Annual report

INFLUENZA VIRUSES RECEIVED AND TESTED BY THE MELBOURNE WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA ANNUAL REPORT, 2015
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Abstract

As part of its role in the World Health Organization’s (WHO) Global Influenza Surveillance and Response System, the WHO Collaborating Centre for Reference and Research on Influenza in Melbourne received a total of 5,557 influenza positive samples during 2015. Viruses were analysed for their antigenic, genetic and antiviral susceptibility properties. In 2015, influenza B viruses predominated over influenza A(H1)pdm09 and A(H3) viruses, accounting for a total of 58% of all viruses analysed. The vast majority of A(H1)pdm09, A(H3) 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 2015. However, phylogenetic analysis of a selection of viruses indicated that the majority of circulating A(H3) viruses were genetically distinct from the WHO recommended strain for 2015, resulting in an update to the recommended vaccine strain for the Southern Hemisphere for 2016. With an increasing predominance of B/Victoria lineage viruses over B/Yamagata lineage viruses through the course of 2015, WHO also updated the recommended influenza B strain in the trivalent influenza vaccine for 2016. Of more than 3,300 samples tested for resistance to the neuraminidase inhibitors oseltamivir and zanamivir, only 1 A(H1)pdm09 virus showed highly reduced inhibition by oseltamivir. The Centre undertook primary isolation of candidate vaccine viruses directly into eggs, and in 2015 a total of 45 viruses were successfully isolated in eggs. Commun Dis Intell 2017;41(2):E150–E160.

Keywords: annual report; influenza

Introduction

The WHO Collaborating Centre for Reference and Research on Influenza in Melbourne 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 its impact through the use of vaccines and antiviral medications. The Centre in Melbourne is one of 5 such Collaborating Centres (the others being in Atlanta, Beijing, London and Tokyo) that monitor the antigenic and genetic changes in circulating human influenza viruses, and makes biannual recommendations on which influenza strains should be included in the influenza vaccine for the upcoming winter season in either the Northern or Southern Hemisphere. This report summarises the results of virological surveillance activities undertaken at the Centre in Melbourne during 2015.

Two types of influenza cause significant disease in humans: types A and B. Influenza A viruses are further classified into subtypes, based on their haemagglutinin (H) surface protein. Globally, there are currently 2 subtypes circulating in human populations: A(H1N1)pdm09 and A(H3N2). Here the N refers to the type of neuraminidase surface protein, of which there are also several types. Influenza B viruses are not classified into subtypes; however, they are 2 lineages, which currently circulate globally, B/Victoria and B/Yamagata. In addition, each year influenza C viruses are detected from humans, but these viruses tend not to cause severe disease and are not a major focus of surveillance.

Methods

Virus isolation

All A(H1)pdm09 and all influenza B viral isolates received at the Centre were re-passaged in cell culture (Madin-Darby Canine Kidney (MDCK) cells), whilst all A(H3) viral isolates were re-passaged in MDCK-SIAT1 cells. Virus isolation was also attempted from a selection of original clinical specimens received. In addition, influenza positive original clinical samples were directly inoculated into eggs as potential candidate vaccine viruses.
**Antigenic analysis**

The antigenic properties of influenza viral isolates were analysed using the haemagglutination inhibition (HI) assay as previously described. In HI assays, viruses were tested for their ability to agglutinate red blood cells in the presence of ferret antisera previously raised against 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 different from the titre of the homologous reference strain. During 2015, results were reported by reference to the A/California/7/2009 (H1N1pdm09)-like, A/Scotland/7475/2013 (H3N2)-like, B/Phuket/3073/2013-like (Yamagata lineage), and B/Brisbane/60/2008-like (Victoria lineage) viruses that were recommended for the 2015 influenza vaccine. In recent years, including 2015, HI assays involving A(H3) viruses have been performed in the presence of oseltamivir carboxylate in order to reduce non-specific binding of the N protein.

**Genetic analysis**

A subset of all influenza viruses analysed at the Centre underwent genetic analysis by sequencing of viral RNA genes; usually the H and N genes as well as the matrix gene for influenza A viruses and non-structural protein genes for influenza B viruses. In addition, the full genomes (all 8 gene segments) of a smaller subset of viruses were sequenced.

