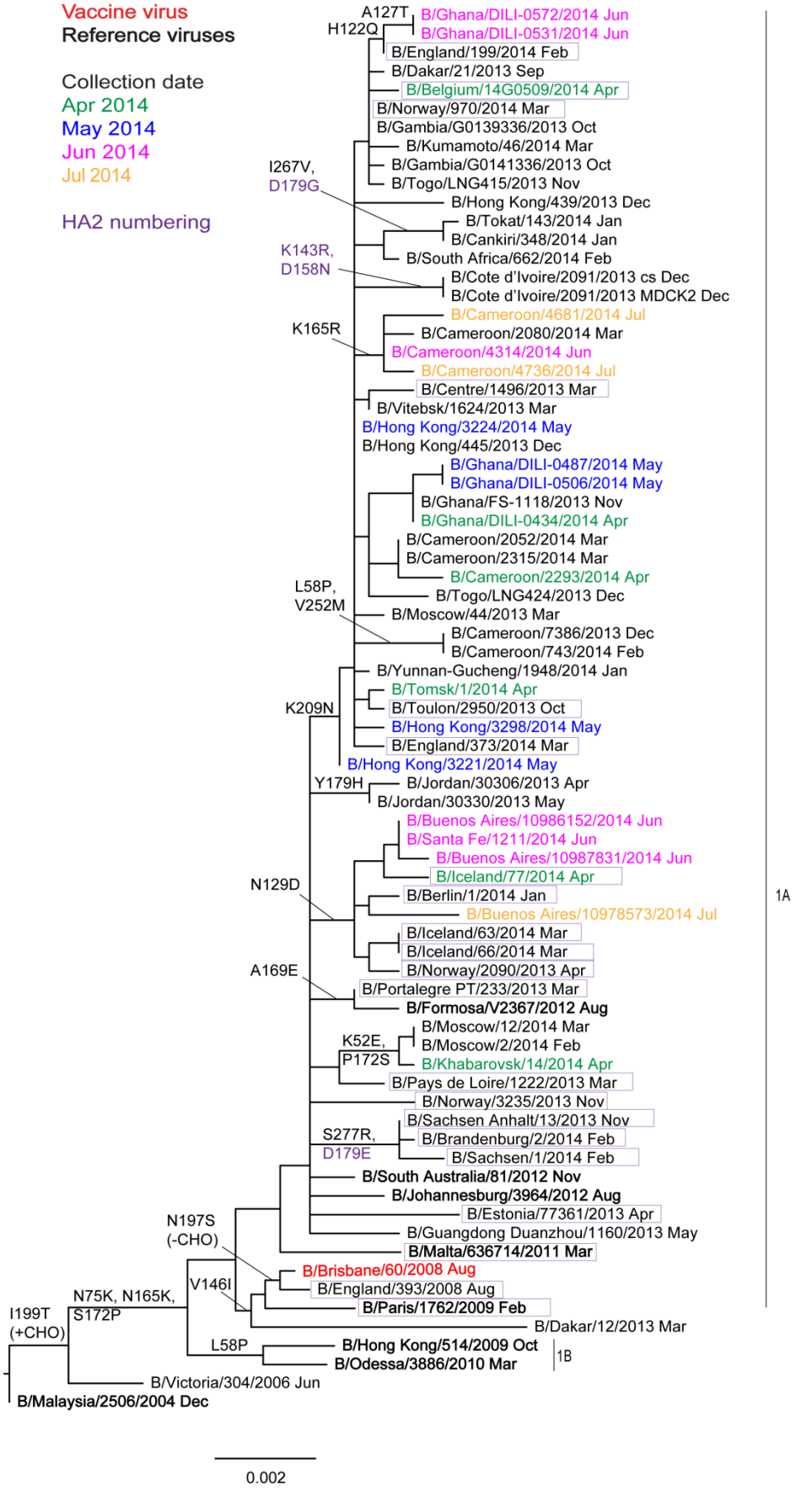
