INFLUENZA IN VULNERABLE POPULATIONS

Dr RASHMI DIXIT

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Primary supervisor:
Professor Robert Booy
• National Centre for Immunisation Research and Surveillance at The
  Children’s Hospital at Westmead
• University of Sydney

Associate Supervisor:
Professor Dominic Dwyer
• Institute of Clinical Pathology & Medical Research (ICPMR) Westmead
  Hospital
• Centre for Infectious Diseases & Microbiology (CIDM) Westmead Hospital
• University of Sydney
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Authorship Attribution Statement

Publication #1/Chapter #1: Dixit R, Khandaker G, Ilgoutz S, Rashid H, Booy R.

I was the first and corresponding author for this study. I conducted the literature review for this publication. Scott Ilgoutz and I reviewed the literature. I analysed and interpreted the literature, with oversight by Gulam Khandaker and Harunor Rashid. I wrote the manuscript. I revised the manuscript with input from co-authors Harunor Rashid and Robert Booy.


I was the first and corresponding author for this randomised controlled trial. Robert Booy and Gulam Khandaker designed the study. I conducted the literature review. Gulam Khandaker, Peter Hay and I recruited cases and obtained the specimens. Some cases were recruited in 2011, and the rest at the commencement of my PhD in 2012, and all analysis was conducted 2012-2013. Dr Gulam Khandaker recruited three hospital cases, and I recruited the others. Dr Peter Hay recruited the community cases from his General Practice; I liaised with him regularly to obtain updated data. Dominic Dwyer and Ken McPhie provided microbiological analysis. I interpreted the clinical and microbiological results, and drafted the manuscript. I revised the manuscript with input from co-authors, especially Leon Heron and Robert Booy.


I was first and corresponding author for this study. I performed the extensive literature review of two databases for this publication, with assistance from Richard Dalton. I analysed the literature and summarized it. I interpreted the findings. I drafted the manuscript and revised it with input from Robert Booy and Jenny Herz.

Chapter #4: Dixit R; Sheppeard V, Corbett S, Dwyer D, Leon Heron L, Lindley R, Bag, S, Conaty, Booy R. A Randomised Controlled Trial of Oseltamivir Treatment and Prophylaxis During Influenza Outbreaks in Aged-care Facilities in the Context of Optimal Influenza Vaccination and Infection Control

I was the first author for this study protocol. I researched current national and international
guidelines and literature. I designed the protocol, based on an earlier study, with the significant addition of a section on active surveillance and expanded discussion on infection control. I am liaising with expert statisticians regarding sample size and statistical analysis of resulting data. Professor Booy and I liaised with professionals in Aged Care and Public Health, who contributed as co-authors to the final protocol. I wrote the manuscript with input from Professor Booy.


I was the first and corresponding author for this study. I designed the study with my co-authors. I was involved in collecting the specimens, with assistance from Marino Festa and Gulam Khandaker. Slade Matthews provided pharmacokinetic analysis. Results were interpreted and summarized by Slade Matthews and me. I wrote the manuscript with input from co-authors.

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Peter McIntyre and I designed the concept, which was refined by Robert Menzies. I was the main author and data analyst. I performed the literature review. Priyanka Ray, Fleur Webster and I retrieved the data. I charted all the data, collated it and statistically analysed it, with guidance from Robert Menzies. I wrote the chapter with revisions by Robert Menzies.

Rashmi Dixit

Robert Booy

December 30, 2017
Statement of originality

This is to certify that, to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any other degree or other purposes. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and all sources have been acknowledged.

Rashmi Dixit

December 30, 2017
Abstract - Influenza in Vulnerable Populations

Influenza is a ubiquitous virus that results in thousands of deaths annually, particularly in susceptible people. Vulnerability is manifested in two different scenarios, with circumstances that render the whole population vulnerable i.e. when resistant or novel strains start to circulate or predominate; and in those prone to influenza, such as the extremes of age, colonised populations, and those with chronic diseases. The first three chapters address situations that have a population impact. Chapter 1 reviews literature on oseltamivir-resistance. Current circulating strains are susceptible, albeit with clusters of oseltamivir-resistance. In the recent past, oseltamivir resistant strains predominated, highlighting the need for alternative strategies. Chapter 2, a randomized controlled trial, demonstrated no clinical or virological advantage of double dose versus standard dose oseltamivir. Chapter 3 reviews the application of polyclonal antibodies to neglected diseases including avian influenza, with promising results in terms of safety and immunogenicity. Chapters 4, 5 and 6 turn attention to those demographically at risk of influenza. Chapter 4 aims to discover which antiviral strategies works best to prevent and manage influenza outbreaks in aged care facilities. A protocol for treatment alone versus treatment plus prophylaxis is proposed. The issue of how to obtain participation consent remains unresolved. In chapter 5, oseltamivir pharmacokinetics in infants is presented, with a paucity of published data. Our results support current dose recommendations. Chapter 6 examines the intersection of higher rates of both background chronic conditions and severe influenza during the 2009 pandemic amongst Indigenous Australians. The first age-standardised national analysis of government-collected data is presented. There was no clear correlation between background chronic disease and influenza in Indigenous Australians. This suggests other factors resulting from colonisation are responsible for higher influenza rates.

I would like to acknowledge the traditional Aboriginal and Torres Strait Islander owners of Australia and commit to working with their interests in mind.

Rashmi Dixit
PhD Student
Faculty of Medicine
Sydney University
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1. Influenza in Vulnerable Populations - Introduction

1.1 Motivation for this PhD Thesis

As an infectious diseases specialist with an interest in the health of vulnerable populations, I undertook this PhD thesis in influenza, as it is not only a ubiquitous respiratory virus, but varies in its clinical impact, depending on the underlying health of the patient. Whilst influenza causes annual seasonal epidemics in temperate climates, certain populations are more prone to severe or intractable disease. This thesis examines the impact of influenza and its antiviral management in vulnerable populations: those who are particularly susceptible to influenza and its complications. These include circumstances where antiviral-resistant strains of influenza are circulating such that treatment options become limited, the elderly, the very young, those with compromised immune responses (particularly due to pre-existing organ system diseases), and Indigenous peoples of colonised countries (1–5). Serological naivety to circulating strains also predisposes the population to infection. This is particularly evident during influenza pandemics, such as the 2009 influenza A H1N1 'swine flu' pandemic, which mostly affected the younger population due to pre-existing immunity from prior exposure to related influenza strains in the older demographic, especially in those over 75 years (6,7). It is also of concern in the event of circulation of new reassorted influenza viruses, such as various avian influenza strains. I have an interest in the intersection between and communicable and non-communicable diseases, the latter which can be the result of proximal social, economic, environmental, and ‘lifestyle’ determinants, such as diet, human movement culture, travel, poverty and health infrastructure and access.

1.2 Thesis Aims

The aims of this thesis are:

1. To examine the extent of influenza antiviral resistance in circulating strains, which place immunologically vulnerable populations at risk – e.g. due to reduced vaccine response

2. To examine strategies to prevent or overcome antiviral resistance – e.g. different dosing regimens and novel antiviral strategies, such as antibody therapies

3. To examine the use of antivirals in the vulnerable extremes of age – infants, about whom there is a paucity of pharmacological data; and the elderly in aged care facilities, in whom immune responses to vaccines are suboptimal and in whom outbreaks occur

4. To examine whether the vulnerability of Indigenous Australians to higher rates of influenza and its complications is due to the health gap between Indigenous and non-Indigenous Australians in chronic conditions
1.3 Introductions to Each Chapter

1.3.1 CHAPTER 1 – A Historical Review of Oseltamivir Resistance

My first paper is a published review of the emergence of oseltamivir resistance in influenza strains that predominantly circulated before, during and soon after the 2009 H1N1 influenza A ‘swine flu’ pandemic (8). Antiviral resistance is relevant in two settings of population vulnerability: when influenza immunisations are less reliable, and when there is serological naivety in the whole population due to a reasserted, novel virus. Effective antiviral agents are an essential part of the influenza management armament because vaccination, the most important strategy for influenza prophylaxis, has limitations. Firstly, the annual influenza vaccine is based on a prospective prediction of the circulating influenza strains, an imperfect science (9). Mismatch between vaccine strains and those that ultimately circulate can result in population vulnerability to influenza. Secondly, vaccine effectiveness is determined by the immune response in the recipient. The same immunocompromising factors that predispose vulnerable people to severe influenza can reduce biological responses to the vaccine, particularly old age and immunodeficiencies (10). Thus, the main additional protective measures for these individuals in whom the vaccine may, afford only partial protection are high levels of community vaccination conferring a ‘herd immunity’ effect; and antiviral medications (11). Herd immunity is limited by the influenza vaccine not being funded for all individuals in Australia by the Pharmaceutical Benefits Scheme; and by lack of vaccine promotion and uptake in those for whom it is funded (12,13).

The main current antivirals recommended for influenza treatment by the World Health Organization (WHO) are the neuraminidase inhibitors oral oseltamivir, inhaled zanamivir, and IV peramivir (14). Neuraminidase inhibitors (NAI) were introduced in 1999, and are the only specific anti-influenza antivirals currently registered in Australia. Other antiviral classes such as the adamantanes (amantadine and rimantadine) now lack utility due to fairly rapid development of resistance amongst influenza A viruses: more than 99% resistance to influenza A (H3N2 and H1N1), and inherent inactivity against influenza B. Thus, continuous vigilance is required to anticipate development of the same fate for NAIs.

I performed a literature search of all reports of resistance rates to oseltamivir in circulating viruses over the last several influenza seasons. Being aware of patterns such as the rate of development of resistance and the geographical distribution of resistant strains provides information to health authorities and the pharmaceutical industry to enable both accurate planning and development of alternative anti-influenza measures. These might include development of new antiviral medications and modes of therapy, and novel dosing and administration regimens of currently registered antivirals to avert or overcome resistance. I analysed utility of oseltamivir by documenting a detailed timeline of circulating influenza strains since the release of oseltamivir in 1999, and the degree to which oseltamivir resistance had developed amongst them. I summarised in table format many published reports and case series from different countries and continents, representing different clusters. Oseltamivir resistance can be conferred by a single missense mutation at the gene coding for the oseltamivir-binding site. Resistance arose and spread quickly in 2007–2008, resulting in a majority of
circulating seasonal influenza A/H1N1 strains being oseltamivir-resistant in 2008. I took a narrative approach, because the studies were heterologous.

Strategies to reduce emergence of resistant strains, such as higher dose oseltamivir regimens, development of new antiviral classes, or even resurrection of historical treatment methods, warranted further examination.