For sequencing, RNA was extracted from isolates or original clinical specimens using either manual QIAGEN QIAamp spin columns method or the automated QIAGEN QIAxtractor robot, followed by reverse transcription polymerase chain reaction (PCR) using the BIOLINE MyTaq one step reverse transcription 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 product using an Applied Biosystems 3500 XL sequencer. Sequence assembly was performed using the SeqPro Pro Module of DNASTAR Lasergene version 13 software (DNASTAR, Madison, WI, USA). Next generation sequencing (NGS) was performed on a selection of viruses using an Applied Biosystems Ion Torrent Personal Genome Machine System according to the manufacturer’s recommendations. These sequences were analysed using a proprietary FluAnalysis pipeline. Phylogenetic analysis was performed using Geneious 9.0.4 software (Biomatters Ltd, Auckland, New Zealand) and FigTree v1.3.1.

**Antiviral drug resistance testing**

As there is potential for influenza viruses to develop resistance to antiviral drugs, 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 in which the susceptibility of test viruses to the antiviral drugs was measured in terms of the drug concentration needed to reduce the neuraminidase enzymatic activity by 50% (IC₅₀), and compared with values obtained with sensitive reference viruses of the same subtype or lineage. NAI assays were performed as previously described with the incorporation of a robotic platform by TECAN EVO200 and Infinite 200Pro for liquid handling and plate reading (Tecan Australia). For the purposes of reporting, reduced inhibition of influenza A viruses was defined as a 10–99-fold increase in IC₅₀, while highly reduced inhibition was defined as a ≥100-fold increase in IC₅₀ in a neuraminidase inhibition assay. For influenza B viruses, these figures were 5–49-fold and ≥50-fold increases, respectively. However, it should be noted that the relationship between the IC₅₀ value and the clinical effectiveness of a neuraminidase inhibitor is not well understood and reduced inhibition may not be clinically significant.

Viruses found to have highly reduced inhibition by either oseltamivir or zanamivir underwent further analysis to determine the presence of amino acid substitutions in the neuraminidase protein that were associated with the reduction of inhibition by NAIs. For example, a change from histidine to tyrosine at position 275 (H275Y) of the neuraminidase protein of A(H1N1)pdm09 viruses is known to reduce inhibition by oseltamivir, as does the H273Y neuraminidase mutation in B viruses.

**Candidate vaccine strains**

The viruses used to produce human vaccines are required to be isolated and passaged in embryo-nated hen's eggs or certified cell lines. The Centre undertook primary isolation of selected viruses from clinical samples directly into eggs, using previously described methods, except for the following modifications. First, the viruses were initially inoculated into the amniotic cavity only, and once growth was established the isolates were then further passaged in the allantoic cavity. This was followed by harvest only from either the amniotic or allantoic cavity, depending on site of inoculation. Egg incubation conditions also differed slightly with A(H1)pdm09 and A(H3) viruses incubated at 35°C for 3 days, and influenza B viruses incubated at 33°C for 4 days.
at 33°C for three days. These isolates were then analysed by haemagglutination assay, HI assay, real time (RT)-PCR and genetic sequencing.

**Results**

During 2015, the Centre received 5,557 clinical specimens and/or virus isolates from 34 laboratories in 14 countries (Figure 1). As in previous years, most samples were submitted by laboratories in the Asia–Pacific region, including Australian laboratories and were received during the Southern Hemisphere influenza season. Figure 2 shows the temporal distribution of samples sent to the Centre by type or subtype and lineage.

Isolation was attempted for 4,911 (88%) of the samples received and was successful in 3,360 cases (68%). Of these, 2,815 were characterised by HI assay in comparison with the 2015 vaccine strains (Table). In addition, 229 samples were characterised by RT-PCR to determine their type, subtype or lineage. Sanger sequencing and NGS techniques were used to sequence the haemagglutinin genes of 957 viruses. The full genomes of 39 viruses were sequenced using either Sanger sequencing or NGS. Of the samples for which results could be obtained via antigenic or genetic analysis, (n=3,503), influenza B viruses predominated, comprising 58% of viruses analysed (30% B/Yamagata and 28% B/Victoria). The remaining portion of viruses were mostly A(H3) (31% of total number of viruses), followed by A(H1)pdm09 (11%). There was 1 sample with mixed A(H3)/B viruses.

