Report on influenza viruses received and tested by the Melbourne WHO Collaborating Centre for Reference and Research on Influenza in 2019

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Abstract

As part of its role in the World Health Organization's (WHO) Global Influenza Surveillance and Response System (GISRS), the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne received a record total of 9,266 human influenza positive samples during 2019. Viruses were analysed for their antigenic, genetic and antiviral susceptibility properties. Selected viruses were propagated in qualified cells or embryonated hen's eggs for potential use in seasonal influenza virus vaccines. In 2019, influenza A(H3N2) viruses predominated over influenza A(H1N1)pdm09 and B viruses, accounting for a total of 51% of all viruses analysed. The majority of A(H1N1)pdm09, A(H3N2) and influenza B viruses analysed at the Centre were found to be antigenically similar to the respective WHO recommended vaccine strains for the Southern Hemisphere in 2019. However, phylogenetic analysis indicated that a significant proportion of circulating A(H3N2) viruses had undergone genetic drift relative to the WHO recommended vaccine strain for 2019. Of 5,301 samples tested for susceptibility to the neuraminidase inhibitors oseltamivir and zanamivir, four A(H1N1)pdm09 viruses showed highly reduced inhibition with oseltamivir, one A(H1N1)pdm09 virus showed highly reduced inhibition with zanamivir and three B/Victoria viruses showed highly reduced inhibition with zanamivir.

Keywords: influenza, vaccines, GISRS, surveillance, laboratory, annual report, WHO

Introduction

The World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza in Melbourne (the Centre) is part of the World Health Organization Global Influenza Surveillance and Response System (WHO GISRS). GISRS is a worldwide network of laboratories that was established in 1952 to monitor changes in influenza viruses circulating in the human population, with the aim of reducing influenza's impact through the use of vaccines and antiviral drugs.[1,2] The Centre in Melbourne is one of five such Collaborating Centres (others are in Atlanta, Beijing, London and Tokyo) that monitor antigenic and genetic changes in circulating human influenza viruses; the Centre participates in making twice-yearly recommendations on which influenza strains should be included in the influenza vaccine for the upcoming influenza season in either the northern or southern hemisphere. This report summarises the results of virological surveillance activities undertaken at the Centre in Melbourne during 2019.

Two types of influenza viruses (A and B) cause significant disease in humans. Influenza A viruses are further classified into subtypes, based on their haemagglutinin (HA) and neuraminidase (NA) surface proteins. Globally, there are currently two influenza A subtypes circulating in the human population – A(H1N1)pdm09 and A(H3N2). Influenza B viruses are not classified into subtypes; however, there are two distinct co-circulating lineages of influenza

B viruses – B/Victoria/2/87 (B/Victoria lineage) and B/Yamagata/16/88 (B/Yamagata lineage). Influenza C viruses are also detected each year from humans, but these viruses do not cause severe disease and are not a major focus of influenza surveillance.

Methods

Virus isolation

All A(H1N1)pdm09 and influenza B viral isolates received at the Centre were re-passaged in cell culture using Madin-Darby Canine Kidney (MDCK) cells, whilst all A(H3N2) viral isolates were re-passaged in MDCK-SIAT-1 cells.[3] Virus isolation in cell culture was also attempted from a selection of original clinical specimens received using the same cell types as described above when the type/subtype was known. A smaller subset of influenza-positive original clinical samples was directly inoculated into eggs and a qualified cell line to generate potential candidate vaccine viruses (CVV).

Antigenic analysis

The antigenic properties of influenza viral isolates were analysed using the haemagglutination inhibition (HI) assay as previously described[4] and by the Focus Reduction Assay (FRA) for a subset of A(H3N2) viruses. The majority of the HI assays were performed using the TECAN Freedom EVO200 robot platform which incorporates a camera (SciRobotics, Kfar Saba, Israel) and imaging software (FluHema™) for automated analysis. In HI assays, viruses were tested for their ability to agglutinate turkey (A(H1N1)pdm09 and B viruses) or guinea pig (A(H3N2)) red blood cells (RBC) in the presence of post-infection ferret antisera raised against several reference viruses. Isolates were identified as antigenically similar to the reference strain if the test samples had a titre that was no more than 4-fold higher than the titre of the homologous reference strain. During 2019, results were reported with reference to the A/Michigan/45/2009 (H1N1pdm09)-like, A/Switzerland/8060/2017 (H3N2)-like,

B/Colorado/06/2017-like (Victoria lineage), and B/Phuket/3073/2013-like (Yamagata lineage) viruses that were recommended for inclusion in the southern hemisphere 2019 influenza vaccine. In recent years (including 2019), HI assays involving A(H3N2) viruses have been performed in the presence of 20 nM oseltamivir carboxylate (OC) to reduce non-specific binding of the NA protein.[5] The addition of OC reduces the number of influenza virus isolates that can be tested by HI because around 11% of viruses lose the ability to bind RBC and therefore cannot be assayed by HI. To test a subset of these viruses, the Centre has employed the Focus Reduction Assay (FRA), a microneutralisation assay which is more specific and sensitive than the HI assay and does not require binding to RBCs.[6] The FRA utilised the same ferret antisera as did the HI assay and was performed as previously described,[7] but with 1.2% Avicell RC591 (IMCD Mulgrave, Australia) replacing the carboxymethyl cellulose.

Genetic analysis

For influenza-positive samples that failed to grow in MDCK cells, real-time reverse transcription polymerase chain reaction (RT-PCR) was performed to determine the influenza type/subtype/lineage using the CDC Influenza Virus Real-Time RT-PCR kit.[i]

A substantial subset of influenza viruses analysed at the Centre underwent genetic analysis by sequencing of viral RNA genes – usually HA and NA genes as well as the matrix gene for influenza A viruses and non-structural protein gene (NS) for influenza B viruses. Whole genome sequencing (WGS) of a smaller subset of viruses was also performed.

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i   The CDC Influenza Virus Real-Time RT-PCR Influenza A/B Typing Panel (RUO) (Catalog No. FluRUO-01), FR-198, was obtained through the International Reagent Resource, Influenza Division, WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Centers for Disease Control and Prevention, Atlanta, GA, USA. https://www.internationalreagentresource.org/.

For sequencing, RNA was extracted from isolates or original clinical specimens using either a manual QIAGEN QIAamp Viral RNA kit or the automated QIAGEN QIAXtractor platform, followed by RT-PCR using the BIOLINE MyTaq one step RT-PCR kit according to the manufacturer's recommendations, with gene-specific primers (primer sequences available on request). Conventional Sanger sequencing was carried out on PCR products using an Applied Biosystems 3500XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequence assembly was performed using the Geneious Prime software version 9.0.4 (Biomatters Ltd, Auckland, New Zealand).