1.3.2 CHAPTER 2 – An Unblinded Randomised Controlled Trial of Standard versus Double Dose Oseltamivir

My second paper explored one of these strategies: I conducted an unblinded controlled trial of differential oseltamivir dosing in healthy children and adults with influenza (15). I compared the current recommended doses to a regimen where the standard dose was doubled (16). Due to limits imposed by ethical considerations, the study was performed on healthy patients without serious comorbidities, in whom side effects would be better tolerated. The strategy of doubling the dose of oseltamivir has been employed in treating hospitalised immunocompromised patients, who shed virus for longer than immunocompetent people, and in those with severe influenza in an attempt to clear symptoms and stop viral shedding more quickly (17–19). Prolonged nasal shedding of the influenza virus can stretch hospital resources due to the requirement to isolate these patients. This strategy of doubling standard doses has been recommended by the WHO for immunosuppressed individuals with severe influenza, with acknowledgment that the evidence base is weak (20). Evidence is additionally lacking for the proposition that increasing the viral kill rate via higher antiviral doses reduces development of strains containing resistance-conferring mutations. However, oseltamivir has a narrow therapeutic index with frequent gastrointestinal side effects, which might limit higher doses (21). While a few studies have examined different dosing regimens of oseltamivir in hospitalised patients, where recognition of efficacy and side effects is enhanced by the ability for close clinical observation, most patients at increased risk of severe influenza are diagnosed and treated in the community, including those with obesity, diabetes, heart disease and asthma (22-25). Our study was unique in addressing patients with influenza in the mild to moderate (non-hospitalised) stages. We aimed to recruit from either a hospital emergency department with most cases discharged straight back into the community, or in a community general practice. The objectives of this study were to investigate the effectiveness of DD (150mg bid for adults or 10mg/kg for children) as compared with SD (75mg bid or 5mg/kg for children) oseltamivir in subjects ≥5 years old. I examined clinical disease, virological outcomes, adverse events and the frequency of detection of oseltamivir-resistant virus. If the results of this study demonstrated a clinical and virological advantage without increased gastrointestinal side effects, it could provide a case for extrapolating such a study to those with predisposing health conditions. The next chapter examined an alternative strategy for antiviral management in the context of oseltamivir resistance and novel strains, which, as I have discussed, place both the general population and those at risk of infection and disease severity at risk.
My third paper explored another of the strategies for management of resistant influenza (and other infections) – heterologous polyclonal antibody therapy. I explored an 130-year-old strategy with modern applications – antibodies against various toxins and pathogens that have been raised in horses, sheep, and other animals, and are then extracted and purified before being applied to humans (42). I performed an extensive, original review. As my first paper demonstrated, currently circulating strains are largely sensitive to oseltamivir, but strains that predominated in previous seasons developed a high level of resistance, e.g. seasonal H1N1 during the 2007–2008 influenza season. Additionally, resistance can develop either de novo or during treatment. Such viruses can then circulate, and become responsible for clusters of oseltamivir-resistant influenza, such as the 29-case cluster in 2011 of oseltamivir-resistant pandemic 2009 A (H1N1) viruses ‘swine flu’, from Hunter New England, New South Wales (Appendix 9.1). More worriedly, highly pathogenic avian viruses such as H5N1 and H7N9, which currently have limited human-to-human transmission ability and to which the population is by and large previously unexposed, may acquire virulence factors that would enable expedient human spread. This sets the scene for another global pandemic – one that may be far more devastating than the 2009 ‘swine flu’ pandemic, to which there was some pre-existing population immunity in the elderly. These avian strains currently remain sensitive to oseltamivir. Were resistance to NAIs to become widespread, there would be no fallback treatment option, with potential to cause widespread morbidity and mortality. A relatively low number of cases of avian types, such as influenza A (H5N1) and influenza A (H7N9), have been reported; mainly in vulnerable groups such as the elderly and those with pre-existing chronic illnesses (26–31). Thus, vulnerable populations – the elderly, and those with underlying illnesses and compromised immune systems – are particularly at risk of avian influenza. There is serological evidence that the incidence of subclinical infection of H5N1 is much higher than clinical infection (32,33). These strains currently have low person-to-person transmissibility, but mutations that can confer this ability can set the scene for a global pandemic (27,34). There is no evidence of background serological immunity to H7N9 prior to 2012, and only of low background rate of immunity to H5N1, rendering the global population vulnerable to an avian influenza pandemic (32,35,36). The WHO and the United States-based Centers for Disease Control and Prevention both currently recommend early initiation of oseltamivir, based on limited data demonstrating a possible mortality benefit (37,38). However, several oseltamivir-resistant H7N9 and H5N1 clinical isolates have emerged (39–43). In the event of an epidemic or pandemic, secondary modes of effective treatment are warranted. Development of a reliable vaccine has been hampered by technical difficulties and poor immunogenicity on testing, requiring at this time a reliance on treatment modalities against avian influenza to address morbidity and mortality (44).

Currently, heterologous polyclonal antibodies are used against snake venom toxin and rabies virus infection, with great success. Historically, however, they have had applications to a range of viruses and bacteria in the pre-antibiotic era, and may be called upon in the advancing era of multi-resistant
bacterial infections. (45,46). These therapies are being reexamined as treatment options for epidemic and pandemic strains of influenza, such as avian influenza (47). They were used in the 1918 ‘Spanish flu’ pandemic, and the WHO Blood Regulators Network has recognised their potential role against pandemic influenza in its ‘Position paper on collection and use of convalescent plasma or serum as an element in pandemic influenza planning; July 2009’ (48). Thus, there is a renewed focus on old technologies that predated the antibiotic era. This review aimed to examine the current utility of heterologous antibodies – their safety and efficacy, and modern ways of overcoming their past limitations, namely hypersensitivity reactions to animal proteins. I reported developments in their production for application to avian influenza, and the result of a phase I trial. This paper concluded my analysis of oseltamivir resistance as a condition that places the general population, particularly those at higher risk of influenza acquisition and severity, at risk of untreatable disease. I proceeded in the rest of my thesis to explore the impact of influenza and its management in various vulnerable sub-populations.

My last three papers focused on influenza in those individuals whose demographics confer vulnerability.

1.3.4 CHAPTER 4 – A Protocol for a Randomised Controlled Trial of Oseltamivir for Management of Influenza Outbreaks in the Elderly Residing in Aged Care Facilities

The first of these investigations of those whose demography predisposes them to influenza, was a study of strategies to prevent of influenza outbreaks amongst the elderly residing in aged care facilities (ACFs). Elderly people are at a heightened risk of acquiring influenza and dying from it. Moreover, communal living arrangements and sharing of both staff and amenities promote influenza outbreaks. Aged care facilities are also a potential source of community outbreaks. The Communicable Diseases Network of Australia guidelines on influenza outbreak management in residential care facilities were published in 2009 and were updated in 2017 (49,50). Both the previous and current guidelines advise ‘consideration’, only, of oseltamivir for treatment of cases plus treatment of contacts (prophylaxis) during outbreaks (as opposed to no prophylaxis). The lack of firm recommendations reflects the paucity of evidence.

I generated a protocol for an extensive, unblinded, randomised controlled trial of influenza treatment of cases (arm 1) versus treatment of cases plus prophylaxis (arm 2) during influenza outbreaks, in up to 100 Western Sydney ACFs. Elderly residents of ACFs are particularly prone to influenza and its complications (51,52). Being frail, the institutionalised elderly person mounts a poorer immune response to the influenza vaccine than younger recipients (53–55). Elderly patients often have chronic illnesses and reduced mobility, both of which predispose them to complications of influenza, such as pneumonia (56,57). Aged care facilities are also a setting for influenza outbreaks amongst both residents and staff due to communal living arrangements; these outbreaks can potentially spread to the community via staff and visitors (51,52)
The role of oseltamivir itself has come under much scrutiny in the published literature, at meetings and conferences, and subsequently amongst health care professionals. A 2014 Cochrane review of randomised controlled trials concluded that oseltamivir treatment reduced symptoms of influenza by 17 hours, and prophylaxis reduced development of influenza (58). Regarding development of complications or death, they reported either no reduction or a lack of firm conclusions. They reported an increase in gastro-intestinal and neuropsychiatric side effects. Significantly, this review did not address institutional outbreaks. The review was criticised for excluding observational data, the main data type collected during the 2009 pandemic, and its findings were controversial. A 2014 review of all Roche-sponsored controlled trials by the United Kingdom-based Multiparty Group for Advice on Science came up with different findings: they showed symptom reduction by 25 hours, as well as a risk reduction in lower respiratory tract infections (pneumonia and bronchitis) of 44%, and a 63% risk reduction of hospital admission (59). They found only gastro-intestinal side effects, and no neuropsychiatric adverse events. Renowned Australian influenza epidemiologists Aeron Hurt and Heath Kelly recently called these secondary outcome findings into question, as hospital admissions were ‘all cause’ rather than specifically for influenza, and definitions of lower respiratory infection were not standardised. Various reviews of observational data of tens of thousands of hospitalised patients by the same Multiparty Group for Advice on Science demonstrated a reduction in mortality amongst those with laboratory-proven influenza in those who received oseltamivir within 48 hours (60,61).

In 2016, Hurt and Kelly reviewed all these conflicting publications and concluded that oseltamivir reduces symptoms by up to 1 day, and possibly mortality in severely ill patients, when given early (within 48 hours) (62).

Booy et al., in a study of 16 ACFs, showed oseltamivir significantly reduced acute influenza attack rates and outbreak duration when used for treatment of cases plus prophylaxis of contacts, compared to treatment of cases alone. It was underpowered to show differences in complications of severe influenza (63). In contrast, a recent study of 42 nursing homes over five seasons demonstrated oseltamivir prophylaxis was ineffective in preventing influenza or influenza-like illness, but this was also underpowered (64). A retrospective comparison of three different prophylaxis approaches in three individual ACFs during one influenza season showed that prophylaxis did not reduce attack rate but did reduce mortality, hospitalisation, and outbreak duration (65). These contrasting findings amongst underpowered studies of oseltamivir use in ACFs highlight the need for better data. An expanded study of 70–100 ACFs would provide the statistical power needed to accurately assess the benefits of antiviral prophylaxis.

Therefore, this enlarged and expanded study protocol was developed with several additional measures proposed. This study introduces several elements not present in the smaller study. It includes information sessions conducted at ACFs to institute enhanced infection control before outbreaks, e.g., promoting vaccination, reinforcing hand hygiene, and instituting contact and droplet precautions. The protocol also introduces a computerised active surveillance system with clinical monitoring and early notification of cases of influenza-like illness, an intervention that could conceivably widened to other infectious outbreaks in ACFs. Influenza-like illness confirmed as
influenza with point-of-care tests for rapid detection of influenza – training and monitoring ACF staff performance of these tests would be a new challenge. Finally, the calculation of sample size and outcome measures required involvement of a statistician; I liaised with Professor Christopher Triggs, Head of Department of Statistics at University of Auckland. I supplied him data from Booy et al’s current study as well as other literature to enable sample size calculations, based on statistical modelling.

I collaborated with a wide range of professionals with expertise in public health, geriatrics, infectious diseases and microbiology. This was required to encourage effective cooperation to enable implementation of an expanded study in terms of scope and size.

In this study, we proposed a cluster-randomised, unblinded controlled trial of residents and staff of 70–100 ACFs with partnership between various stakeholders: ACFs, clinical researchers, general practitioners, government public health bodies, and relevant industry partners. Over one influenza season, screening of symptoms to identify influenza-like illness would be followed up by rapid point-of-care tests to diagnose influenza. Aged care facilities would be randomised to receive either oseltamivir treatment of cases or oseltamivir treatment of cases plus prophylaxis. We hoped to compare the attack rate of influenza in treatment vs. treatment and prophylaxis groups, and hospital admission incidence. Secondary outcome measures would be the rates of case fatality, lower respiratory tract infections, and adverse events as well as influenza outbreak duration.