**Figure 2:** Number of samples received at the WHO Collaborating Centre for Reference and Research on Influenza, 2015, by week of sample collection

**Figure 1:** Geographic spread of influenza laboratories sending viruses to the WHO Collaborating Centre for Reference and Research on Influenza during 2015
A(H1N1)pdm09

Of the 395 A(H1)pdm09 isolates analysed by HI assay using ferret antisera in 2015, the majority (98.7%) were antigenically similar to the vaccine reference strain A/California/7/2009 (Table).

Sequencing and phylogenetic analysis of haemagglutinin genes from 67 viruses showed that A(H1)pdm09 viruses sent to the Centre during 2015 fell mainly into the 6B clade with a smaller number of viruses in subclades 6B.1 and 6B.2 (Figure 3). No antigenic differences were detectable between viruses from the 6B, 6B.1 or 6B.2 genetic clades in ferret antisera HI assays and the majority of viruses reacted in a similar manner to the reference and 2015 vaccine virus A/California/7/2009.

Figure 3: Phylogenetic tree of representative haemagglutinin genes of A(H1)pdm09 viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015

* Reference virus is indicated in red text.
† 2015 Southern Hemisphere vaccine strain is presented in capital letters.
e: egg isolate
Scale bar represents 0.3% nucleotide sequence difference between viruses.
} Braces indicate clades
Twenty-three viruses were inoculated into eggs for vaccine candidate strain isolation. Of these, 9 (39%) were successfully isolated, including viruses from each of the 2 emerging subclades (6B.1 and 6B.2).

Of 380 A(H1)pdm09 viruses tested 1 exhibited highly reduced inhibition by oseltamivir. This virus was from Australia and had the H275Y mutation in the N gene, which is known to reduce inhibition by oseltamivir. No A(H1)pdm09 viruses received during 2015 showed highly reduced inhibition to zanamivir.

A(H3N2)

Antigenic analysis of 651 A(H3) subtype isolates showed that only 1.7% were low reactors to the cell-propagated reference strain A/Switzerland/9715293/2013 (Table). However, 47% of viruses were low reactors to the egg-propagated strain A/Switzerland/9715293/2013 (data not shown). An additional 232 A(H3) viruses were inoculated and/or isolated by cell culture but did not reach sufficient titres for antigenic analysis, whilst a further 503 were successfully isolated but did not reach sufficient titres when tested by HI assay in the presence of oseltamivir.

A total of 673 haemagglutinin genes from A(H3) viruses were sequenced. Phylogenetic analysis indicated that the majority of circulating viruses fell into clade 3C.2a, which is a genetically different clade to the 2015 vaccine strain A/Switzerland/9715293/2013 (clade 3C.3a) and is represented by the A/Hong Kong/4801/2014 virus (Figure 4). A small number of H3 viruses fell into the 3C.3b clade.

Fifty-four viruses were inoculated into eggs, of which 21 (39%) grew successfully. These viruses included at least 17 viruses from clade 3C.2a and 4 viruses from clade 3C.3b.

None of the 1,339 A(H3) viruses tested had highly reduced inhibition by either oseltamivir or zanamivir.

Influenza B

Similar proportions of viruses from both B lineages (B/Victoria and B/Yamagata) were received at the Centre during 2015. A total of 1,769 influenza B viruses were characterised by HI assay. All but 3 of the 837 B/Victoria viruses received and analysed were similar to B/Brisbane/60/2008 (Table). Only 4 of the 932 B/Yamagata lineage viruses analysed were low reactors to the B component of the 2015 vaccine, B/Phuket/3073/2013 vaccine strain (these comparisons were made to ferret antisera generated to the MDCK-isolated virus induced infections).

Sequencing was performed on 217 haemagglutinin genes from B viruses, the majority being B/Yamagata viruses. All of the viruses of B/Victoria lineage were genetically similar to the B/Brisbane/60/2008 reference virus (Figure 5). The majority of B/Yamagata lineage viruses belonged to Clade 3, which was represented by the 2015 vaccine strain B/Phuket/3073/2013 as well as B/Wisconsin/1/2010 (the 2013 Southern Hemisphere influenza vaccine strain) (Figure 6).

Egg isolation was attempted for 26 B/Victoria and 15 B/Yamagata viruses, with 8 (31%) B/Victoria viruses and 7 (47%) B/Yamagata viruses successfully isolated in eggs. At least 1 representative from the major clades of both B lineages was among the successful isolates.

