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Effectiveness of seasonal influenza vaccine in Australia, 2015: An epidemiological, antigenic and phylogenetic assessment

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ABSTRACT

Background: A record number of laboratory-confirmed influenza cases were notified in Australia in 2015, during which type A(H3) and type B Victoria and Yamagata lineages co-circulated. We estimated effectiveness of the 2015 inactivated seasonal influenza vaccine against specific virus lineages and clades.

Methods: Three sentinel general practitioner networks conduct surveillance for laboratory-confirmed influenza amongst patients presenting with influenza-like illness in Australia. Data from the networks were pooled to estimate vaccine effectiveness (VE) for seasonal trivalent influenza vaccine in Australia in 2015 using the case test-negative study design.

Results: There were 2443 eligible patients included in the study, of which 857 (35%) were influenza-positive. Thirty-three and 19% of controls and cases respectively were reported as vaccinated. Adjusted VE against all influenza was 54% (95% CI: 42, 63). Antigenic characterisation data suggested good match between vaccine and circulating strains of A(H3); however VE for A(H3) was low at 44% (95% CI: 21, 60). Phylogenetic analysis indicated most circulating viruses were from clade 3C.2a, rather than the clade included in the vaccine (3C.3a). VE point estimates were higher against B/Yamagata lineage influenza (71%; 95% CI: 57, 80) than B/Victoria (42%, 95% CI: 13, 61), and in younger people.

Conclusions: Overall seasonal vaccine was protective against influenza infection in Australia in 2015. Higher VE against the B/Yamagata lineage included in the trivalent vaccine suggests that more widespread use of quadrivalent vaccine could have improved overall effectiveness of influenza vaccine. Genetic characterisation suggested lower VE against A(H3) influenza was due to clade mismatch of vaccine and circulating viruses.

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1. Background

Since 2010, the number of notified laboratory confirmed influenza cases in Australia increased by an average of 63% annually to a record 100,582 in 2015 [1]. Notified cases in 2015 were predominantly type B, which was unusual for two reasons. First, there

is a widely held belief that seasons dominated by influenza B viruses are mild. However, while this was somewhat reflected in outpatient surveillance data, the large number of notified cases suggested otherwise [2]. Second, influenza seasons are only dominated by B viruses every 7–10 years and this last occurred in Australia in 2008. Interestingly, in both 2008 and 2015 there was a switch in influenza B lineage [3]. This has implications for vaccine strain composition and vaccine effectiveness. In 2015, quadrivalent influenza vaccine (QIV) was introduced in Australia but was not widely used because it was not included in the

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Australian government's National Immunisation Program (NIP). This program provides free influenza vaccine to certain groups, which in 2015 included all adults aged 65 years and older, Aboriginal and Torres Strait Islander people aged six months to less than five years or 15 years of age and over, pregnant women and individuals aged six months and older with medical conditions predisposing to severe influenza [4]. Approximately 71% of target groups receive their seasonal influenza vaccine under the NIP [5]. Individuals were also able to be vaccinated outside the NIP by private purchase at pharmacies or through workplace vaccination programs. Indeed, QIV was only available for private purchase in Australia or as part of the healthcare immunisation program in Western Australia [6].

The 2015 southern hemisphere trivalent influenza vaccine (TIV) comprised an A/California/7/2009 (H1N1)pdm09-like virus; an A/Switzerland/9715293/2013 (H3N2)-like virus; and a B/Phuket/3073/2013-like virus (of the B/Yamagata/16/88 lineage) [7]. The QIV also contained a B/Brisbane/60/2008-like virus (of the B/Victoria/2/87 lineage). Phylogenetic data from the northern hemisphere suggested that the H3 component of this vaccine might be genetically mismatched. By February 2015, Canada reported that the majority of circulating viruses fell in genetic group 3C.2a, not the 3C.3a group in which the Switzerland virus lay [8]. Moreover, early in the season it was apparent that Victoria lineage B viruses were circulating in high frequency [9], and by mid-season Victoria lineage viruses had taken over Yamagata-lineage viruses as the dominant B viruses [3]. These two observations were therefore expected to result in compromised vaccine effectiveness.

The 2015–16 northern hemisphere influenza vaccine had the same composition as the 2015 southern hemisphere vaccine [10]. Although influenza activity at the time of writing was low, circulating B viruses were predominantly Victoria lineage and H3 viruses were predominantly from genetic group 3C.2a [11]. Thus, the northern hemisphere had entered its influenza season with a vaccine that may have been suboptimally matched to circulating strains. In the present study, we estimate influenza vaccine effectiveness using Australian data. Having established the validity of pooling data from three sentinel general practitioner (GP) networks across the country [12], we were provided the opportunity to estimate the effectiveness of the 2015 seasonal influenza vaccine against specific virus lineages and clades.