WGS was performed on a selection of viruses using the SuperScript-III One-Step Platinum Hi-Fi PCR kit according to the manufacturer's recommendations, with universal primers (primer sequences available on request). WGS was conducted using an Applied Biosystems Ion Torrent™ Personal Genome Machine™ (PGM) System according to the manufacturer's recommendations. These sequences were analysed using a proprietary pipeline, FluLINE.[8] Phylogenetic analysis was performed using Geneious 9.0.4 and FigTree v1.3.1 software.

Antiviral drug resistance testing

Circulating viruses were tested for their sensitivity to the currently-used neuraminidase inhibitors oseltamivir (Tamiflu) and zanamivir (Relenza). The neuraminidase inhibition (NAI) assay used was a functional fluorescence-based assay using the substrate 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA), in which the susceptibility of test viruses to the antiviral drugs was measured in terms of the drug concentration needed to reduce the influenza neuraminidase enzymatic activity by 50% (IC_{50}), and compared to values obtained with sensitive reference viruses of the same subtype or lineage. NAI assays were performed as previously described,[9] with the incorporation of a robotic platform by Tecan EVO 200 and Infinite 200Pro for liquid handling and plate reading (Tecan

Australia). For reporting purposes, 'highly reduced inhibition' of influenza A viruses has been defined by WHO as a ≥ 100-fold increase in IC_{50} in a NAI assay. For influenza B viruses, this figure was a ≥ 50-fold increase. However, it should be noted that the relationship between the IC_{50} value and the clinical effectiveness of a neuraminidase inhibitor is not yet well understood and a small or medium reduction in inhibition may not be clinically significant.

Viruses found to have highly reduced inhibition by either oseltamivir or zanamivir underwent genetic analysis using pyrosequencing, Sanger sequencing or WGS to determine the presence of amino acid substitutions in the NA protein that were associated with the reduction of inhibition by neuraminidase inhibitors. For example, a change from histidine to tyrosine at position 275 (H275Y) of the NA protein of A(H1N1)pdm09 viruses is known to reduce inhibition by oseltamivir, as does the H273Y NA mutation in B viruses.[9] Pyrosequencing was also performed on original clinical specimens of selected viruses which may have contained a known mutation such as H275Y if no isolate was available for phenotypic testing. Pyrosequencing was performed as previously described[10] using the MyTaq One-Step RT-PCR Kit (QIAGEN, Hilden, Germany) for virus amplification, with pyrosequencing reactions performed using the PyroMark instrument (QIAGEN, Hilden, Germany).

Candidate vaccine strains

The viruses used in production of human influenza vaccines are required by regulatory authorities to be isolated and passaged in embryonated hen's eggs or qualified cell lines[11–13] directly from human clinical respiratory samples. The Centre has undertaken primary isolation of selected viruses from clinical samples directly into eggs, using previously described methods.[14] Briefly, the viruses were inoculated into the amniotic cavity of embryonated eggs and, once virus growth was established, isolates were passaged in the allantoic cavity until a sufficient titre was obtained. Egg incubation conditions differed

slightly, with A(H1N1)pdm09 and A(H3N2) viruses incubated at 35 °C for three days, and influenza B viruses incubated at 33 °C for three days. In addition, selected clinical samples were inoculated into the qualified cell line MDCK 33016PF (Seqirus Limited, Holly Springs, NC, USA)[15] and incubated at 35 °C for three days, with viral growth monitored by haemagglutination of turkey or guinea pig RBC. These isolates were then analysed by HI assay, real time RT-PCR and genetic sequencing using the methods described above.

Results

During 2019, the Centre received 9,266 samples (including 7,687 clinical specimens, 1,014 virus isolates and 569 paired specimens and isolates) from 40 laboratories in 17 countries (Figure 1). As in previous years, most samples were submitted by laboratories in the Asia-Pacific region,[16–19] with the majority received from Australia (7,410 samples; 80%). However, unlike previous years, a large number of samples were received between January and April, which is before the typical Southern Hemisphere temperate regions influenza season. Figure 2 shows the weekly temporal distribution of samples sent to the Centre by type and subtype/lineage. During 2019, influenza A(H3N2) was the predominant circulating strain, followed by A(H1N1)pdm09 and B. For samples received from Australia where the lineage was confirmed, B/Victoria viruses predominated significantly over B/Yamagata viruses (93% and 7% of B viruses respectively). Overall, isolation and re-passaging was attempted for 9,146 (99%) of the samples received and this yielded 6,101 isolates (overall isolation rate of 67%). Isolation rates were lower for clinical specimens (60.5%) than for virus isolates received (94.9%). Of the viruses for which type and subtype could be confirmed, isolation rates by cell propagation were 75% (1,767/2,350) for A(H1N1)pdm09; 81% (3,150/3,911) for A(H3N2); and 81% (1,142/1,407) for influenza B. However, amongst these viruses, 15% (464/3,150) of A(H3N2) isolates did not reach sufficient titres for antigenic analysis. A total of 4,445 viral isolates were successfully characterised by HI assay, and were compared to the 2019 reference viruses (Table 1). In addition, 991 samples were characterised by real-time RT-PCR to determine their type/subtype or lineage. Sanger sequencing and WGS techniques were used to sequence the HA genes of 2,487 viruses. The full genomes of 415 viruses were also sequenced using either Sanger sequencing or WGS. Of the samples for which results could be obtained by antigenic or genetic analysis (n = 6,368), influenza A(H3N2) viruses predominated, comprising 49% (n = 3,133) of viruses received and analysed.

Figure 1: Geographic spread of influenza laboratories sending viruses to the Centre during 2019
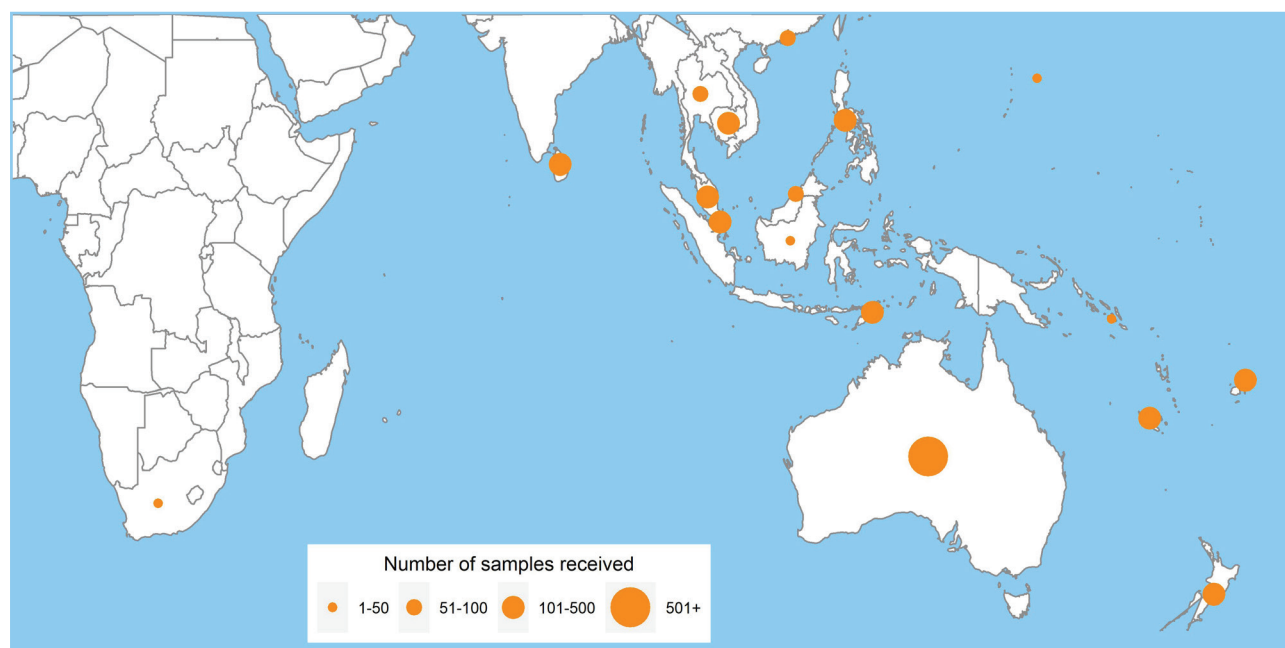
Table 1: Antigenic analysis of viruses received by the Centre in 2019, by geographic region of origin