Hurt and Kelly commented that randomised controlled trials of oseltamivir are unlikely to be conducted on severe laboratory-confirmed influenza due to the ethical constraints (62). They recommended ongoing evaluation of prospectively collected observational data. We certainly found that we were subject to concerns about ethics in obtaining buy-in and consensus from prospective co-authors and stakeholders to a randomised controlled trial of oseltamivir use in ACFs. Obtaining consent became the sticking point, as noted in my discussion. Ultimately, we were unsuccessful in obtaining consensus within the time frame for this PhD. However, there have been some positive discussions with prospective funding bodies for this study; the question will remain as to how the issue of consent will be resolved. Funding has been procured for an observational study of oseltamivir in management of outbreaks in ACFs with Professor Booy as lead.

1.3.5 CHAPTER 5 – Pharmacokinetics Of Oseltamivir in Infants

Extremes of age predispose individuals to influenza and its complications. This does not limit itself to the elderly, but also includes infants and neonates. I published an oseltamivir pharmacokinetic study in infants for my 5th chapter. Infants less than a year old are at greater risk of influenza infection than older children and adults (66,67). Vaccinating pregnant mothers is one strategy for protecting neonates and infants, via transplacental transfer of antibodies, but has not categorically confirmed a reduction in the risk of infant hospitalisation from influenza (5,68–70). When infants become very unwell with influenza, then treatment with oseltamivir is often attempted to reduce morbidity and mortality. In Australia, oseltamivir is registered for treatment from infancy and prevention from 1 year
of age (71). There is a paucity of pharmacokinetic data for oseltamivir use in infants under 1 year of age. The Center for Disease Control and Prevention's Advisory Committee on Immunization Practices advise dosing regimens for those under the age of 1 year based on extrapolation of doses in older children and adults (72). This may or may not be appropriate, given differences in developmental physiology of infants affecting parameters such as absorption, volume of distribution and clearance (73).

There is only published data from one series of infants examining serum/plasma oseltamivir levels at the recommended dosing regimens of 3-3.5 mg/kg/dose, and the results confirmed that the dosing recommendations reached target concentrations without excessive side effects (74). We conducted a case series of oseltamivir levels in infants receiving oseltamivir at doses decided by the treating teams (73). This study undertook to confirm these recommendations by analysing pharmacokinetic data from infants who received oseltamivir. We sampled blood at set time points post-oseltamivir administration, usually when blood was being collected for other purposes to reduce the frequency of sampling; additionally, an ethical restriction required babies be cannulated for another purpose for inclusion in the study. Recruitment of cases was complicated by the fact that, as oseltamivir is an oral agent, many babies didn't require cannulation. Another issue that arose was that some babies were cannulated for only a few hours, too short for our sampling time frame. One of the influenza seasons over which the study was conducted resulted in no suitable cases admitted to the paediatric infective care unit from which we were recruiting. On one occasion we were unable to obtain blood through the cannula. As a result, we were able to recruit 4 infants and sent the samples to a laboratory in the United States to measure oseltamivir serum levels.

1.3.6 CHAPTER 6 – The Role of Chronic Diseases in Influenza Incidence and Severity Differential Between Indigenous Australians and Non-Indigenous Australian Populations During the 2009 Influenza Pandemic in Australia

My final paper is central to my thesis for a PhD in Sydney, Australia. No analysis of vulnerable Australians would be complete without attention to the responsibility health professionals have towards Indigenous Australians. The first people of this land, Aboriginal Australians, have been proven to be vulnerable to both communicable and non-communicable diseases introduced after colonisation (75). Chronic conditions, such as diabetes mellitus, cardiovascular disease, tobacco-related respiratory conditions, malignancies, and alcohol-related diseases are often nominated as risk factors for severe influenza, and vaccination is subsidised for those who suffer from these (5). However, rarely is prevention of non-communicable diseases discussed as a central strategy in reducing propensity to severe influenza. It is theorised that Indigenous Australian peoples, like many hunter-gatherer populations, evolved physiological mechanisms, such as relative insulin resistance, to efficiently utilise food resources – the so called, albeit controversial, ‘thrifty phenotype’ phenomenon (76,77). Many Indigenous peoples have lost traditional dietary and activity patterns. Game, fibrous foods and roots, paired with high levels of activity to procure these, were the basis of the traditional pre-European lifestyle; this has been replaced with a processed, high-carbohydrate, high fat diet, and
poor access to modern economic systems, leading to inactivity (78). This has resulted in an epidemic of metabolic dysfunction with its sequelae of obesity, cardiovascular disease, and diabetes. Add the introduction of tobacco and alcohol, and psychosocial intergenerational trauma from being displaced and oppressed, and Aboriginal and Torres Strait Islander peoples have higher rates of modern health ailments than other Australians; diseases that they successfully avoided for tens of thousands of years.

Severe influenza occurs more often in Aboriginal and Torres Strait Islander peoples – but so do non-communicable, chronic diseases (79). We theorised that it is the disparity in the rates of these modern ailments that contribute to higher rates of severe influenza in Aboriginal and Torres Strait peoples, but a data analysis would help to confirm or refute our theory. If our theory is correct, it provides yet more impetus for focus on closing the gap in chronic conditions between Indigenous and non-Indigenous Australians (80).

We conducted a data analysis of de-identified state and territory group aggregate data on Indigenous Australian and non-Indigenous Australian patients who developed influenza in 2009, who also had various background chronic illnesses. This is the first age-standardised analysis of Australian national data sets; this is in contrast to smaller local analyses, which were usually not age-standardised. We examined a national data set for notifications, hospitalisations, Intensive Care Unit admissions and deaths from influenza by Indigenous status and by presence of a one of five common background conditions, as coded by the International Classification of Diseases and Health Related Problems (ICD-11) (81). These were chronic lower respiratory conditions, renal disease, cardiac disease, diabetes mellitus, and obesity. We used data on influenza reported to the Communicable Diseases Network of Australia on Indigenous and non-Indigenous Australians, and information on background rates of chronic illness in these groups from national health surveys reported in both the National Aboriginal and Torres Strait Islander Health Survey, and in the National Health Survey. Population rates of chronic conditions were calculated using rates obtained from these surveys and population data from the 2011 Australian Bureau of Statistics census. Correlating this data allowed us to examine the impact differential background chronic disease rates between Indigenous and non-Indigenous Australians had on the incidence and severity of influenza A (H1N1) in 2009. There were some data limitations: data was only available for adults; there was no separation of data into those with influenza who had none, one or multiple background chronic conditions. Therefore, the comparator was all reported cases of influenza, which comprised a combination of those with any and all chronic conditions. We theorised that, if higher rates of influenza infection in Indigenous versus non-Indigenous people are largely or solely due to the higher prevalence of chronic non-communicable diseases, then this disparity would be largely or completely eliminated by comparing infection rates only amongst those with chronic conditions. Further, if chronic diseases predispose patients to more severe influenza disease, then the increasing disparity between Indigenous and non-Indigenous people with increasing influenza severity would be largely or solely eliminated by comparing influenza disease rates only amongst those with chronic disease. Our results were interesting and highlighted a number of potential interpretations, as I will discuss.
1.4 Summary

In summary, I examined influenza both in situations of vulnerability to the general population, and when demographics confer specific vulnerability to certain populations. I researched epidemiological patterns and management strategies, via literature reviews, data analyses, protocol generation and a randomised clinical trial.

References


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Emergence of Oseltamivir Resistance: Control and Management of Influenza before, during and after the Pandemic

Only one class of antivirals are currently utilised for influenza due to resistance emerging to other antiviral classes: neuraminidase inhibitors (NAIs). Resistant strains of NAIs were introduced in 1999, and are the only specific anti-influenza antivirals currently registered in Australia. Oseltamivir resistance can be conferred by a single missense mutation at the gene coding for it's binding site. Oseltamivir resistance renders those with suboptimal immunity due to chronic conditions, extremes of age or specific immune deficiencies at high-risk of severe influenza disease; many of these same risk groups have suboptimal vaccine responses. NAI-dependence is thus problematic for these vulnerable populations. The otherwise healthy population is also vulnerable in the case of antigenic shifted strains such as avian influenza in which vaccines are not yet registered, and in whom background population immunity is low, were they to acquire more efficient person-to-person transmission as well as mutations that confer oseltamivir resistance. In this review, I analysed utility of oseltamivir by documenting a detailed timeline of circulating influenza strains since the release of oseltamivir in 1999, and the degree to which oseltamivir resistance had developed amongst them. I did this to highlight the vulnerability of reliance on neuraminidase-inhibitors, alone, as treatment for influenza. I summarised in table format many published reports and case series from different countries and continents, representing different clusters. I took a narrative approach, because the studies were heterologous.
Emergence of Oseltamivir Resistance: Control and Management of Influenza before, during and after the Pandemic

Rashmi Dixit¹, Gulam Khandaker¹, Scott Ilgoutz², Harunor Rashid¹ and Robert Booy¹,³*

¹National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, The Children’s Hospital at Westmead and The University of Sydney, New South Wales, Australia; ²Monash University Medical School, Melbourne, Victoria, Australia; ³Sydney Emerging Infections and Biosecurity Institute, The University of Sydney, New South Wales, Australia

Abstract: Neuraminidase inhibitors (NAIs), such as oseltamivir and zanamivir, are the medicines of choice against influenza A or B. Oseltamivir resistance can be conferred by a single point missense mutation from histidine to tyrosine at position 275 (H275Y) of the neuraminidase gene. Oseltamivir resistance in seasonal influenza A/H1N1 strains rose markedly during the 2007-2008 season. Furthermore, oseltamivir resistant (OsR) strains of pandemic influenza A/H1N1 2009 (influenza A(H1N1)pdm09) have been increasingly isolated, although the majority remain sensitive. These OsR strains retain virulence, replicative fitness and transmissibility from person to person, with outbreaks reported. Treatment options in those at risk of severe or complicated disease are limited to zanamivir which is only licenced in those over the age of 5 years; of further concern, strains demonstrating low level resistance to both oseltamivir and zanamivir have been reported. Strategies to reduce emergence of resistant strains, such as higher dose oseltamivir regimens, need further examination.

Keywords: Influenza, neuraminidase inhibitors, oseltamivir, pandemic, resistance.

1. INFLUENZA A RESISTANCE TO NEURAMINIDASE INHIBITORS

Neuraminidase inhibitors (NAIs), such as oseltamivir and zanamivir, are the drugs of choice against seasonal influenza A or B. NAIs bind to the surface protein neuraminidase (NA) of influenza A and B viruses, interfering with the release of viral progeny from host cells thereby reducing spread of infection to adjacent cells [1]. As part of pandemic planning, many developed nations acquired large stockpiles of oseltamivir. It’s advantages over zanamivir include being licensed for use as early as the neonatal period [2]. Wide-scale use of antiviral agents, such as during a pandemic, may promote drug resistance.