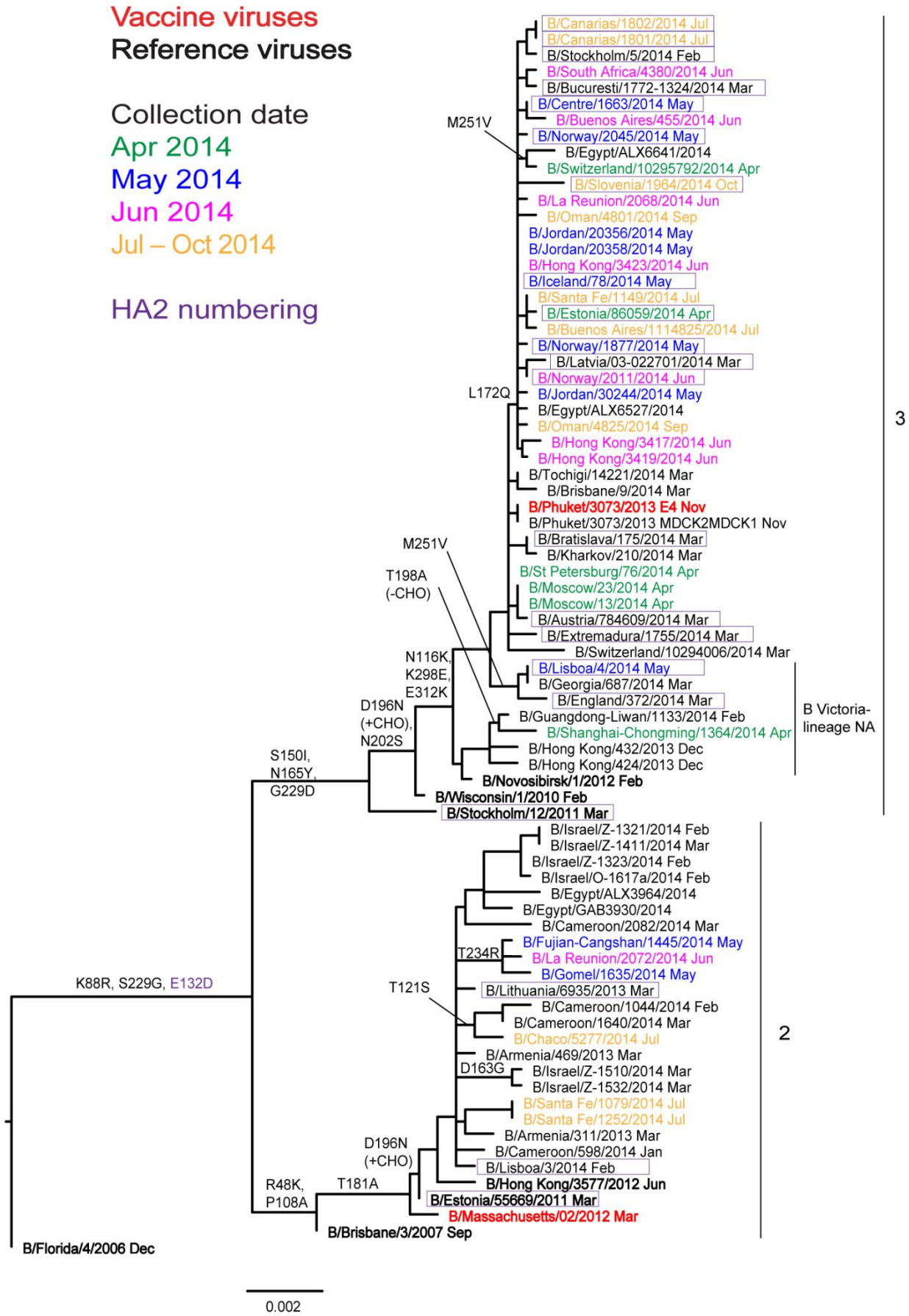