Region	A(H1N1)pdm09 reference strain: A/Michigan/45/2015 (cell)		A(H3N2)[a] reference strain: A/Switzerland/8060/2017 (cell)		B/Victoria reference strain: B/Colorado/06/2017 (cell)		B/Yamagata reference strain: B/Phuket/3073/2013 (cell)	
	Like	Low reactor (%)	Like	Low reactor (%)	Like	Low reactor (%)	Like	Low reactor (%)
Australasia	1,136	31 (2.7%)	1,214	347 (22.2%)	503	64 (11.3%)	41	1 (2.4%)
South East Asia	298	26 (8%)	58	96 (62.3%)	118	48 (28.9%)	79	0
Pacific	80	34 (29.8%)	25	5 (16.7%)	99	22 (18.2%)	2	0
Africa	3	0	4	11 (73.3%)	0	0	0	0
South Asia	13	0	0	5 (100%)	18	0	4	0
East Asia	31	3 (8.8%)	4	1 (20%)	11	9 (45%)	1	0
Total	**1,561**	**94 (5.7%)**	**1,305**	**465 (26.3%)**	**749**	**143 (16%)**	**127**	**1 (0.8%)**

a   Note that a small number of A(H3N2) virus isolates that were obtained could not be analysed by HI assay due to low haemagglutination assay (HA) titre in the presence of oseltamivir.

Figure 2: Number of samples received at the Centre by week of sample collection 2019
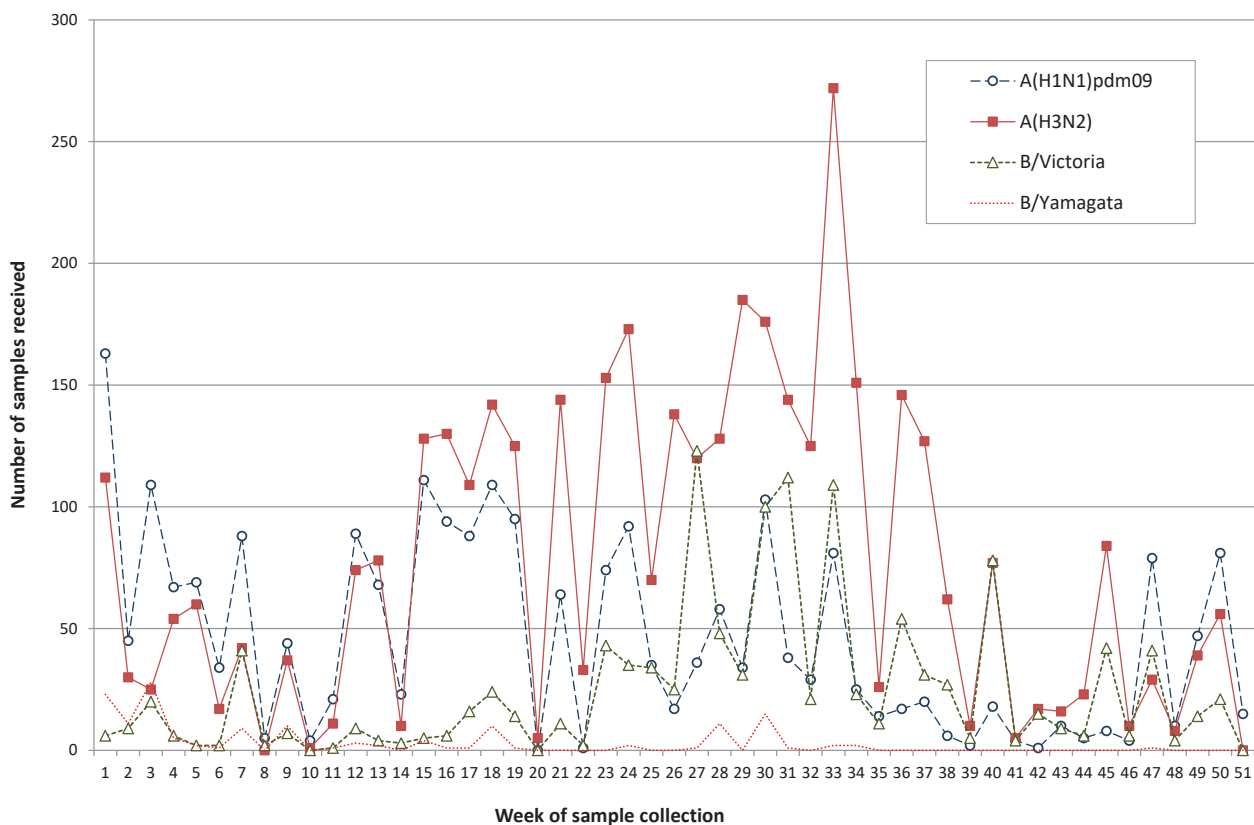
A(H1N1)pdm09

Of the 1,655 A(H1N1)pdm09 isolates analysed by HI assay using ferret antisera in 2019, a majority (94%) were antigenically similar to the vaccine reference strain A/Michigan/45/2015 (Table 1).

Sequencing and phylogenetic analysis of HA genes from 644 viruses showed that all A(H1N1)pdm09 viruses sent to the Centre during 2019 fell into the 6B.1 clade (Figure 3). The majority of these viruses reacted in a similar manner to the 2019 vaccine virus A/Michigan/45/2015 in HI assays using ferret antisera.