NAI resistance most often results from substitution of the amino acid histidine to tyrosine at position 275 (H275Y) in the neuraminidase (NA) gene of H1N1 affecting the NA binding site [3]. Certain factors favour its selection, particularly the pressure of using oseltamivir. There are increasing numbers of oseltamivir resistant (OsR) strains with mutations other than H275Y [4-9]. These amino-acid substitutions confer variable degrees of reduced oseltamivir susceptibility.

From the introduction of the new antivirals in 1999 until 2007-08, the proportion of influenza viruses resistant to NAIs among circulating influenza viruses in children was lower than 6% in clinical trials [10]. The detection of increased oseltamivir resistance was quite often not linked to increased oseltamivir use. For example, in Norway, where the reported frequency of OsR strains of pre-pandemic H1N1 was highest, data indicated that prior to emergence of the OsR strain, between 2004-2007, oseltamivir usage was low (0.17–1.64 courses sold/1000 inhabitants) [11]. In Japan, rates of OsR H1N1 strains increased from 1.5%–2.6% in the 2007-2008 season to almost 100% in the 2008-2009 season. The influenza A/H1N1 Yamagata lineage Japanese strains of 2007-2008 were phylogenetically distinct from those found in Europe and low-level oseltamivir resistance emerged in the context of wide usage of oseltamivir in clinical settings. In contrast the OsR strains found during the 2008-2009 Japanese season appeared to have spread from Europe [12].

2. OSELTAMIVIR RESISTANCE IN THE PRE-PANDEMIC PERIOD

During the 2007-2008 influenza season, OsR influenza A/H1N1 viruses, such as OsR A/Brisbane/59/07(H1N1) virus, were first detected in continental Europe (about 56% viruses being resistant by week 19 of 2008), particularly in Norway (67% of 272 viruses tested being resistant) [13, 14]. In the same season in the USA, 12.3% of influenza A/H1N1 strains were resistant to oseltamivir [5]. A clinical trial showed emergence of oseltamivir resistant pre-pandemic influenza A (H1N1 & H3N2) virus in up to 27% of children in the UK [15].

Spread to the southern hemisphere subsequently occurred, with OsR H1N1 increasing from <1% to >90% in less than 12 months in 2008 throughout South Africa, South East Asia and Oceania [16].
Global and local surveillance data from USA, Europe, South Africa, Japan, Hong Kong, and Taiwan demonstrated that oseltamivir resistance predominated in pre-pandemic influenza A H1N1 during the 2008-2009 season [17]. For instance, 2008-09 surveillance data from USA showed 99.4% of influenza A H1N1 strains were resistant to oseltamivir [18]. No difference in clinical severity was noted between the OsR and oseltamivir sensitive (OsS) viruses [19]. Table 1 shows the most recent trial and surveillance data from different parts of the world on oseltamivir resistant influenza [8, 13-57].

3. OSELTAMIVIR RESISTANCE IN THE PANDEMIC PERIOD

The pandemic influenza A /H1N1 virus was first detected in Mexico in March 2009 and in the USA in April 2009 (influenza A(H1N1)pdm09) [58]. It spread rapidly around the world, with the WHO declaring a pandemic on 11 June 2009 [59]. Oseltamivir was used liberally for both treatment and post-exposure prophylaxis [12]. In Australia, recorded oseltamivir prescribing increased from approximately 25,000 units in 2008 to over 350,000 in 2009 [40]. This increased drug pressure heightened the potential for oseltamivir resistance to develop in the influenza A(H1N1)pdm09 strain.

3.1. Global Data

Global data from WHO revealed 598 cases of OsR influenza A(H1N1)pdm09 cases as of 21 September 2011 [59]. In the 78% from whom clinical information was available (468/598), 29% (133/468) of OsR strains were found in severely immunocompromised patients and 71% (335/468) in non-immunocompromised patients. Amongst the immunocompetent, 63% (211/335) were associated with oseltamivir, and/or peramivir, treatment or prophylaxis. All of these viruses demonstrated the same H275Y mutation that confers resistance to oseltamivir, but not to zanamivir. On the other hand, worldwide, more than 10,000 clinical specimens of the influenza A(H1N1)pdm09 virus were tested and found to be sensitive to oseltamivir [60].

Data from the CDC in the USA demonstrated an OsR influenza A(H1N1)pdm09 rate of 1.1% in 2009-2010 [24]. In the 2009-2010 season, most (76%) of the people in whom OsR viruses were found were immunocompromised and 89% of them had been treated with oseltamivir before the OsR viruses were detected; the latter figure may reflect, in part, collection of specimens in those who received oseltamivir who were clinically suspected to have developed resistance. In contrast, only 11% of those with OsS virus were immunocompromised and 14% had received oseltamivir treatment, with none having received oseltamivir prophylaxis [46]. In the USA 2010-11 season, influenza A(H3N2) predominated slightly over influenza A(H1N1)pdm09 and influenza B, although some influenza A(H1N1)pdm09 and influenza B peaked. Of almost 5800 strains, 41 (0.7%) from all subtypes were OsR; 39 were the 2009 pandemic influenza A strain and 2 influenza A/H3N2 [25]. Another source gave a rate of 1% (35/3652) in 2010-2011, comparable to the rate for the 2009-2010 influenza season [49]. In contrast to the 2009-2010 season, in the 2010-2011 season only 26% of those with OsR virus had received oseltamivir, and only 24% were immunocompromised [49].

Multi-drug resistant strains of influenza A(H1N1)pdm09 have been reported in association with I223R (isoleucine to arginine) mutation in the NA protein, mainly with immunocompromised patients, but also from case reports of immunocompetent patients from North America [5-7, 61]. The clinical and public health significance of this mutation is undetermined as the IC50 (the concentration of drug required to inhibit 50% of NA activity) for each of oseltamivir and zanamivir remain below serum levels achieved by standard dosing regimens.

Influenza B infection rates have been higher in recent seasons and some oseltamivir resistance has been reported. Sleeman et al. reported a cluster of 59 influenza B viruses isolated from patients in North Carolina that displayed oseltamivir resistance as a result of an isoleucine to valine substitution at position 221 (I221V) [50]. This mutation is at an analogous site to the S247N mutation (substitution of serine with asparagine at position 247) found in influenza A virus. As the IC50 for oseltamivir of influenza B viruses is already far greater than that of influenza A viruses, the I221V mutation may tip the viruses into clinically significant oseltamivir resistance. The IC50 for zanamivir for influenza B viruses is low; accordingly no zanamivir resistant isolates have been identified [62]. One OsR resistant influenza B case has also been isolated in the UK, as well as one in the Philippines and two in New Zealand. Influenza B does not generally cause epidemics and usually is a milder illness, however it can cause significant myositis, paediatric encephalomyelitis and more severe disease as well as complications in the debilitated and immunocompromised [63, 64].

3.2. Australian Clusters

Co-circulation of different influenza strains, primarily influenza A(H1N1)pdm09 strain, influenza B and influenza A/H3N2 characterised the influenza seasons of the northern and southern hemispheres for 2010/2011, 2011, 2011/2012 and 2012. Within Australia, the majority of influenza virus detections in 2011 were influenza A(H1N1)pdm09 with some co-circulation of influenza B [56]. At the start of the 2011 season, high levels of influenza A/H3N2 were seen. More than 6% of positive influenza samples for the 2011 season in Australia were sent to the World Health Organisation Collaborating Centre (WHO CC) in Melbourne. The WHO detected an influenza A(H1N1)pdm09 virus isolate that was resistant to oseltamivir, harbouring the H275Y mutation. Initially two resistant viruses were detected in Australia, in January 2011 and March 2011 respectively, prior to a cluster of 25 OsR influenza A(H1N1)pdm09 cases identified from the Newcastle region in New South Wales between May and August 2011, none of whom had received oseltamivir [54]. A further two OsR influenza A(H1N1)pdm09 viruses which were virologically related to the Newcastle cluster, but geographically further afield, were detected in untreated children in July and August: one was from Sydney (160 kilometres from Newcastle) and the other was from Orange (380 kilometres away). This Newcastle cluster represents, globally, the largest cluster of oseltamivir resistant viruses reported by the WHO to date [65]. However, spread of oseltamivir resistant influenza A(H1N1)pdm09 strains to Sydney was limited, with only 2/143 (1.2%) of pre-treatment
Table 1. Recent data on oseltamivir resistance across the world.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Nature of Study</th>
<th>Site</th>
<th>Duration</th>
<th>Subtype</th>
<th>Percentage of resistant viruses % (resistant cases/tested samples)</th>
<th>Neuraminidase mutations</th>
<th>Susceptibility to zanamivir (R/S)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stephenson et al. [15]*</td>
<td>Clinical trial</td>
<td>United Kingdom</td>
<td>2005-07</td>
<td>Pre-pandemic seasonal H1N1 H3N2 B</td>
<td>Pre-pandemic seasonal H1N1: 27.3% (3/11) H3N2: 2.9% (1/34) B: 0% (0/19)</td>
<td>H275Y (H1N1) R292K (H3N2)</td>
<td>R292K (H3N2): 10 fold reduction in susceptibility H275Y (H1N1): S</td>
<td></td>
</tr>
<tr>
<td>Dharn et al. [19]</td>
<td>Surveillance</td>
<td>USA</td>
<td>Sep 07-08 Sep 08-Feb 09</td>
<td>Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 12.3% (142/1155) Pre-pandemic seasonal H1N1 08-09: 98.5% (264/268)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Cheng et al. [22]</td>
<td>Surveillance</td>
<td>Hong Kong</td>
<td>Jan 08-Jun 08</td>
<td>Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 63.6% (168/264)</td>
<td>H275Y</td>
<td>Not specified</td>
<td>A/Brisbane/59/2007</td>
</tr>
<tr>
<td>Dia et al. [23]</td>
<td>Surveillance</td>
<td>Senegal</td>
<td>Jul 08-Sept 08</td>
<td>Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 100% (86/86)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Hurt et al. [16]</td>
<td>Surveillance</td>
<td>South Africa, Oceania and south-east Asia</td>
<td>2008</td>
<td>Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 63.6% (168/264)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>CDC, 2009-2013 [18,24-27]</td>
<td>Surveillance</td>
<td>USA</td>
<td>2009-10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 1.1% (53/4811)</td>
<td>Not specified</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2010-11</td>
<td>A(H1N1)pdm09 H3N2 B</td>
<td>Overall: 0.7% (41/5758) A(H1N1)pdm09: 0.9% (39/4229) H3N2: 0.2% (2/806) B: 0% (0/723)</td>
<td></td>
<td>Not specified</td>
<td>S</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2011-12</td>
<td>A(H1N1)pdm09 H3N2 B</td>
<td>Overall: 0.6% (16/2756) A(H1N1)pdm09: 1.4% (16/1164) H3N2: 0% (0/1275) B: 0% (0/317)</td>
<td></td>
<td>Not specified</td>
<td>S</td>
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<td></td>
<td></td>
<td></td>
<td>2012-13</td>
<td>A(H1N1)pdm09 H3N2 B</td>
<td>Overall: 0.1% (4/2768) A(H1N1)pdm09: 0.5% (2/427) H3N2: 0.1% (2/1692) B: 0% (0/649)</td>
<td>H275Y</td>
<td>S</td>
<td>No resistance amongst B or H3N2 strains</td>
</tr>
<tr>
<td>Reference</td>
<td>Nature of Study</td>
<td>Site</td>
<td>Duration</td>
<td>Subtype</td>
<td>Percentage of resistant viruses % (resistant cases/tested samples)</td>
<td>Neuraminidase mutations</td>
<td>Susceptibility to zanamivir (R/S)</td>
<td>Comment</td>
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<tr>
<td>Alfaresi et al. [32]</td>
<td>Surveillance</td>
<td>United Arab Emirates</td>
<td>July 09 - Nov 09</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 1% (1/96)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Harvala et al. [33]</td>
<td>Surveillance</td>
<td>England and Scotland</td>
<td>Nov 09 - Dec 09</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.6% (10/1608)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Suppiah et al. [34]</td>
<td>Surveillance</td>
<td>Malaysia</td>
<td>2009</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0% (0/67)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Zhou et al. [31]</td>
<td>Surveillance</td>
<td>China</td>
<td>Jan 08 - Aug 09</td>
<td>Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 47.8% (107/224) A(H1N1)pdm09: 0% (0/221)</td>
<td>H3N2: 0% (0/194) B: 0.4% (1/234)</td>
<td>H275Y</td>
<td>2008: A/Brisbane/59/2007</td>
</tr>
<tr>
<td>EuroFlu [30]</td>
<td>Surveillance</td>
<td>Europe</td>
<td>2009-10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 2.0% (40/1974)</td>
<td>Not specified</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>HPA [29]</td>
<td>Surveillance</td>
<td>United Kingdom</td>
<td>May 09 - Apr 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.8% (45/5587)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Kawai et al. [28]</td>
<td>Surveillance</td>
<td>Japan</td>
<td>2007-08 Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 0% (0/44)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>2008-09 Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 100% (32/32)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2009-10 A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 2.4% (2/82)</td>
<td>H275Y</td>
<td>Not specified</td>
<td>The resistance developed post oseltamivir therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2010-11 influenza season</td>
<td>A(H1N1)pdm09: 0% (0/44)</td>
<td>H275Y</td>
<td></td>
<td>S</td>
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<td></td>
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<td></td>
<td>2011-12 influenza season</td>
<td>A(H1N1)pdm09: 0% (0/44)</td>
<td>H275Y</td>
<td></td>
<td>S</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2012-13 influenza season</td>
<td>A(H1N1)pdm09: 2 resistant A(H1N1)pdm09 isolates 1 resistant B isolate</td>
<td>A: Not specified B: I221T</td>
<td>A: S</td>
<td>B I221T: S</td>
<td>Several hundred viruses tested (exact data not given)</td>
</tr>
<tr>
<td>EuroFlu [30]</td>
<td>Surveillance</td>
<td>Europe</td>
<td>2009-10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 2.0% (40/1974)</td>
<td>Not specified</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>HPA [29]</td>
<td>Surveillance</td>
<td>United Kingdom</td>
<td>May 09 - Apr 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.8% (45/5587)</td>
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<td>S</td>
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<tr>
<td>Kawai et al. [28]</td>
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<td>Not specified</td>
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<td></td>
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<td></td>
<td>2008-09 Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1: 100% (32/32)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2009-10 A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 2.4% (2/82)</td>
<td>H275Y</td>
<td>Not specified</td>
<td>The resistance developed post oseltamivir therapy</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2010-11 influenza season</td>
<td>A(H1N1)pdm09: 0% (0/44)</td>
<td>H275Y</td>
<td></td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2011-12 influenza season</td>
<td>A(H1N1)pdm09: 0% (0/44)</td>
<td>H275Y</td>
<td></td>
<td>S</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2012-13 influenza season</td>
<td>A(H1N1)pdm09: 2 resistant A(H1N1)pdm09 isolates 1 resistant B isolate</td>
<td>A: Not specified B: I221T</td>
<td>A: S</td>
<td>B I221T: S</td>
<td>Several hundred viruses tested (exact data not given)</td>
</tr>
</tbody>
</table>