2. Methods

The Australian Sentinel Practices Research Network (ASPREN), the Victorian Sentinel Practice Influenza Network (VicSPIN), and the Sentinel Practitioners Network of Western Australia (SPNWA) constitute Australia's three sentinel general practice (GP) networks for influenza surveillance. SPNWA and VicSPIN operate in the respective states of Western Australia and Victoria, whilst ASPREN GPs are primarily located in the other six states and territories, except for several in Victoria.

The sentinel networks use similar data collection methods, which have been described previously [13–15]. Briefly, participating general practitioners submitted weekly reports of the number of patients presenting with influenza-like illness (ILI – defined as cough, fever and fatigue) and the number of total consultations. Nose/throat swabs were collected from a sample of ILI patients along with demographic data, date of ILI onset, influenza vaccination status and presence of comorbid conditions for which influenza vaccination is indicated. Vaccination status is by self-report and verified from GP records, if available. ASPREN GPs were instructed to systematically swab 20% of ILI patients, whilst ILI

patients in SPNWA and VicSPIN were selected for swabbing at the discretion of the GP.

2.1. Laboratory analyses

Swabs were tested for influenza, influenza subtypes A(H1) and A(H3) [16,17], and type B lineages Victoria and Yamagata [18], by reverse transcriptase polymerase chain reaction (RT-PCR). All positive samples from ASPREN and VicSPIN, and SPNWA samples that tested positive by culture at PathWest Laboratory Medicine WA, were referred to the WHO Collaborating Centre for Reference and Research on Influenza (Melbourne, Australia) for antigenic characterisation and sequencing. Viruses were isolated using Madine-Darby canine kidney (MDCK) cells. Antigenic characterisation was performed on all virus isolates by haemagglutinin inhibition (HI) assay [19], using turkey erythrocytes for A(H1) and B viruses. For A(H3) viruses, guinea pig erythrocytes were used in the presence of oseltamivir. Assays were performed against the 2015 vaccine strains using cell-derived strains, and for A(H3), viruses were also tested against the egg-derived strain.

The haemagglutinin (HA) and neuraminidase (NA) genes of all A (H3) isolates were characterised genetically by next generation sequencing (NGS). Briefly, RNA was extracted from H3 viruses cultured in MDCK cells using QIAxtractor (Qiagen) according to the manufacturer's instruction. HA and NA genes were amplified by multiplex RT-PCR using a novel method with mixed HA and NA primers (unpublished). The amplicons were fragmented and ligated to bar-coded sequencing adaptors using Ion Xpress fragment library kit (Life Technologies, Carlsbad, CA). The library was then clonally amplified and sequenced on the Ion Torrent Personal Genomic Machine (PGM) as per the manufacturer's instruction. After sequencing, the data were processed and consensus sequences were generated using a custom-made pipeline compiling different open source software programs for streamlined analysis (unpublished). Phylogenetic maximum likelihood (ML) trees were constructed with Geneious 9.0.4 (Biomatters, Auckland, New Zealand) and visualized with Figtree v1.4 (<http://tree.bio.ed.ac.uk/software/figtree>). Key amino acid substitutions including the antigenic sites described by Webster and Laver [20], Skehel et al. [21], and Koel et al. [22] and potential changes in glycosylation sites were also identified.

The HA sequences obtained in this study and used in the phylogenetic analysis were deposited in EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAI) under the following accession numbers: EPI197966, EPI197988, EPI198096, EPI194516, EPI206572, EPI194517, EPI194518, EPI202450, EPI206570, EPI206571, EPI197967, EPI194525, EPI198094, EPI202452, EPI202453, EPI198083, EPI197968, EPI202455, EPI197987, EPI197392, EPI202457, EPI197879, EPI194545, EPI194546, EPI197973, EPI197979, EPI198023, EPI198024, EPI202460, EPI198086, EPI198090, EPI198088, EPI198089, EPI198087, EPI198091.

2.2. Estimation of vaccine effectiveness

Vaccine effectiveness (VE) was estimated using the case test-negative design [23]. VE was calculated as $(1 - \text{adjusted odds ratio}) \times 100\%$, where the odds ratio compares the odds of vaccination among patients testing positive for influenza with patients testing negative. Odds ratios were adjusted for week of specimen collection (cubic spline with 4 knots), and age group (<18 years, 18–64 years \geq 65 years) using logistic regression with a fixed effect for network. Estimates were calculated separately for each age group and influenza type/subtype/lineage. Heterogeneity among networks was assessed by the I^2 statistic. Patients were excluded from the analysis if vaccination status or the date of

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