Of 780 B/Victoria and 825 B/Yamagata viruses tested, none displayed highly reduced inhibition by either oseltamivir or zanamivir.

Table: Antigenic analysis of viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015, by country of origin

<table>
<thead>
<tr>
<th>Region</th>
<th>A(H1N1)pdm09 reference strain: A/California/7/2009 (cell)</th>
<th>A(H3N2)* reference strain: A/Switzerland/975193/2013 (cell)</th>
<th>B/Victoria reference strain: B/Brisbane/60/2008 (cell)</th>
<th>B/Yamagata reference strain: B/Phuket/3073/2013 (cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australasia</td>
<td>224 Like 4 Low reactor (%)</td>
<td>459 Like 9 Low reactor (%)</td>
<td>809 Like 3 Low reactor (%)</td>
<td>766 Like 2 Low reactor (%)</td>
</tr>
<tr>
<td>Pacific</td>
<td>18 Like 0 Low reactor (%)</td>
<td>7 Like 0 Low reactor (%)</td>
<td>12 Like 0 Low reactor (%)</td>
<td>51 Like 0 Low reactor (%)</td>
</tr>
<tr>
<td>South East Asia</td>
<td>102 Like 1 Low reactor (%)</td>
<td>133 Like 2 Low reactor (%)</td>
<td>13 Like 0 Low reactor (%)</td>
<td>87 Like 2 Low reactor (%)</td>
</tr>
<tr>
<td>East Asia</td>
<td>14 Like 0 Low reactor (%)</td>
<td>25 Like 0 Low reactor (%)</td>
<td>0 Like 0 Low reactor (%)</td>
<td>18 Like 0 Low reactor (%)</td>
</tr>
<tr>
<td>South Asia</td>
<td>25 Like 0 Low reactor (%)</td>
<td>12 Like 0 Low reactor (%)</td>
<td>0 Like 0 Low reactor (%)</td>
<td>2 Like 0 Low reactor (%)</td>
</tr>
<tr>
<td>Africa</td>
<td>7 Like 0 Low reactor (%)</td>
<td>4 Like 0 Low reactor (%)</td>
<td>0 Like 0 Low reactor (%)</td>
<td>4 Like 0 Low reactor (%)</td>
</tr>
<tr>
<td>Total</td>
<td>390 Like 5 (1.3%)</td>
<td>640 Like 11 (1.7%)</td>
<td>834 Like 3 (0.4%)</td>
<td>928 Like 4 (0.4%)</td>
</tr>
</tbody>
</table>

* Note that many A(H3N2) virus isolates that were obtained could not be analysed by haemagglutination inhibition assay due to low haemagglutination assay titre in the presence of oseltamivir.
Figure 4: Phylogenetic tree of representative haemagglutinin genes of A(H3) viruses received by the Centre during 2015

* Reference virus is indicated in red text.
† 2015 Southern Hemisphere vaccine strain is presented in capital letters.
e: egg isolate
Scale bar represents 0.3% nucleotide sequence difference between viruses.
} Braces indicate clades
Figure 5: Phylogenetic tree of representative haemagglutinin genes of B/Victoria viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015

- Sydney/1071/2015 Sep
- Victoria/1009/2015 Aug
- Brisbane/46/2015e Apr
- Christchurch/521/2015 Jul
- Darwin/83/2015 Oct
- Newcastle/1012/2015 Aug
- SouthAustralia/156/2015 Nov
- Brisbane/69/2015 May
- SouthAuckland/32/2015 Sep
- Dunedin/7/2015 Oct
- Townsville/7/2015 Jul
- Victoria/861/2015 Aug
- Wellington/14/2015 Jul
- Victoria/861/2015e Aug
- Singapore/SP895/2015e Jun
- Brisbane/310/2015 Nov
- Newcastle/7/2015 May
- Sydney/11/2015 Jun
- SouthAuckland/16/2015 Jun
- Tasmania/30/2015 Aug
- Wellington/3/2015 May
- Auckland/3/2015 Jul
- Wellington/71/2015 Sep
- Victoria/849/2015e Aug
- Brisbane/4/2015 Feb
- SouthAustralia/118/2015 Sep
- Victoria/524/2015 Jul