(Table 1) contd....
(Table 1) contd....

<table>
<thead>
<tr>
<th>Reference</th>
<th>Nature of Study</th>
<th>Site</th>
<th>Duration</th>
<th>Subtype</th>
<th>Percentage of resistant viruses % (resistant cases/tested samples)</th>
<th>Neuraminidase mutations</th>
<th>Susceptibility to zanamivir (R/S)</th>
<th>Comment</th>
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<tbody>
<tr>
<td>Burrel et al. [35]</td>
<td>Surveillance</td>
<td>South-western France</td>
<td>2007-09</td>
<td>Pre-pandemic seasonal H1N1 A(H1N1)pdm09</td>
<td>H3N2 07-08 Pre-pandemic seasonal H1N1: 47.6% (10/21) 08-09 Pre-pandemic seasonal H1N1: 100% (5/5) 08-09; A(H1N1)pdm09: 0% (0/129) 08-09 H3N2: 0% (0/92)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
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<tr>
<td>Souza et al. [36]</td>
<td>Surveillance</td>
<td>Brazil</td>
<td>Apr 09-Dec 09; sporadic cases 2010</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: &lt;10% quoted (exact data not given)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Shin et al. [37]</td>
<td>Surveillance</td>
<td>South Korea</td>
<td>May 09-Jan 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 16% (11/67)</td>
<td>H275Y</td>
<td>Not specified</td>
<td>All those tested were suspected OR (e.g. Immunosuppression, young age)</td>
</tr>
<tr>
<td>Yang et al. [17]</td>
<td>Surveillance</td>
<td>Taiwan</td>
<td>May 09-Jan 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.7% (8/1187)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Sheu et al. [38]</td>
<td>Surveillance</td>
<td>Global</td>
<td>Oct 08-Sept 09 Oct 09-Jan 10</td>
<td>Pre-pandemic seasonal H1N1</td>
<td>Pre-pandemic seasonal H1N1 08-09: 92.7% (1328/1432) 09-10: 4% (1/25)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ujike et al. [39]</td>
<td>Surveillance</td>
<td>Japan</td>
<td>May 09-Feb 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 1.4% (61/4307)</td>
<td>H275Y</td>
<td>3/482 just outside reference range</td>
<td></td>
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<tr>
<td>Hurt et al. [21]</td>
<td>Surveillance</td>
<td>Oceania, Asia and Africa: World Health Organization Collaborating Centre for Reference and Research on Influenza (WHO CC), Melbourne, Australia</td>
<td>Mar 09-Mar 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09 All countries: 1.1% (16/1488) A(H1N1)pdm09 Australia: 1.3% (12/961)</td>
<td>H275Y</td>
<td>S</td>
<td>9/16 immunocompromised</td>
</tr>
<tr>
<td>Longtin et al. [41]</td>
<td>Surveillance</td>
<td>Ontario, Canada</td>
<td>Jun 09-Mar 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.6% (5/804)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Ramirez-Gonzalez et al. [42]</td>
<td>Surveillance</td>
<td>Mexico</td>
<td>May 09-April 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.1% (1/692)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Calatayud et al. [43]</td>
<td>Surveillance</td>
<td>England and Scotland</td>
<td>April 09-April 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.6% (36/6379) A(H1N1)pdm09 amongst hospitalised patients: 1.0% (36/3515)</td>
<td>H275Y</td>
<td>S</td>
<td>Imunosuppression risk factor for OR</td>
</tr>
<tr>
<td>Reference</td>
<td>Nature of Study</td>
<td>Site</td>
<td>Duration</td>
<td>Subtype</td>
<td>Percentage of resistant viruses % (resistant cases/tested samples)</td>
<td>Neuraminidase mutations</td>
<td>Susceptibility to zanamivir (R/S)</td>
<td>Comment</td>
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<tr>
<td>Meijer et al. [44]</td>
<td>Surveillance</td>
<td>Netherlands</td>
<td>April 09-May10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: Maximum 5.7% (63/1100)</td>
<td>H275Y</td>
<td>R</td>
<td>&gt;1100 viruses tested (exact data not given) One OR case (H275Y) developed zanamivir resistance post zanamivir treatment</td>
</tr>
<tr>
<td>Ledesma et al. [45]</td>
<td>Surveillance</td>
<td>Spain</td>
<td>April 09-May10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.7% (8/1229)</td>
<td>H275Y</td>
<td>Not specified</td>
<td>All OR cases inpatients, nil in community</td>
</tr>
<tr>
<td>Graitcer et al. [46]</td>
<td>Surveillance</td>
<td>USA</td>
<td>April 09-June 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.5% (37/7400)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Payungporn et al. [47]</td>
<td>Surveillance</td>
<td>Thailand</td>
<td>Apr 09-Oct 10</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0.31% (4128)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
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<tr>
<td>Hurt et al. [8]</td>
<td>Surveillance</td>
<td>Asia-Pacific region</td>
<td>Apr 09-Dec 10</td>
<td>H1N1 not otherwise specified</td>
<td>A(H1N1)pdm09: Maximum 1.6% (45/2900)</td>
<td>H275Y</td>
<td>S247N</td>
<td>H275Y: Not specified</td>
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<tr>
<td>Kouri et al. [48]</td>
<td>Hospital Surveillance</td>
<td>Greece</td>
<td>2010-11 influenza season</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 1% (2/50)</td>
<td>H275Y</td>
<td>S</td>
<td>All subjects pretreated with oseltamivir prior to hospitalisation</td>
</tr>
<tr>
<td>Storms et al. [49]</td>
<td>Surveillance</td>
<td>USA</td>
<td>2010-11 influenza season</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 1.0% (35/3652)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Sleeman et al. [50]</td>
<td>Surveillance</td>
<td>USA</td>
<td>2010-11 influenza season</td>
<td>B</td>
<td>B: 12.3% (59/478)</td>
<td>I221V</td>
<td>S</td>
<td>B/North Carolina/11/2010</td>
</tr>
<tr>
<td>Miller H. et al. [51]</td>
<td>Surveillance</td>
<td>Hawaii and US-affiliated Pacific islands</td>
<td>Jun 09-Jul 11</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 0% (0/263)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Hurt et al. [52]</td>
<td>Surveillance</td>
<td>Australia</td>
<td>May 11-Aug 11</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 15.9% (29/182)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Wang et al. [53]</td>
<td>Surveillance</td>
<td>Australia</td>
<td>Jun 11-Aug 11</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09 Untreated cases: 1.4% (2/143) A(H1N1)pdm09 Treated cases: 13.0% (3/23)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>Hurt et al. [54]</td>
<td>Surveillance</td>
<td>Hunter New England, Australia</td>
<td>May 11-Sept 11</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 15.2% (29/191)</td>
<td>H275Y</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Leung et al. [55]</td>
<td>Surveillance</td>
<td>Asia-Pacific</td>
<td>2011</td>
<td>A(H1N1)pdm09</td>
<td>A(H1N1)pdm09: 2.9% (42/1435) H3N2: 0% (0/795) B Victoria: 0.3% (3/1073) B Yamagata: 0% (0/52)</td>
<td>H275Y</td>
<td>1/3355 resistant, lab artefact.</td>
<td>Novel OR influenza B detected. Not isolated in previous seasons</td>
</tr>
</tbody>
</table>
strains surveyed exhibiting the H275Y mutation [53]. All of the resistant viruses were similar antigenically to the H1N1 strain contained in that season’s trivalent seasonal influenza vaccine, indicating that vaccination would provide protection. Overall, the vast majority of the influenza A(H1N1)pdm09 viruses received by Australia’s WHO CC by August 2011 were sensitive to oseltamivir. The other two circulating influenza viruses (H3N2 and B) were also sensitive to oseltamivir.