Eighteen A(H1N1)pdm09 viruses were inoculated into eggs for isolation of candidate vaccine strains with 16 (89%) successfully isolated; all fell into the genetic subclade 6B.1A. Thirty-nine viruses were inoculated into the qualified cell line MDCK 33016PF, of which 25 (64%) grew successfully and all also fell into the genetic subclade 6B.1A.

Of 1,701 A(H1N1)pdm09 viruses tested, three viruses exhibited highly reduced inhibition by oseltamivir and one virus exhibited highly reduced inhibition by zanamivir. The viruses showing highly reduced inhibition by oseltamivir (two from Australia and one from Malaysia) were confirmed to contain the H275Y substitution in their NA genes, a known mutation that is associated with highly reduced inhibition by oseltamivir. Another NA substitution linked to the reduction in inhibition by zanamivir was also identified in one of the viruses from Australia.[20] This virus was confirmed to contain the E119G substitution in its NA gene.

Figure 3: Phylogenetic tree of haemagglutinin genes of A(H1N1)pdm09 viruses received by the Centre during 2019

Legend

#2019 SOUTHERN HEMISPHERE VACCINE STRAIN

e: egg isolate

^REFERENCE VIRUS

| Braces indicate clades

(+/-) Indicates gain/loss of a potential glycosylation site.

Scale bar represents 0.3% nucleotide sequence difference between viruses. Amino acid changes relative to the outgroup sequence (A/CALIFORNIA/07/2009e) are shown.

A/Victoria/265/2019 oct
A/Ratchaburi/435/2019 nov
A/Lopburi/4448/2019 dec
A/Perth/182/2019 dec
niidA/Okinawa/93/2019 aug
A/Perth/1156/2019 dec
A/South Australia/476/2019 dec
A/Sydney/1237/2019 nov
A/Brisbane/146/2019 oct
A/Canberra/411/2019 nov
A/Perth/1154/2019 oct
A/Victoria/2569/2019 nov
A/Philippines/39/2019 oct
A/Chanthaburi/443/2019 nov
A/Malaysia/RP2260/2019 oct
R45K
T120I
A/Philippines/34/2019 oct
A/Sydney/454/2019 oct
A/Brisbane/149/2019 oct
A/Brisbane/144/2019 oct
A/Philippines/37/2019 oct
A/Canberra/415/2019 nov
A/South Australia/1128/2019 dec
A/Perth/178/2019 nov
D187A
Q189E
A/Brisbane/142/2019 oct
A/Malaysia/UM015/2019 oct
A/Sydney/1235/2019 nov
D187V
A/Guangdong-Maonan/SWL1536/2019e jun
A/Victoria/2455/2019e aug
A/Victoria/2454/2019e aug
A/Malaysia/RP2253/2019 oct
A/Lopburi/440/2019 nov
A/Sydney/451/2019 oct
A/Malaysia/RP2255/2019 oct
A/Chiang Rai/1391/2019 dec
A/Victoria/267/2019 oct
A186T
A/Darwin/739/2019 oct
A/Malaysia/RP2261/2019 oct
A/Nakhonphanom/487/2019 dec
A/Bangkok/426/2019 oct
A/Nakhonsithammarat/425/2019 oct
A/South Australia/466/2019 nov
A/South Africa/R05624/2019 may
A/South Australia/451/2019 oct
A/Victoria/2568/2019 nov
A/Cambodia/D1216368/2019 nov
mohA/Singapore/GP2220/2019 nov
A/Victoria/2570/2019 nov
A/Philippines/31/2019 oct
K504R
A/Sri Lanka/15/2019 apr
A/Darwin/764/2019 nov
A/Brunei/8/2019 mar
A/Macau/600148/2019 jan
A/Cambodia/d0619361/2019 may
A/New Caledonia/87/2019 may
A/South Australia/477/2019 dec
A/Timor Leste/41/2019 apr
A/New Caledonia/67/2019 jun
A/New Caledonia/95/2019 jun
A/Sri Lanka/40/2019 aug
N129D
T185I
A/Victoria/214/2019e aug
A/Darwin/639/2019e aug
A/Victoria/255/2019 sep
A/Victoria/212/2019e aug
^A/DARWIN/6/2018e
A/Darwin/123/2018e
A/Darwin/122/2018e
A/Darwin/125/2018e
A/Darwin/1024/2019 nov
A/Perth/193/2019 nov
A/Canberra/417/2019 nov
K520A
A/Darwin/738/2019 oct
N260D
A/CANBERRA/337/2019e aug
A/Canberra/394/2019 oct
A/Canberra/400/2019 oct
A/South Australia/456/2019 oct
A/Darwin/784/2019 dec
A/South Australia/471/2019 nov
K130N, K160M
T216K, H296M
A/Philippines/27/2019 sep
A/Wellington/36/2019 jun
A/Brisbane/147/2019 oct
A/Iowa/12/2019e jun
^crickA/SWITZERLAND/3330/2017e
A/Fiji/54/2018e
A/South Australia/49/2018e sep
A/Darwin/4/2018e oct
A/Brisbane/59/2018e
P183S
^A/SOUTH AUSTRALIA/272/2017e
A/Fujian Gulou/SWL1884/2018e
T120A
A/Newcastle/31/2019 mar
A/South Africa/R03973/2019 apr
^A/BRISBANE/157/2018
A/Canberra/13/2019e jan
A/Darwin/102/2019e mar
A/Newcastle/46/2019e mar
A/Brunei/5/2019e feb
A/Brunei/54/2019 apr
A/Fiji/26/2019 mar
A/Fiji/51/2019 mar
A/Brisbane/70/2018e
A/Brisbane/72/2018e
L233I
V520A
A/Brisbane/68/2018e
^A/CHRISTCHURCH/506/2018e
A/Cambodia/FSS40727/2019 jun
R45G, P282A
I298V
^A/TOWNSVILLE/21/2018
A/Christchurch/515/2018e
^A/PERTH/183/2018 nov
^A/BRISBANE/02/2018e
A/Newcastle/45/2018e
A/BeijingXicheng/SWL1633/2018e
K302T, I404M
N496S, E506D
A/Timor-Leste/94/2019 apr
A/Timor Leste/34/2019 mar
A/Victoria/700/2019 mar
^A/INDIANA/30/2019
A/Macau/600080/2019 jan
P83S, S84N, D97N, S162N (+)
K163Q, S185T, S203T, I216T
A256T, K283E, I321V, E374K
S451N, E499K
S74R, S164T, S183P
R223Q, I295V
^cdcA/MEXICO/2549/2018
^A/MICHIGAN/45/2015e
^A/BRISBANE/37/2017
^A/CALIFORNIA/07/2009e

183P-5A

6B.1A/183P-5

183P-5B

183P-6

183P-2

6B.1A

183P-4

183P-1

183P-7

183P-3

E235D
V520A

0.003

A(H3N2)

Antigenic analysis of 2,104 A(H3N2) subtype isolates using the HI assay showed that 26% were low reactors to the ferret antisera prepared against the cell-propagated reference strain A/Switzerland/8060/2017/2017 (Table 1). However, 99% of viruses were low reactors to the ferret antisera prepared against the egg-propagated strain A/Switzerland/8060/2017 (data not shown). An additional 464 A(H3N2) viruses were inoculated and isolated by cell culture but did not reach sufficient titres for antigenic analysis, whilst a further 334 were successfully isolated but did not reach sufficient titres when tested by HI assay in the presence of oseltamivir carboxylate.