- Texas/02/2013e*
- Newcastle/1005/2015 Jun
- Canberra/29/2015 Jul
- Perth/24/2015 May
- Darwin/17/2015 Jul
- Sydney/256/2015 Sep
- Perth/201/2015 Aug
- Darwin/97/2015 Dec
- NewCaledonia/6/2014e Nov
- Canberra/23/2014 Dec
- Victoria/502/2015e Mar
- Darwin/11/2015 Apr
- SouthAustralia/3/2015 Feb
- Brisbane/15/2015 Mar
- Townsville/9/2014 Dec
- Brisbane/78/2014 Dec
- Vietnam/1/2014 Jan
- NewCaledonia/27/2015e Apr
- SouthAustralia/48/2015 May
- Brisbane/18/2013e Jun*
- Brisbane/63/2014e Oct
- Newcastle/28/2015 Jul
- Sydney/137/2015 Aug
- SouthAustralia/11/2012e Mar*
- Darwin/40/2012 Jun*
- SouthAustralia/81/2012e Nov*
- BRISBANE/60/2008e Aug†
- Brisbane/33/2008e*
- Malaysia/2506/2004e Dec†
- HongKong/90/2008 Feb†
- Philippines/6363/2009†
- Sydney/508/2010e Aug†
- Wellington/14/2015 Jul
- Victoria/861/2015e Aug
- Singapore/SP895/2015e Jun
- Brisbane/310/2015 Nov
- Newcastle/7/2015 May
- Sydney/11/2015 Jun
- SouthAuckland/16/2015 Jun
- Tasmania/30/2015 Aug
- Wellington/3/2015 May
- Auckland/3/2015 Jul
- Wellington/71/2015 Sep
- Victoria/849/2015e Aug
- Brisbane/4/2015 Feb
- SouthAustralia/118/2015 Sep
- Victoria/524/2015 Jul

* Reference virus is indicated in red text.
† 2015 Southern Hemisphere vaccine strain is presented in capital letters.
e: egg isolate
Scale bar represents 0.3% nucleotide sequence difference between viruses.
} Braces indicate clades
Figure 6. Phylogenetic tree of representative haemagglutinin genes of B/Yamagata viruses received by the WHO Collaborating Centre for Reference and Research on Influenza, 2015

* Reference virus is indicated in red text.
† 2015 Southern Hemisphere vaccine strain is presented in capital letters.
e: egg isolate

Scale bar represents 0.4% nucleotide sequence difference between viruses
} Braces indicate clades
Discussion

As in previous years, the vast majority of samples received at the Centre in 2015 were from Australia. Data from the National Notifiable Diseases Surveillance System indicated that in 2015, Australia reported the highest number of notifications of laboratory-confirmed cases (n=100,584) on record, which was at least partly due to increased influenza testing. This was also reflected in the large quantity of samples received at the Centre in 2015, the greatest annual number since 2009, the year of the A(H1N1)pdm09 pandemic, when 6,435 samples were received. In addition, a total of 45 candidate vaccine viruses were isolated in eggs during 2015, the largest number and highest rate of successful isolations since 2010.

Data from national influenza centres indicated that influenza B predominated over influenza A viruses in Australia during 2015, which was also reflected in the viruses analysed by the Centre. When considering only samples received from Australia and for which the lineage could be confirmed, there were slightly more B/Victoria viruses than B/Yamagata viruses (52%–48%). However, when samples submitted to the Centre from all countries were included, there were a larger number of B/Yamagata viruses (53%) compared with B/Victoria viruses (47%). This can be attributed to more B/Yamagata viruses being received from all non-Australian countries, especially New Caledonia, New Zealand and Singapore; this is also reflected in a larger number of B/Yamagata infections than B/Victoria infections recorded by the New Zealand surveillance system during 2015.

In Australia, there was a rapid rise in the number of B/Victoria lineage viruses detected from May onwards. The predominance of influenza B over influenza A viruses in any given year occurs relatively infrequently at a rate of approximately once a decade. The last year in which this occurred in Australia was 2008, and a similar switch in predominance from B/Yamagata to B/Victoria viruses was also observed in that year. The reasons for this change between the 2 lineages within the season are not clear, but may be due to subtle phylogenetic differences between the 2 lineages, differential population susceptibility or a combination of these factors. The change in the relative predominance of B lineage viruses from B/Yamagata to B/Victoria during 2015 was also reflected in the change in recommended B strain for the 2016 Southern Hemisphere trivalent influenza vaccine (TIV) to the B/Victoria-lineage reference virus B/Brisbane/60/2008.