In 2010 an influenza A(H1N1)pdm09 strain was detected, which demonstrated low-level resistance to both oseltamivir (6 fold higher IC50) and zanamivir (3 fold higher IC50), in 10% of community samples from Singapore and in 30% of community samples from Darwin, Northern Australia [8]. Such strains have also been found at lower levels in Western Australia, Brunei and rarely in the United States, Europe and Asia since 2009. This is due to a S247N neuraminidase mutation (substitution of serine with aspartagine at position 247). The maximum drug levels achieved via the recommended dose are much greater than the IC50, in 10% of community samples from Singapore and in 30% of community samples from Darwin, Northern Australia [8]. Dual H275Y/S247N strains have been detected. An immunocompromised patient from Perth, Western Australia, was diagnosed with a strain containing both H275Y and S247N mutations, which conferred a 7000-fold increase in IC50 to oseltamivir but only a fivefold increase in IC50 to zanamivir, which would therefore remain effective.

3.3. At-risk Patients and OsR Influenza

The propensity to development of oseltamivir resistance whilst on treatment has been repeatedly demonstrated for both immunosuppressed patients and those with underlying chronic medical conditions [39, 46, 66-69]. Whilst annual influenza vaccinations are recommended, generation of an immune response can be suboptimal in these populations [70, 71]. Vaccination of close contacts such as family and health care workers, who are the usual sources of transmission, is an alternative strategy. From March 2009 to March 2010 the Australian WHO CC isolated 16 oseltamivir resistant influenza A(H1N1)pdm09 strains from the Asia-Pacific region [40]. Nine of these 16 cases were from immunocompromised subjects receiving oseltamivir. Some strains demonstrated prolonged viral shedding of up to nine weeks, and received multiple courses of both single and double-dose oseltamivir. A case-control study from the United Kingdom revealed that those infected with oseltamivir resistant influenza A(H1N1)pdm09 virus were more likely to have one or more underlying medical conditions than those that were infected with an OsS strain (93.3% vs. 58.9%). Those with OsR strains were more often immunosuppressed than those infected with OsS strains (75% vs. 6.9%). This difference was attributed to selective drug pressure amongst immunocompromised patients [43]. Viral shedding for up to 18 months in immunosuppressed patients has been reported [72].

Prolonged viral shedding and thus increased viral replication provide conditions that promote emergence of drug resistance [68]. Immunosuppressed patients with prolonged viral shedding may serve as a reservoir for NAI resistant H1N1 virus in the community [66, 73, 74]. Given the H275Y mutation is of the oseltamivir-binding site, a higher dose will not overcome the resistance. However, whether either a higher dose and/or longer duration of oseltamivir treatment would reduce the emergence of resistance is unresolved. It has been suggested that zanamavir be considered first line therapy in patients who are immunocompromised in certain populations with known oseltamivir resistance [39, 75-77].

4. OSELTAMIVIR RESISTANCE IN THE POST-PANDEMIC PERIOD

The predominating strains for the southern hemisphere influenza season 2012 and northern hemisphere 2012–13 influenza season have been influenza A(H3N2) and influenza B.

4.1. Global data

In the USA 2011-12 season, 86% of almost 20,000 surveyed strains were influenza A viruses of which 74% were influenza A (H3N2) and 26% were influenza A(H1N1)pdm09 [26]. OsR strains were 0.6% of a selection of almost 2800 strains from all subtypes sampled, all were influenza A(H1N1)pdm09 viruses.

Influenza B (e.g. 73.5% in week 12) predominated in the USA 2012-2013 season, followed by influenza A/H3N2

<table>
<thead>
<tr>
<th>Reference</th>
<th>Nature of Study</th>
<th>Site</th>
<th>Duration</th>
<th>Subtype</th>
<th>Percentage of resistant viruses % (resistant cases/tested samples)</th>
<th>Neuraminidase mutations</th>
<th>Susceptibility to zanamivir (R/S)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DoHA [56]</td>
<td>Surveillance</td>
<td>Australia</td>
<td>2012</td>
<td>H3N2 B</td>
<td>Overall: 0.1% (1/1314)</td>
<td>H275Y</td>
<td>Not specified</td>
<td>Numbers of each subtype not specified. The resistant case was an A(H1N1)pdm09 strain virus.</td>
</tr>
<tr>
<td>WHO [57]</td>
<td>Surveillance</td>
<td>Europe</td>
<td>2012-13 influenza season</td>
<td>A(H1N1)pdm09 H3N2 B</td>
<td>A(H1N1)pdm09: 1.0% (1/100) H3N2: 0% (0/95)</td>
<td>H275Y</td>
<td>Not specified</td>
<td></td>
</tr>
</tbody>
</table>

* This study (Stephenson et al. [15]) dealt with children aged 1-12 years, all other studies dealt with ‘all age groups’.
(8.7% of isolates tested in week 12). Of the remaining positive isolates in week 12, only 1.5% were influenza A(H1N1)pdm09, with 16% being untyped influenza A. This low level of the 2009 pandemic strain is reflected in only four cases of OsR influenza isolated on surveillance up to the week ending 23 March 2013 (0.1% of almost 2700 strains tested: 2/1693 (0.1%) influenza A/H3N2 and 2/427 (0.5%) influenza A(H1N1)pdm09, nil influenza B) [27].

In Europe during 2012-2013, reasonably equal rates for co-circulation of all three strains were reported, with influenza A(H1N1)pdm09 representing 31% of surveyed strains, as opposed to the 2011-2012 season when influenza A (H3N2) predominated [30]. In the 2012-13 season, resistance to oseltamivir in Europe remained low with only 2 cases of OsR isolates reported to surveillance agencies, both influenza A(H1N1)pdm09; there were no OsR cases reported in the 2011-12 season, reflecting the low numbers of 2009 pandemic influenza A circulating during that season [30].

4.2. Australian Data

During 2012 in Australia, influenza A/H3N2 and influenza B were the predominant circulating strains, with a low level of influenza A(H1N1)pdm09 detected on surveillance [56]. During this season no strains of influenza H3N2 tested by the WHO CC were resistant to oseltamivir. The single OsR strain detected of 1,314 tested was an influenza A(H1N1)pdm09. Compared to 2011, this low-level oseltamivir resistance detected in 2012 likely reflects the overall lower circulation of influenza A(H1N1)pdm09.

5. PUBLIC HEALTH SIGNIFICANCE OF RESISTANT STRAINS

Selection and subsequent spread of an NA-resistant strain are dependent upon the transmission fitness of that strain [78]. Before the 2007-2008 influenza seasons, detection of oseltamivir-resistant viruses in humans had typically been reported only among persons treated with oseltamivir and human-to-human transmission of an NA-resistant virus had never been documented [79-81]. From 2007 until March 2008, 1182 of 7530 pre-pandemic influenza A(H1N1) viruses (16%) tested and reported to the WHO were resistant to oseltamivir [82]. By 2009 virtually all pre-pandemic influenza A(H1N1) viruses globally were oseltamivir-resistant [38]. This suggests the mutant virus retains transmission fitness [83].

Studies of the transmissibility of the OsR influenza A(H1N1)pdm09 yield mixed results. A study of infected ferrets revealed fitness, transmissibility and pathogenicity were not compromised [84]. However, some studies do demonstrate loss of replicative fitness in OsR strains in vitro and in vivo [85, 86]. The authors of one such study concluded that the OsR virus would require further modifications, such as antigenic drift, additional mutations or intense selection pressure to spread and predominate. Whether any additional genetic polymorphisms have been acquired to compensate for any conferred loss of replicative fitness in OsR strains is presently unclear. Permissive mutations in the neuraminidase gene, V234M (replacement of valine by methionine) and R222Q (replacement of arginine by glutamine) appeared to have enabled pre-pandemic influenza A strains with the H275Y mutation to retain viral fitness [87]. Neither of these compensatory mutations has yet been detected in any influenza A(H1N1)pdm09 virus. One possible mechanism for retention of viral fitness may be inherent to the H275Y substitution. An earlier mutation of the NA protein that increased its receptor affinity may have actually compromised the “fitness” of the influenza strain by disrupting the functional balance between its haemagglutinin and neuraminidase proteins. The H275Y mutation, which reduces neuraminidase substrate affinity, may have restored this balance, and also enhanced the NA cleaving function [83, 88]. Hayden et al. suggested that such mutations may not require drug pressure in order to be selected and may explain those cases and clusters of untreated OsR influenza A [88].

Human-to-human transmission has recently been identified around the world. Reports emerged initially in 2009, with the CDC reporting acquisition of OsR influenza A(H1N1)pdm09 amongst two adolescents who received oseltamivir during a mass chemoprophylaxis drive during an outbreak of influenza-like illness at a summer camp; whether the mutations developed de-novo or whether the strain spread from one to the other was unclear [89]. Ujike et al. reported that 2/61 cases of laboratory-confirmed OsR influenza A(H1N1)pdm09 were suspected to be due to human to human transmission between close contacts [39]. However, there was no evidence of sustained spread of OsR influenza A(H1N1)pdm09 in Japan. Person to person transmission of OsR strains have also been reported from Wales and Vietnam; similarly, transmission was not sustained [40]. Moore et al. report person-to-person spread of OsR influenza A(H1N1)pdm09 in a haematology unit in the United Kingdom [90]. Of seven cases of OsR influenza in immunocompetent patients from the Oceania WHO surveillance, four had not received oseltamivir and additionally they developed influenza after the peak of the pandemic period, suggesting possible establishment of OsR viruses [40]. The largest report involves the Newcastle, New South Wales, cluster described above and suggested a wide region of spread but limited establishment of the OsR influenza A(H1N1)pdm09 [53, 91].

That a greater proportion of OsR influenza A(H1N1)pdm09 cases in the USA received pre-treatment with oseltamivir in the 2009-2010 (89%) season versus the 2010-2011 season (26%) suggests establishment of a low level of community transmission of oseltamivir-resistant influenza A(H1N1) pdm09 [24, 49].