A total of 75 A(H3N2) viruses that could not be characterised by HI assay were analysed using the FRA assay. The FRA assay indicated that 16% of these viruses showed greater than four-fold difference in titre compared to the cell-propagated reference strain A/Switzerland/8060/2017; however, the majority of viruses (93%) had a greater than four-fold difference in titre compared to the egg-propagated strain (data not shown).

The HA genes of 1,309 A(H3N2) viruses were sequenced. Phylogenetic analysis indicated that the majority of circulating viruses fell into subclade 3C.2a1b based on their HA genes, whereas the 2019 vaccine strain, A/Switzerland/8060/2017 fell into subclade 3C.2a2 (Figure 4). A small number of A(H3N2) viruses fell into the 3C.3a and 3C.2a3 clades.

Forty-seven viruses were inoculated into eggs, of which 27 (57%) grew successfully and consisted of 21 viruses from genetic subclades 3C.2a1b+131K and two from 3C.2a1b+135K. Additionally, four subclade 3C.3a viruses were isolated. Fifty-seven viruses were inoculated into the qualified cell line MDCK 33016PF, of which 38 (67%) grew successfully, with 23 viruses from genetic subclades 3C.2a1b+131K, four from 3C.2a1b+135K and 11 from 3C.3a.

None of the 2,323 A(H3N2) viruses tested by NAI assay showed highly reduced inhibition by oseltamivir or zanamivir.

Influenza B

Amongst influenza B viruses received at the Centre during 2019, B/Victoria-lineage viruses were predominant over B/Yamagata-lineage viruses (Figure 2). A total of 1,020 influenza B viruses were characterised by HI assay. Almost all B/Yamagata-lineage viruses were antigenically similar to the B/Phuket/3073/2013-like vaccine virus, while 84% of B/Victoria-lineage viruses were antigenically similar to the B/Colorado/06/2017 vaccine virus (Table 1).

Sequencing was performed on HA genes from 367 B/Victoria viruses and 88 B/Yamagata viruses. A small number of B viruses (51/367; 14%) were genetically similar to the B/Colorado/06/2017 reference virus, with a two-amino-acid deletion in the HA protein (positions 162–163) (Figure 5); however, the majority of viruses (272/367; 74%) had a three-amino-acid deletion in the HA protein at positions 162–164 and formed a separate phylogenetic clade from the B/Colorado/06/2017 clade. All B/Yamagata-lineage viruses belonged to genetic clade 3 (or Y3), which is the same genetic clade as the 2019 vaccine virus B/Phuket/3073/2013 (Figure 6).

Egg isolation was attempted for 15 B/Victoria and eight B/Yamagata-lineage viruses, resulting in the successful isolation of 11 (73%) B/Victoria-lineage viruses and four (50%) B/Yamagata-lineage viruses. All B/Victoria-lineage viruses isolated were from genetic subclade V1A, two of which were further grouped into V1A.1 and eight others in V1A.3. All B/Yamagata-lineage viruses isolated were from genetic clade Y3. For viruses inoculated into the qualified cell line MDCK 33016PF, nine of 16 (56%) B/Victoria-lineage viruses and four (80%) of five B/Yamagata-lineage viruses were isolated. At least one representative from the major clades of each B lineage was isolated. A total of 21 viruses were inoculated into the qualified cell line MDCK 33016PF, of which 13 (62%) grew successfully.

Figure 4: Phylogenetic tree of haemagglutinin genes of A(H3N2) viruses received by the Centre during 2019
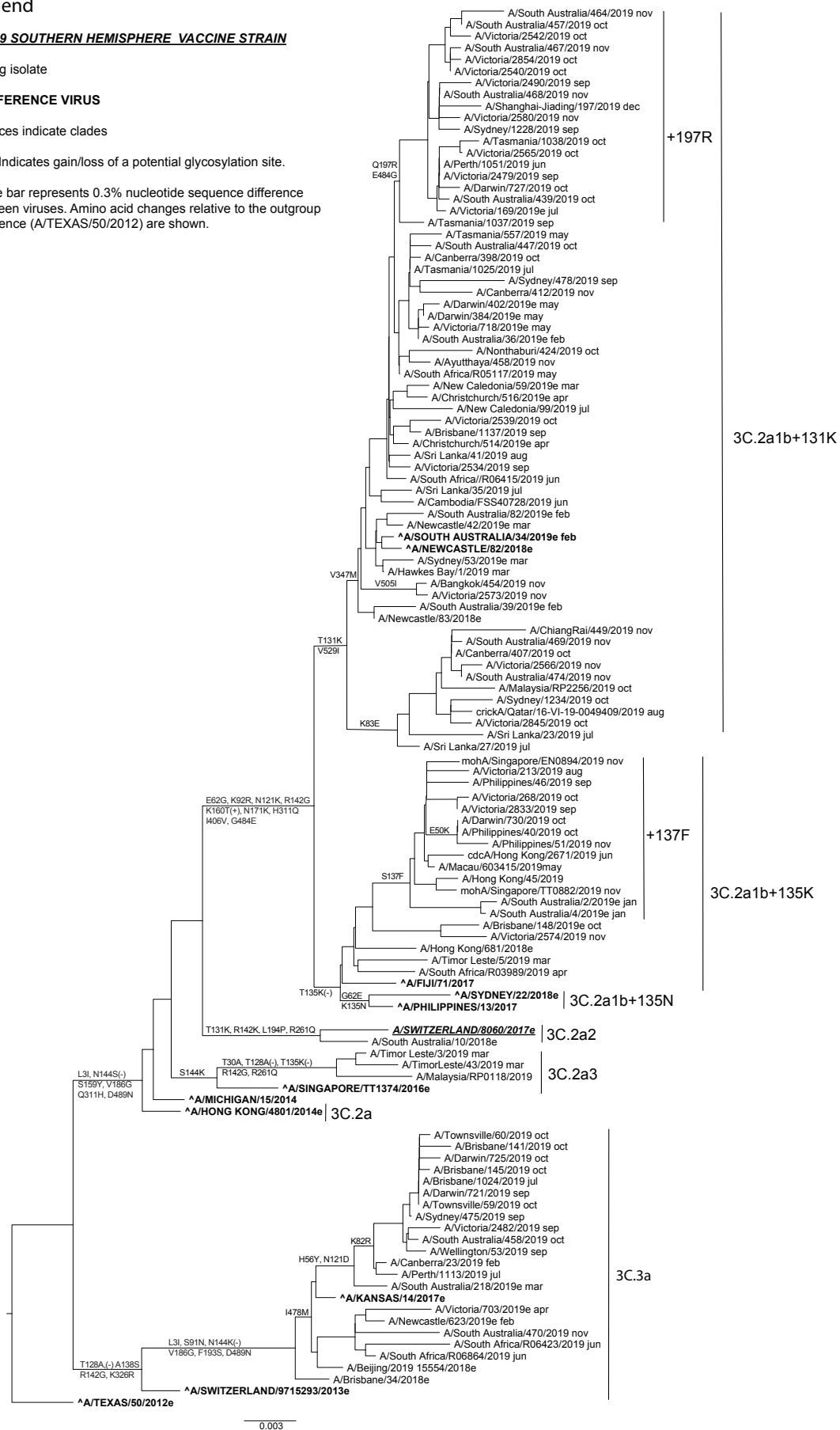
Figure 5: Phylogenetic tree of haemagglutinin genes of B/Victoria-lineage viruses received by the Centre during 2019