Almost all B/Yamagata lineage viruses tested were antigenically similar to the 2015 vaccine virus B/Phuket/3073/2013 and this was reflected in the overall vaccine effectiveness (VE) estimates for Australia (VE 71%, 95% CI: 57–80). Similarly, the majority of B/Victoria lineage viruses tested were antigenically similar to the reference virus B/Brisbane/60/2008. Lower VE estimates were reported for B/Victoria lineage viruses (VE 42%, 95% CI: 13–61) in Australia in 2015, however this is unsurprising as these estimates were calculated for the trivalent influenza vaccine (TIV), which included only a B/Yamagata lineage virus. Vaccine effectiveness against both influenza B lineages may be improved through the use of the quadrivalent influenza vaccine (QIV) which was introduced in Australia in 2015, but not widely used as it was not included in the National Immunisation Program and only available for private purchase.

Despite the predominance of influenza B during 2015, influenza A(H3N2) predominated in Tasmania, and co-circulation of A(H3N2) and influenza B was observed in Western Australia. The majority of circulating A(H3) viruses were antigenically similar to the cell derived A/Switzerland/9715293/2013 reference strain in the vaccine. However, more than half of the viruses analysed at the Centre were low reactors to the egg derived A/Switzerland/9715293/2013 reference strain. The difficulties in detecting antigenic changes in A(H3) subtype viruses using the HI assay reported in recent years, continue to present an ongoing challenge for surveillance. This factor and poor viral growth reduced the number of A(H3) viruses received during 2015 that could be analysed by the HI assay by 44%. The Centre is continuing to work on the development of other assays (e.g. virus neutralisation) to characterise the antigenicity of recent A(H3) viruses, however at this time such assays remain time- and labour-intensive and would most likely complement rather than replace the HI assay. In light of this, genetic analysis remains an important tool for detecting both minor and major changes in A(H3) viruses.

Genetic data from the Centre indicated that many of the A(H3) viruses in 2015 fell into clade 3C.2a. Viruses from this clade have been circulating in Australia since 2014 and are somewhat antigenically distinct to the A/Switzerland/9715293/2013 reference strain in the 2015 Southern Hemisphere vaccine, which lies in clade 3C.3a. Phylogenetic data from the Northern Hemisphere suggested that the A(H3) component of the 2015 TIV may not be ideally matched and VE estimates for Australia were indeed low to moderate for A(H3N2) (VE 44%; 95% CI: 21–60). In the WHO recommended vaccine for the southern hemisphere TIV/QIV in 2016, the A(H3) vaccine virus was updated from A/Switzerland/9715293/2013 to A/Hong Kong/4801/2014, which lies in clade 3C.2a.
Finally, antigenic and genetic data for A(H1)pdm09 viruses analysed at the Centre in 2015 indicated an overall good match with the recommended vaccine strain A/California/7/2009. Whilst phylogenetic analysis indicated the emergence of 2 distinct subclades. These changes did not affect the antigenic behaviour of the viruses in HI assays using ferret antisera. As such, A/California/7/2009 was recommended for inclusion again in the TIV/QIV for 2016. This was also reflected in the Australian vaccine effectiveness estimates for A(H1)pdm09 viruses in 2015 (VE 79%, 95% CI: 33–93), as well as interim vaccine effectiveness estimates from the northern hemisphere for the 2015–2016 season that indicated that vaccines containing A/California/7/2009 were also effective against viruses in the emerging 6B.1 clade (VE 64% (95% CI: 44%–77%).

With continual change and evolution in influenza viruses and the absence of a universal vaccine, there remains a need for ongoing influenza surveillance for the foreseeable future. In light of this, the work performed by the Centre in Melbourne is crucial to the efforts of the global surveillance community to ensure that viruses recommended for the influenza vaccine remain updated and as closely matched to circulating viruses as possible.

Acknowledgements

The authors would like to thank all of the National Influenza Centres and other contributing laboratories who sent influenza positive samples to the Centre during 2015. We also thank Ian Barr for his comments and input to this report. The FluAnalysis pipeline was developed by October Sessions and Uma Kamaraj from Duke-NUS Medical School, Singapore. The Melbourne WHO Collaborating Centre for Reference and Research on Influenza is supported by the Australian Government Department of Health.