Figure 4. Phylogenetic comparison of influenza B/Yamagata-lineage HA genes



Summary of genetic data submitted to TESSy

As of 17 December 2014, the majority of influenza viruses identified genetically since week 40/2014 were A(H3N2) viruses (79%), with lower numbers of influenza B viruses (13%) and A(H1N1)pdm09 viruses (8%) being reported.

All influenza A(H1N1)pdm09 viruses fell into genetic clade 6B, represented by A/South Africa/3626/2013. Influenza B viruses of the B/Yamagata lineage outnumbered those of the B/Victoria lineage by 4 to 1. The majority of influenza A(H3N2) viruses belonged to genetic subgroup 3C.2a (56%), represented by A/Hong Kong/5738/2014; smaller proportions were in genetic subgroup 3C.3 (28%), represented by A/Samara/73/2013, genetic subgroup 3C.3a (8%), represented by A/Switzerland/9715293/2013, and genetic subgroup 3C.1 (8%), represented by A/Texas/50/2012, the vaccine virus for the 2014/2015 northern hemisphere influenza season.

Antiviral susceptibility

Between weeks 40–50/2014, based on reports to TESSy, 88 influenza viruses (70 A(H3N2), 17 A(H1N1)pdm09 and one type B) were subjected to phenotypic or genotypic testing for neuraminidase inhibitor susceptibility. None showed evidence of reduced susceptibility to either oseltamivir or zanamivir. Fifty-three A(H3N2) viruses and two A(H1N1)pdm09 viruses were assessed for M2 ion channel blocker (adamantane) susceptibility; all were resistant due to the S31N amino acid substitution in the M2 protein.

Viruses with collection dates after 31 September 2014 have not been assessed for antiviral susceptibility at the London WHO CC. However, 356 viruses recovered at the London WHO CC, with collection dates after 31 January 2014, have been subjected to phenotypic testing against oseltamivir and zanamivir: 157 A(H1N1)pdm09, 171 A(H3N2) and 28 influenza B viruses. All viruses showed normal inhibition (NI) by zanamivir. A single (0.6%) A(H1N1)pdm09 virus showed highly reduced inhibition (HRI) by oseltamivir due to NA H275Y amino acid substitution, and three (1.8%) A(H3N2) viruses showed reduced inhibition by oseltamivir (RI; fold increases in IC50 of 10–26) with the three viruses having NA S331R amino acid substitution in common. All influenza B viruses showed NI by oseltamivir. M-gene sequencing of A(H1N1)pdm09 and A(H3N2) viruses showed all to carry a S31N amino acid substitution in the M2-ion channel, which has been associated with resistance to adamantanes.

Influenza A(H7N9) virus

On 1 April 2013, the World Health Organization (WHO) Global Alert and Response [1] reported that the China Health and Family Planning Commission notified the WHO of three cases of human infection with influenza A(H7N9). The cases were confirmed by laboratory testing on 29 March 2013 by the Chinese CDC. A description of the characteristics of H7N9 viruses can be found on the WHO website [2]. Increased numbers of cases were reported over the course of the 2013–14 season. A revised ECDC Rapid Risk Assessment [3] for these A(H7N9) viruses was posted on 27 January 2014, and an updated summary of human infection was posted by WHO on 31 January 2014 [4] followed by an updated risk assessment on 27 June 2014 [5]. The most recent update of the epidemiological situation published by WHO was posted on 18 November 2014; five cases with symptom-onset since 1 September 2014 have been reported. WHO summarised the numbers of cases of human infection and their geographic location on 14 July 2014 [6].

WHO CC reports

A description of results generated by the WHO Collaborating Centre for Reference and Research on Influenza at the MRC National Institute for Medical Research in London, and evaluated at the WHO Vaccine Composition Meetings held at WHO Geneva on 17–19 February 2014 and 22–24 September 2014, can be found at:

http://www.nimr.mrc.ac.uk/documents/about/NIMR-report-Feb2014-web.pdf

http://www.nimr.mrc.ac.uk/documents/about/NIMR-VCM-report-Sep-14-web.pdf

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 ECDC countries are highlighted within boxes. Sequences for many viruses from non-EU/EEA countries were recovered from GISAID. We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's 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 the London WHO Collaborating Centre.

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