Legend

#2019 SOUTHERN HEMISPHERE VACCINE STRAIN

e: egg isolate

^REFERENCE VIRUS

| Braces indicate clades

Scale bar represents 0.3% nucleotide sequence difference between viruses. Amino acid changes relative to the outgroup sequence (B/BRISBANE/60/2008e) are shown.

(+/-) Indicates gain/loss of a potential glycosylation site.

2 DEL and 3 DEL indicate subclades containing 2 or 3 amino acid deletions at the positions162/163 and 162-164 respectively. Deletions are also noted in:
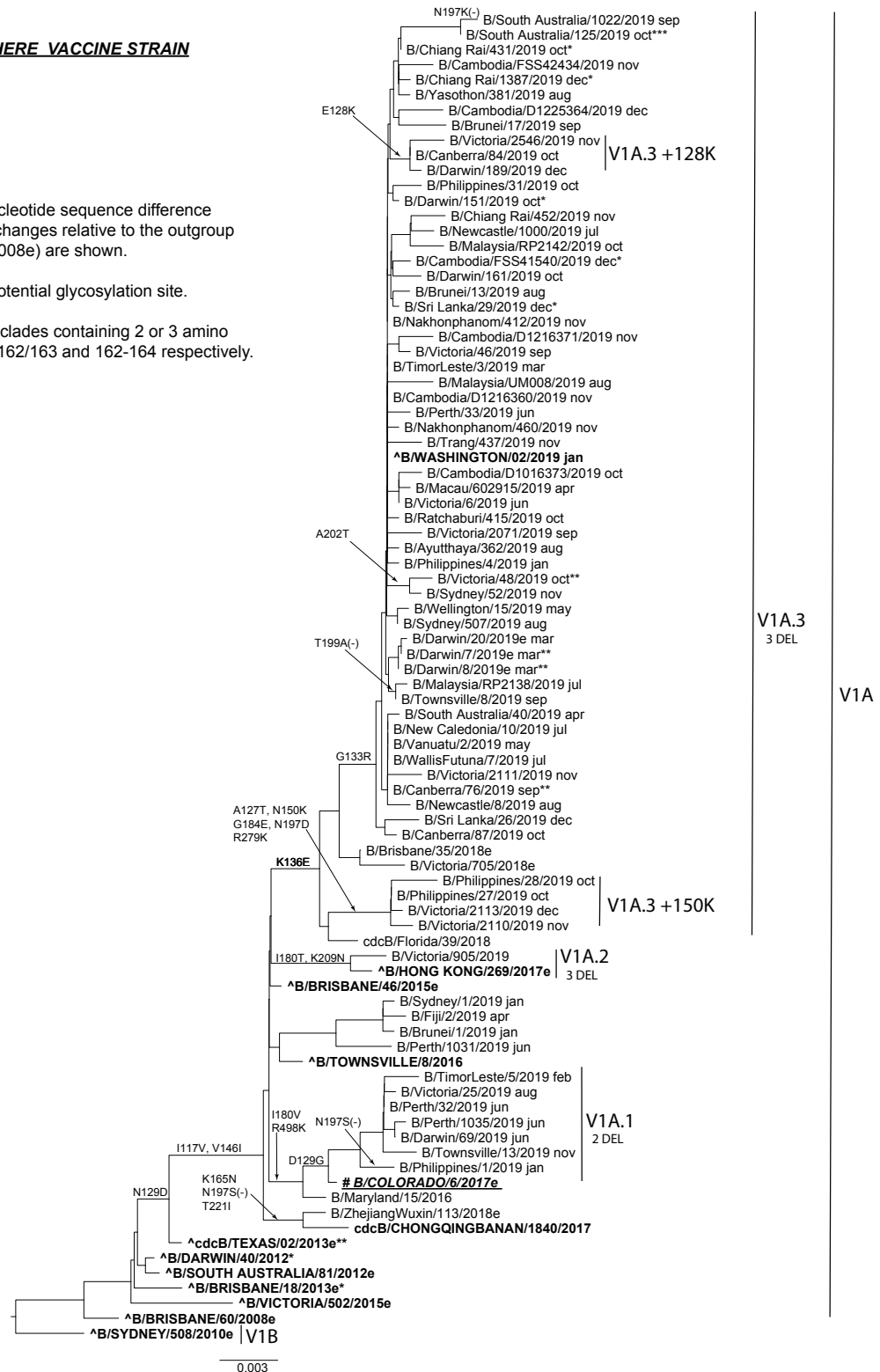* = N197X
** = N199X
*** =N197X & N199X