6. CLINICAL SIGNIFICANCE OF RESISTANT STRAINS

Norwegian surveillance data reveal that overall, the observed clinical manifestations associated with OsR pre-pandemic influenza viruses A (H1N1) were as expected for pre-pandemic influenza [14]. No differences were noted for virus shedding, primary symptoms, complications or hospitalisation rates amongst those infected with either OsS or OsR viruses. Likewise, neither USA nor UK surveillance data reported any prolongation or increase of symptoms in those with OsR versus OsS pre-pandemic influenza viruses [15, 19]. The OsR influenza A(H1N1)pdm09 strain has also
been reported to be no more virulent than the sensitive strain [92]. Due to pre-existing immunity (perhaps from exposure to a similar strain in those aged over 65 years) [93], influenza A(H1N1)pdm09 caused more morbidity and mortality in younger adults and children, in contrast to the usual pattern for seasonal influenza which disproportionately affects the elderly [94]. However, OsR influenza A(H1N1)pdm09 strains were associated with a significantly higher risk for complications, particularly respiratory, in the 2009-2010 UK influenza season, even after adjusting for chronic illness and immunosuppression [43].

Management of OsR influenza viruses is unclear. The influenza A(H1N1)pdm09 strain retains sensitivity to zanamivir whilst being resistant to oseltamivir and peramivir [95]. As noted, inhaled or intravenous zanamivir in high risk patients may be considered first line therapy [39, 75-77]. Zanamivir is licensed only for those over 5 years of age. However, there are isolated reports of use of zanamivir in those under the age of 5 years. Dulek et al. report the use of intravenous zanamivir in a profoundly neutropenic 18 month old girl with a haematological malignancy who had a lower respiratory tract infection with influenza A(H1N1)pdm09 repeatedly isolated from her respiratory tract in the context of clinical deterioration [77]. A twenty day course of intravenous zanamivir was utilised with reduction in viral load from endotracheal aspirates. The regimen was well tolerated. Nonetheless respiratory deterioration progressed and the patient died.

7. CONCLUSIONS

In conclusion, in the 2 years before the 2009 pandemic due to H1N1 influenza A, the circulating pre-pandemic H1N1 strains had largely become resistant to oseltamivir; resistance is now emerging in the influenza A(H1N1)pdm09 strain, which remains prevalent and at times the predominant community strain of influenza A. Dual low level resistance to oseltamivir and zanamivir has been detected in various strains, which sets the scene for further new mutations inducing clinical resistance to both of these agents. Ongoing, careful surveillance is important to monitor the incidence of resistant virus and inform recommendations for changes to first-line therapy in the event that resistant strains begin to predominate. There are limited treatment options in high risk patients.

Whether a higher dose and/or longer duration of oseltamivir treatment, for example doubling the dose of the standard oseltamivir regimen, reduces the emergence of resistance is unresolved. This question is being addressed in a randomised controlled trial conducted by this authorship team and the results may help inform the current ad-hoc use of such regimens in certain settings.

CONFLICTS OF INTEREST

RB has received financial support from pharmaceutical companies CSL, Sanofi, GSK, Novartis, Roche, and Wyeth to conduct influenza control research and attend and present at scientific meetings. Any funding received is directed to an NCIRS research account at The Children’s Hospital at Westmead and is not personally accepted by RB. The other authors have no conflict of interest in relation to this work.

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Declared none.

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Emergence of Oseltamivir Resistance

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Emergence of Oseltamivir Resistance


2.3 CHAPTER 1: Synopsis

Emergence of Oseltamivir Resistance: Control and Management of Influenza before, during and after the Pandemic - Synopsis

Over 2007 – 2008, influenza strains became predominantly oseltamivir resistant, and these strains was both arising independently and locally, and spreading globally, amongst pre-pandemic influenza A (H1N1). The potential detrimental effects of limitation of treatment options can be compounded failures in preventative strategies, such as reduced vaccine effectiveness. During the 2017 season, influenza vaccine effectiveness only 33%, with resultant larger national influenza epidemics [Sullivan SG, Chilver MB, Carville KS, Deng Y-M, Grant KA, Higgins G, et al. Low interim influenza vaccine effectiveness, Australia, 1 May to 24 September 2017. Eurosurveillance [Internet]. 2017 Oct 26;22(43)].

Acquisition of additional mutations that conferred a selective advantage over wild-type strains enabled oseltamivir-resistant strain of influenza to dominate in the 2007-8 season, despite the mutation compromising replicative fitness. Intense selection pressure from widespread oseltamivir use, e.g. during a future pandemic, may result in predominance of oseltamivir resistance in current circulating strains.

Strategies to reduce emergence of resistant strains, such as higher dose oseltamivir regimens, development of new antiviral classes, or even resurrection of historical treatment methods, need further examination. Two of these strategies are explored: in a clinical trial of double dose oseltamivir in chapter 2, and through a review of the application of an old treatment modality and it’s modern reworking – heterologous polyclonal antibody therapy, chapter 3.
3.1 CHAPTER 2: Preamble

A Randomized Study of Standard versus Double Dose Oseltamivir for Treating Influenza in the Community

Neuraminidase inhibitors (NAIs, e.g., oseltamivir, zanamivir) are the treatment of choice against seasonal influenza. Given the paucity of other specific anti-influenza agents, higher dose regimens have been tried to maximise clinical resolution and reduce development of antiviral resistance via more rapid virological clearance. Whilst influenza is usually self-limiting, it is important to have a treatment modality when it is severe, or to curb outbreaks. As noted elsewhere, antiviral resistance confers vulnerability to the general population in the context of a novel strain circulating, and to those in whom, due to a variety of clinical conditions, vaccine effectiveness is reduced or vaccines are contra-indicated.

Double-dose (DD) regimens of oseltamivir have been used in both immunocompromised and artificially ventilated patients, without a strong evidence base. Our study was unique in addressing patients with influenza in the mild to moderate (non-hospitalised) stages. The objectives of this study were to investigate the effectiveness of DD (150mg bid for adults or 10mg/kg for children) compared with SD (75mg bid or 5mg/kg for children) oseltamivir in subjects ≥5 years old. I examined clinical disease, virological outcomes, adverse events and the frequency of detection of oseltamivir-resistant virus.
Original article

A randomized study of standard versus double dose oseltamivir for treating influenza in the community

Rashmi Dixit1*, Gulam Khandaker1, Peter Hay2, Kenneth McPhie3, Janette Taylor3, Harunor Rashid1,
Leon Heron1, Dominic Dwyer3, Robert Booy1

1NCIRS, The Children's Hospital, Westmead, NSW, Australia
2Castlehill Medical Centre, Castle Hill, NSW, Australia
3ICPMR, Westmead Hospital, Wentworthville, NSW, Australia

*Corresponding author e-mail: rashmid@uni.sydney.edu.au

Background: The neuraminidase inhibitors are the treatment of choice for influenza virus infection. Oseltamivir-resistant (OsR) strains of influenza A(H1N1)pdm09 are described, but the effect of higher dose oseltamivir on efficacy, safety and emergence of resistance has not been addressed in the developed setting in outpatients. The objectives of the study were to compare standard dose (SD) versus double dose (DD) oseltamivir regimens for frequency of detecting OsR influenza virus, clinical disease resolution, virological clearance and adverse events.

Methods: This was an unblinded randomized controlled trial of community-based patients with confirmed influenza. Participants were randomized to a 5-day regimen of either SD or DD oseltamivir.

Results: Of 52 participants (aged 4.8–54.8 years), 25 received SD and 27 DD oseltamivir. Clinical resolution did not differ by dosing regimen ($P=0.43$); neither did virological clearance differ for either influenza A ($P=0.20$) or B ($P=0.70$). Adverse events, predominantly gastrointestinal, were greater with DD than SD ($P=0.04$). One OsR strain was detected prior to treatment and two individuals developed OsR strains during treatment, one each on SD and DD. Those with OsR strains did not appear to have a different clinical course.

Conclusions: DD oseltamivir did not appear to provide a clinical or virological advantage, nor reduce the emergence of oseltamivir resistance, but our study was underpowered. Adverse events occurred more frequently on DD compared to SD oseltamivir.

Introduction

Neuraminidase inhibitors (NAIs; for example, oseltamivir and zanamivir) are the treatment of choice against seasonal influenza. Since NAIs were introduced in 1999 and until 2007, ≤1% of viruses tested had mutations conferring oseltamivir resistance [1,2]. Oseltamivir-resistant (OsR) influenza A/H1N1 was first detected in Europe during the northern hemisphere winter of 2007–2008 and spread globally, such that by the 2008–2009 northern hemisphere winter, most strains of seasonal influenza A/H1N1 were OsR [3–6]. No difference in clinical severity was noted between the OsR and oseltamivir-sensitive (OsS) viruses [7]. Influenza A(H1N1)pdm09 was first detected in Mexico in March 2009, with the World Health Organization (WHO) declaring a pandemic on 11 June 2009 [8]. Surveillance has detected an increasing minority of influenza A(H1N1)pdm09 viruses to be OsR, almost all bearing the H27Y mutation in the neuraminidase (NA) gene. Between 2009–2011 approximately 600 of >30,000 influenza A(H1N1)pdm09 viruses were reported by the WHO as OsR [9–11]. Both the circulating influenza A/H3N2 and influenza B strains have generally remained OsS.

In preparation for an influenza pandemic many nations stockpiled oseltamivir, preferring it to zanamivir for its oral administration, systemic bioavailability and licensure for use from ≥1 year of age, as opposed to ≥5 years with zanamivir. Filled oseltamivir prescriptions in Australia increased from approximately 25,000 in 2008 to more than 350,000 in 2009, a process that may potentially promote the emergence of OsR viruses [6].

NAIs bind to the surface protein NA of the influenza virus, preventing release of viral progeny from host cells. Oseltamivir may select drug-resistant strains of influenza, especially in the immunocompromised, in...
whom there is reduced viral clearance and prolonged viral replication [12–15]. The most common resistance mutation for oseltamivir is an amino acid tyrosine substituting histidine (H275Y) in the NA gene, altering the NAI binding site. This results in high-level resistance to oseltamivir but not zanamivir [16,17]. In clinical trials, OsR pre-pandemic influenza virus strains developed more frequently in children (5–27%) than in adults (1–2%) [18–23].

Higher doses of oseltamivir may more rapidly reduce viral load, averting the selection of resistant strains, but evidence of this is lacking. Double dose (DD) regimens of oseltamivir have been used in both immunocompromised and artificially ventilated patients [6,24,25]. This study aimed to investigate whether this would be an effective strategy to reduce oseltamivir resistance, particularly amongst children (≤15 years), recruited from both the children’s hospital and a general practice.

The objectives of this study were to investigate the effectiveness of DD (150 mg twice daily for adults or 10 mg/kg for children) compared with standard dose (SD; 75 mg twice daily or 5 mg/kg for children) oseltamivir in subjects ≥5 years old on the frequency of detection of OsR virus, clinical disease and adverse events (AEs).

Methods

Trial design
This was an unblinded, randomized, parallel 1:1 study.

Participants
Participants were those aged ≥5 years old, presenting within 48 h with fever ≥37.8°C, with at least one respiratory symptom and a positive QuickVue point-of-care test (POCT; Quidel, San Diego, CA, USA), for influenza A or B presenting to either a family practice or a children’s hospital emergency department in West Sydney [26,27]. Given the higher false-negative rate of rapid POCT in older children and adults compared to younger children, a rapid nucleic acid test (NAT) using PCR was implemented for POCT-negative cases: the GenXpert FLU assay (Cepheid, Sunnyvale, CA, USA), with results available within 4 h [28–30]. Those positive for either test were offered inclusion in the study.