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References


AUSTRALIAN NATIONAL ENTEROVIRUS REFERENCE LABORATORY ANNUAL REPORT, 2014

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Abstract

Following the World Health Organization (WHO) recommendation, Australia conducts surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years of age as the main method to monitor its polio-free status. Cases of AFP in children are notified to the Australian Paediatric Surveillance Unit or the Paediatric Active Enhanced Disease Surveillance System and faecal specimens are referred for virological investigation to the National Enterovirus Reference Laboratory. In 2014, no cases of poliomyelitis were reported from clinical surveillance and Australia reported 1.4 non-polio AFP cases per 100,000 children, meeting the WHO performance criterion for a sensitive surveillance system. Non-polio enteroviruses can also be associated with AFP and enterovirus A71 and echovirus types 6 and 7 were identified from clinical specimens from cases of AFP. Globally, 359 cases of polio were reported in 2014, with the 3 endemic countries, Afghanistan, Nigeria and Pakistan, accounting for 95% of the cases. In May 2014, the WHO declared the international spread of wild poliovirus to be a public health emergency of international concern and has since maintained recommendations for polio vaccination of travellers from countries reporting cases of wild polio. Commun Dis Intell 2017;41(2):E161–E169.

Keywords: poliovirus, acute flaccid paralysis, surveillance, enterovirus, poliomyelitis, eradication, vaccination

Introduction

Australia has established clinical and virological surveillance schemes to monitor its polio-free status. The clinical surveillance follows the World Health Organization (WHO) recommendation of investigating cases of acute flaccid paralysis (AFP) in children less than 15 years of age as an age group at high risk of poliovirus infection. AFP cases are ascertained either by clinicians notifying the Australian Paediatric Surveillance Unit (APSU) via a monthly report card or through the Paediatric Active Enhanced Disease Surveillance System (PAEDS) at 5 sentinel tertiary paediatric hospitals. The WHO recommends that 2 faecal specimens be collected at least 24 hours apart and within 14 days of the onset of paralysis from cases of AFP for virological investigation to exclude poliovirus as the causative agent. It is a requirement of the WHO polio eradication program that the specimens are tested in a WHO accredited laboratory, which for Australia is the National Enterovirus Reference Laboratory (NERL) at the Victorian Infectious Diseases Reference Laboratory (VIDRL). The clinical and laboratory data from AFP cases in children are reviewed by the Polio Expert Panel (PEP) and reported to the WHO as evidence of Australia’s continued polio-free status.

Clinical specimen and environmental surveillance programs were established as virological surveillance for poliovirus, to complement the clinical surveillance program focused on AFP cases in children. Enteroviruses other than poliovirus have been associated with AFP and poliovirus infection may manifest clinically without paralysis, such as meningitis. The Enterovirus Reference Laboratory Network of Australia (ERLNA) involves public diagnostic virology laboratories reporting enterovirus typing results from clinical specimens to exclude poliovirus involvement and to establish the epidemiology of non-polio enteroviruses (NPEVs) in Australia. Most poliovirus infections are asymptomatic with the virus shed for weeks in the faeces of infected persons. WHO supports the testing of environmental or raw sewage samples as a means of detecting the presence of wild poliovirus in polio-free countries. In 2014, the testing of environmental samples commenced at a sentinel site in metropolitan Melbourne.

The number of wild polio cases worldwide decreased from 416 in 2013 to 359 in 2014. Pakistan reported 306 of the cases and has become the major source of wild poliovirus transmission, this also being the source of cases in neighbouring Afghanistan. Nigeria is the 3rd remaining endemic country with only 6 wild polio cases reported there in 2014, the lowest number since the global polio eradication program started in 1988. Only wild poliovirus serotype 1 was detected in 2014, with the last report of wild poliovirus type 3 being in Nigeria in November 2012 and of wild poliovirus type 2 being in India in 1999. This latter achievement led to the removal of the Sabin 2 serotype from oral polio vaccine (a live vaccine) along with laboratory containment of this serotype, involving restricted access at a limited number of facilities worldwide in 2016. All 3 serotypes will still be incorporated in the inactivated polio vaccine. In May 2014, the WHO Director-General declared...