N197K(-)
B/South Australia/1022/2019 sep
B/South Australia/125/2019 oct***
B/Chiang Rai/431/2019 oct*
B/Cambodia/FSS42434/2019 nov
B/Chiang Rai/1387/2019 dec*
B/Yasothon/381/2019 aug
E128K
B/Cambodia/D1225364/2019 dec
B/Brunei/17/2019 sep
B/Victoria/2546/2019 nov
B/Canberra/84/2019 oct
B/Darwin/189/2019 dec
V1A.3 +128K
B/Philippines/31/2019 oct
B/Darwin/151/2019 oct*
B/Chiang Rai/452/2019 nov
B/Newcastle/1000/2019 jul
B/Malaysia/RP2142/2019 oct
B/Cambodia/FSS41540/2019 dec*
B/Darwin/161/2019 oct
B/Brunei/13/2019 aug
B/Sri Lanka/29/2019 dec*
B/Nakhonphanom/412/2019 nov
B/Cambodia/D1216371/2019 nov
B/Victoria/46/2019 sep
B/TimorLeste/3/2019 mar
B/Malaysia/UM008/2019 aug
B/Cambodia/D1216360/2019 nov
B/Perth/33/2019 jun
B/Nakhonphanom/460/2019 nov
B/Trang/437/2019 nov
^B/WASHINGTON/02/2019 jan
B/Cambodia/D1016373/2019 oct
B/Macau/602915/2019 apr
B/Victoria/6/2019 jun
B/Ratchaburi/415/2019 oct
B/Victoria/2071/2019 sep
A202T
B/Ayutthaya/362/2019 aug
B/Philippines/4/2019 jan
B/Victoria/48/2019 oct**
B/Sydney/52/2019 nov
B/Wellington/15/2019 may
B/Sydney/507/2019 aug
B/Darwin/20/2019e mar
B/Darwin/7/2019e mar**
T199A(-)
B/Darwin/8/2019e mar**
B/Malaysia/RP2138/2019 jul
B/Townsville/8/2019 sep
B/South Australia/40/2019 apr
B/New Caledonia/10/2019 jul
B/Vanuatu/2/2019 may
B/WallisFutuna/7/2019 jul
B/Victoria/2111/2019 nov
G133R
B/Canberra/76/2019 sep**
B/Newcastle/8/2019 aug
B/Sri Lanka/26/2019 dec
A127T, N150K
G184E, N197D
R279K
B/Canberra/87/2019 oct
B/Brisbane/35/2018e
K136E
B/Victoria/705/2018e
B/Philippines/28/2019 oct
B/Philippines/27/2019 oct
B/Victoria/2113/2019 dec
V1A.3 +150K
B/Victoria/2110/2019 nov
cdcB/Florida/39/2018
I180T, K209N
B/Victoria/905/2019
V1A.2
^B/HONG KONG/269/2017e
3 DEL
^B/BRISBANE/46/2015e
B/Sydney/1/2019 jan
B/Fiji/2/2019 apr
B/Brunei/1/2019 jan
B/Perth/1031/2019 jun
^B/TOWNSVILLE/8/2016
B/TimorLeste/5/2019 feb
B/Victoria/25/2019 aug
B/Perth/32/2019 jun
I180V
R498K
N197S(-)
B/Perth/1035/2019 jun
V1A.1
B/Darwin/69/2019 jun
2 DEL
B/Townsville/13/2019 nov
I117V, V146I
D129G
B/Philippines/1/2019 jan
B/COLORADO/6/2017e
K165N
N197S(-)
T221I
B/Maryland/15/2016
N129D
B/ZhejiangWuxin/113/2018e
cdcB/CHONGQINGBANAN/1840/2017
^cdcB/TEXAS/02/2013e**
^B/DARWIN/40/2012*
^B/SOUTH AUSTRALIA/81/2012e
^B/BRISBANE/18/2013e*
^B/VICTORIA/502/2015e
^B/BRISBANE/60/2008e
^B/SYDNEY/508/2010e
V1B

V1A.3
3 DEL

V1A

0.003

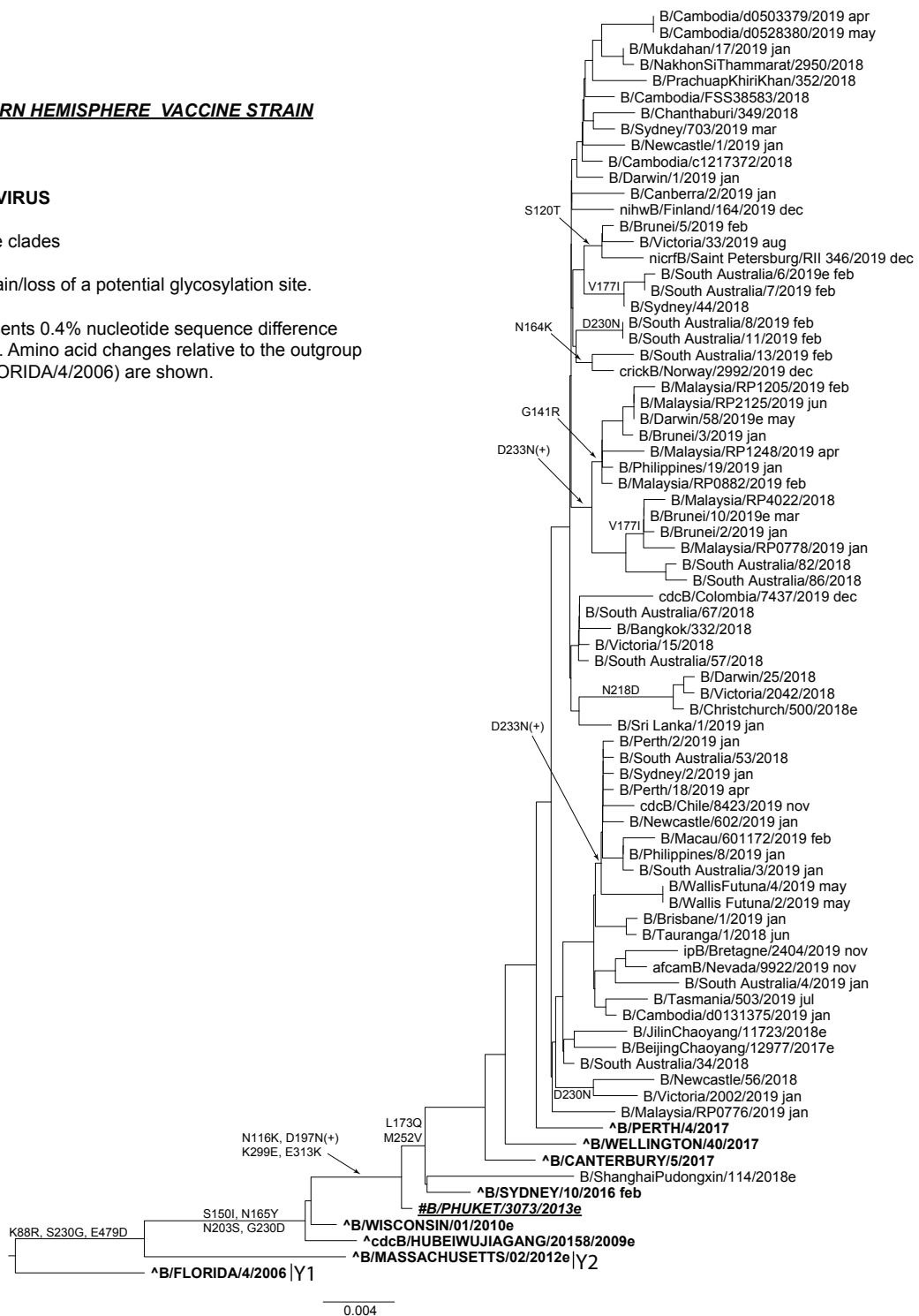Legend

#2019 SOUTHERN HEMISPHERE VACCINE STRAIN

e: egg isolate

^REFERENCE VIRUS

| Braces indicate clades

(+/-) Indicates gain/loss of a potential glycosylation site.

Scale bar represents 0.4% nucleotide sequence difference between viruses. Amino acid changes relative to the outgroup sequence (B/FLORIDA/4/2006) are shown.

Of 54 B/Yamagata-lineage viruses tested, none displayed highly reduced inhibition by oseltamivir or zanamivir. However, three of the 1,223 B/Victoria-lineage viruses tested showed highly reduced inhibition by zanamivir. Two of the viruses, one each from Malaysia and Australia, contained an E105K substitution in their NA genes, which has been shown to reduce susceptibility to neuraminidase inhibitor drugs.[16] A B/Victoria-lineage virus from Malaysia was found to contain gene sequence coding for a novel dual-amino-acid substitution, T146P + N169S, in its NA gene.

Discussion

During 2019 the Centre received a larger number of samples than in any previous single year,[17–19,21,22] with the majority of viruses received (80%) from Australia. The high volume of samples received correlated with a record number of laboratory-confirmed influenza notifications in Australia (313,365 cases) during the 2019 Australian influenza season.[23] There was a continuation of intense inter-seasonal activity, with notifications of laboratory-confirmed influenza at the end of May twelve times higher than during the same time period in the previous three years.[24] The 2019 Australian season was characterised by an early peak and extended activity, with jurisdictions such as the Australian Capital Territory and Tasmania displaying multiple peaks of activity.[25] Unlike previous seasons, where influenza samples received at the Centre typically peaked in August, the Centre saw a dramatic increase in samples received early in the year, peaking in July 2019, with over 1,500 samples received in that month alone.

The geographic spread of influenza around Australia began with a large epidemic of A(H1N1)pdm09 in the Northern Territory, in December 2018. This was followed by large and sustained outbreaks of influenza in eastern Australian jurisdictions, most notably South Australia, in the first quarter of 2019. The Australian Capital Territory and Western Australia had comparatively lower levels of influenza notification but even these both had record notifications in 2019.[25]

The predominant circulating virus in January and February 2019 across Australia was A(H1N1)pdm09, while influenza A(H3N2) virus submissions began to increase rapidly in March 2019, subsequently becoming the predominant circulating strain in 2019. Influenza A(H3N2) viruses accounted overall for 51% of samples submitted to the Centre, followed by A(H1N1)pdm09 (30%), while influenza B viruses comprised 18% of samples received. Globally, influenza A viruses were predominant in 2019, with co-circulation of A(H1N1)pdm09 and A(H3N2). In the southern hemisphere, A(H1N1)pdm09 predominated in South America, followed by A(H3N2). By contrast, A(H3N2) predominated in South Africa with very few detections of A(H1N1)pdm09, while in tropical Asia, A(H1N1)pdm09 was the dominant circulating strain followed by B/Victoria viruses.[26]

Older adults are typically more affected in years when A(H3N2) viruses predominate, while younger populations are usually more affected by A(H1N1)pdm09.[27] Consistent with this observation, high notification rates occurred in 2019 among children aged 5–9 years (2,646 per 100,000 population) and adults aged ≥ 85 years (2,370 per 100,000 population) in Australia.[23] Deaths associated with influenza and pneumonia were the ninth leading cause of death in 2019, with 4,124 deaths recorded.[28]

Most of the influenza A(H1N1)pdm09 viruses received were antigenically and genetically similar to the vaccine strain, A/Brisbane/02/2018, which is in the 6B.1A/183P-1 genetic clade. A number of A(H1N1)pdm09 viruses had amino acid changes in the HA gene, most significantly at residues 156 and 250; the former mutation has been previously associated with significant antigenic changes in the haemagglutination inhibition (HI) assay. These viruses were first detected in Fiji and Brunei in early 2019 and were subsequently seen in some returned travellers from these regions to Australia. These antigenically-distinct viruses comprised 5.7% of the A(H1N1)pdm09 viruses tested and were in the 6B.1A/183-P2 genetic clade. Mid-season vaccine effectiveness (VE) estimates suggest

that vaccine protection in Australia for all age groups was highest against A(H1N1)pdm09 viruses among both primary care (62%, 95% CI: 39,78) and hospitalised patients (70%, 95% CI: 49, 82).[29]

Antigenic analysis of A(H3N2) viruses showed that the majority of viruses displayed similar antigenic characteristics to the cell-propagated vaccine strain, A/Switzerland/8060/2017. As in previous years, there have been ongoing difficulties in the antigenic characterisation of A(H3N2) viruses. Evolutionary changes in this subtype continue to pose challenges in detecting antigenic changes using the HI assay.[5,30] Oseltamivir carboxylate was used in the HI assay to prevent binding of the NA protein to red blood cells, which resulted in a small number of A(H3N2) viruses having insufficient titre to be tested in the HI assay (16% of A(H3N2) viruses isolated). The FRA continued to be used as complementary antigenic assay but remains time- and labour-intensive. Of the viruses analysed by FRA, 75% and 7%, respectively, were antigenically similar to the cell- and egg-propagated reference strain (A/Switzerland/8060/2017).

There were several distinct genetic clades amongst circulating A(H3N2) viruses, with the majority of viruses belonging to the 3C.2a1b+131K HA clade, followed by the 3C.2a1b+135K HA clade. Interestingly, 3C.2a2 viruses, which were represented by the 2019 Southern Hemisphere vaccine strain A/Switzerland/8060/2018, only accounted for a very small proportion of circulating viruses during 2019 and also led to a change in the 2020 Southern Hemisphere A(H3N2) vaccine component to an A/South Australia/34/2019-like virus.[31]

The majority of influenza B isolates analysed at the Centre belonged to the B/Victoria-lineage (86%). These B/Victoria-lineage viruses were mostly antigenically similar to the vaccine strain B/Colorado/6/2017; however, the majority were phylogenetically similar to a new variant that had previously emerged in the Northern Hemisphere. This variant contained a three-amino-acid deletion in the HA gene, from HA positions 162–164, and made up 64% of viruses genetically analysed at the Centre. The increase in circulation of this deletion variant also contributed to an increase in low-reacting viruses in the HI assay, indicating a corresponding antigenic change compared to the vaccine virus. This led to the subsequent change in the recommendation for the 2020 Southern Hemisphere B/Victoria-lineage vaccine component to a B/Washington/02/2019-like triple HA deletion virus.[31] B/Yamagata viruses comprised only 10% of influenza B viruses analysed at the Centre, and all these viruses were antigenically and genetically similar to the vaccine strain, B/Phuket/3073/2013.

With the ongoing evolution of influenza viruses and the absence of an effective universal vaccine, there remains a need for continuous influenza surveillance and regular updating of influenza vaccines. The work performed by the Centre in Melbourne is part of the ongoing efforts of the WHO GISRS to perform these tasks in an effort to better control the disease burden of influenza.

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