Those excluded were patients with a secondary bacterial infection, a poorly controlled underlying medical condition as determined by the treating doctor, immunosuppression (for example, malignancy, transplant or immunosuppressive agents), pregnant or lactating females, known oseltamivir allergy, participation in another clinical trial with an investigational drug or device, or insufficient English language skills to give informed consent.

Interventions
Participants were randomized in equal numbers to either SD or DD of oseltamivir for 5 days. The study did not prescribe clinical management beyond oseltamivir.

Doses were as follows, and standard dosing adhered to international guidelines for children (≤15 years; Table 1) [31]. Adult SD (>15 years) was 75 mg twice daily and DD 150 mg twice daily.

Outcomes
The end points of this study were the difference in the percentage of OsR influenza viruses in patients treated with SD versus DD oseltamivir at day 5 of follow-up, rates of clinical resolution and of the shedding of influenza virus at day 5 ±1 and adverse event profiles between dosing regimens. Clinical resolution was determined by patient or caregiver nominating date of cessation of all originally reported symptoms.

Adverse events
AEs were defined as symptoms developing during therapy that were not present at baseline or a symptom as reported on the subject daily record as present at baseline which resolved for one or more days but subsequently reappeared during therapy.

Sample size
Allowing for a 20% drop-out rate, the original target was 125 patients, yielding 100 completed subjects, conferring an 80% probability of detecting a difference in the frequency of OsR virus emergence from 25% to 5% under a single-tailed test with α=0.05, based on a 2009 study of OsR emergence in 27% of children treated with oseltamivir for influenza A H1N1 [23].

Randomization and sequence generation
We used block randomization to allocate interventions. A colleague not involved in the trial used the Excel random number generator to generate the allocation sequence consisting of randomly permuted blocks of four. Randomization was unblinded to patients and clinicians but blinded to laboratory researchers.

Implementation
The study was carried out at The Children’s Hospital at Westmead and at a nearby general practice, during the Southern hemisphere winter 2011.

First visit (day 1)
The procedures at the first visit (the initial clinical presentation) included collecting nose and throat specimens using a flocked cotton swab, with performance of a rapid POCT and a rapid NAT if POCT was negative (instituted 12 July 2011, one-third of the way through recruitment to increase sensitivity) and dispensing
Continuous variables with non-normal distribution and a normal distribution, Wilcoxon's rank sum test for confirmed by a two-sample t-test for continuous data with demographics, virological outcomes and AEs were performed by intention-to-treat, using SPSS version 20 (2011, IBM, New York, NY, USA) for statistical analysis. Comparisons between treatment groups, and recruitment sites, for a significant difference (P<0.05) in baseline demographics, virological outcomes and AEs were performed by a two-sample t-test for continuous data with normal distribution, Wilcoxon’s rank sum test for continuous variables with non-normal distribution and a two-sample \( \chi^2 \) test for binomial data. A Kaplan–Meier analysis was used to estimate ‘mean time to recovery’ (in days) and its corresponding 95% CI, for selected subgroups of the cohort. The Mantel Cox ‘log rank’ test was used to compare ‘mean times to recovery’ for different cohort sub-groups at the level of \( P<0.05 \).

Ethics

The Sydney Children’s Hospitals Network Human Research Ethics Committee approved the study. All patients or caregivers (for subjects aged <18 years) signed informed consent forms.

Results

Recruitment

A total of 52 participants were recruited over the 2011 influenza season April to August 2011 (Figure 1). Two patients, both in the standard dose group, had short hospital admissions; the rest were outpatients.

Baseline data

Neither age nor gender differed between the two treatment groups, with 62% of recruits being children (≤15 years). The general practice site recruited 20 adults (13 female) and 15 children (6 male); the Children’s Hospital recruited 17 children (4 female; Table 2). Almost all participants were healthy amongst the SD group, one child had uncomplicated, stable sickle cell anaemia and one adult had well-controlled epilepsy. In the DD group, one child had a treated Wolff–Parkinson–White syndrome, one child had a stable neurological condition with an unspecified myopathy and autism, one child had episodes of syncope that were under investigation but, inter-episodically, was neurologically normal.

Of the 50 cases for which an influenza type was determined by POCT or NAT, 21 (42%) had influenza A and 29 (58%) influenza B. Of the influenza A cases, 4 (19%, all adults) had A/H3N2 subtype (Table 3).

Outcomes

Clinical resolution

Outcomes for all 52 patients were analysed by intention-to-treat. There was no significant difference in time to clinical resolution between the two dosing regimens (Figures 2 and 3). A total of 8 of 25 (32%) on SD and 6 of 27 (22%) on DD oseltamivir had residual symptoms by treatment day 5 (\( P=0.43 \)). Children recovered an average of one day faster than persons aged >15 years; this difference was significant comparing within the SD regimen, the DD regimen and across both regimens (Figure 2). A 3-year-old boy with influenza B, who received the DD regimen, was given only 3 days of treatment due to nausea and was later admitted to hospital 10 days post last dose of oseltamivir with pneumonia.

### Table 1. Paediatric dosing regimens

<table>
<thead>
<tr>
<th>Standard dose oseltamivir</th>
<th>Double dose oseltamivir</th>
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<tbody>
<tr>
<td>&lt;15 kg</td>
<td>120 mg per day divided into 2 doses</td>
</tr>
<tr>
<td>15–23 kg</td>
<td>180 mg per day divided into 2 doses</td>
</tr>
<tr>
<td>24–40 kg</td>
<td>240 mg per day divided into 2 doses</td>
</tr>
<tr>
<td>&gt;40 kg</td>
<td>300 mg per day divided into 2 doses</td>
</tr>
</tbody>
</table>

Doses are shown and standard dosing adhered to international guidelines for children (≤15 years). Adult standard (≥15 years) dose 75 mg twice daily and double dose 150 mg twice daily.
requiring intravenous antibiotics. His viral culture and NAT for influenza were negative at 5 days after first dose of oseltamivir; thus it is unlikely that incomplete viral treatment led to the development of pneumonia.

**Virological outcome**

Of the 48 cases that were NAT-positive on visit 1, the dosing regimen did not significantly affect virological clearance as determined by NAT-negative swabs by visit 2 (SD 11/23, 47.8% versus DD 17/25, 68%; \( P = 0.16 \)). Of those recruited, 44 were culture-positive on visit one (SD 21/25, 84% versus DD 23/27, 85.2%; \( P = 0.91 \)). There was no difference by dose in virological clearance as determined by culture-negative swabs by visit 2 (SD 20/21, 95.2% versus DD 21/23, 91.3%; \( P = 0.61 \)).

Clearance was not lower in the SD than the DD group for either by influenza A by NAT (SD 3/11, 27.3% versus DD 6/10, 60.0%; \( P = 0.20 \)) or by culture (SD 9/10, 90% versus DD 7/8, 87.5%; \( P = 0.87 \)), nor for influenza B by NAT (SD 8/12, 66.7% versus DD 11/15, 73.3%; \( P = 0.70 \)) or by culture (SD 11/11, 100% versus DD 14/15, 93.3%; \( P = 0.76 \)). Influenza subtype was not significantly associated with virological clearance by NAT (influenza A 9/21, 42.9% versus influenza B 19/27, 70.4%; \( P = 0.08 \)) or culture (influenza A 16/18, 88.9% versus influenza B 25/26, 96.2%; \( P = 0.35 \)). There was

### Table 2. Participant characteristics by dose regimen

<table>
<thead>
<tr>
<th></th>
<th>Standard dose</th>
<th>Double dose</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>13.7 (4.8–51.9)</td>
<td>9.0 (5.0–54.8)</td>
<td>0.20</td>
</tr>
<tr>
<td>Male gender, ( n ) (%)</td>
<td>15 (60.0)</td>
<td>11 (40.7)</td>
<td>0.27</td>
</tr>
<tr>
<td>Female gender, ( n ) (%)</td>
<td>10 (40.0)</td>
<td>16 (59.2)</td>
<td></td>
</tr>
</tbody>
</table>

Neither age nor gender differed between the two treatment groups, with 62% of recruits being children (≤15 years). The general practice site recruited 20 adults (13 female) and 15 children (6 male); The Children's Hospital recruited 17 children (4 female).

### Table 3. Virological result by dosing regimen (POCT, NAT or isolation) at recruitment

<table>
<thead>
<tr>
<th>Influenza type and subtype</th>
<th>Standard dose (( n = 25 ))</th>
<th>Double dose (( n = 27 ))</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A and influenza A(H1N1)pdm09</td>
<td>9 (36)</td>
<td>8 (29.6)</td>
<td>0.77</td>
</tr>
<tr>
<td>Influenza A H3N2</td>
<td>2 (8)</td>
<td>2 (7.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Influenza B</td>
<td>13 (52)</td>
<td>16 (59.2)</td>
<td>0.78</td>
</tr>
<tr>
<td>No strain documented</td>
<td>1 (4)</td>
<td>1 (3.7)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Data are \( n \) (\%) unless otherwise indicated. Of the 50 cases for which an influenza type was determined by point-of-care test (POCT) or nucleic acid test (NAT), 21 (42%) had influenza A and 29 (58%) influenza B. Of the influenza A cases, 4 (19%, all adults) had A(H1N1)pdm0 subtype.
no significant difference in mean time between swabs 1 and 2 for SD cases (4.65 days; 95% CI 4.17, 5.13) versus DD (4.92 days; 95% CI 4.44, 5.40) nor for those with influenza A (4.86 days; 95% CI 4.36, 5.36) versus influenza B (4.72 days; 95% CI 4.22, 5.22), thus the lack of difference in virological clearance was not confounded by length of time between collection of the two swabs.

Three influenza A(H1N1)pdm09 viruses were OsR, one pre-treatment and two post-treatment (Table 4), all carrying the H275Y mutation. OsR was detected by NAT post-treatment in one case in each of the SD and DD groups (SD 1/23, 4.3% SD versus DD 1/25, 4.0%; P=0.95). A mixture of oseltamivir sensitive and resistant viruses was detected in the case receiving the SD regimen, whereas in the case receiving DD only resistant virus was detected. The case that was OsR at enrolment was genotypically related to a cluster of 31 OsR influenza cases, all carrying the H275Y mutation, from the Newcastle region in New South Wales, Australia between May and August 2011 [10,33,34]. No cases reported recent contact with Newcastle.
Adverse events

There was a difference in frequency of AEs reported between the two dose regimen groups (SD 4/25, 25% versus DD 13/27, 48%; \( P = 0.02 \)); gastrointestinal (GI) AEs occurred in 16 patients (nausea, vomiting or diarrhoea), with one case of insomnia (DD). The difference remained when solely GI events were analysed (\( P = 0.04 \)). More adults than children reported AEs (\( P = 0.01 \)). Amongst the children from whom AEs were reported, all were \( \leq 6 \) years of age. Two children older than 6 years were recruited (both 14 years old) and neither reported AEs. This age-based difference was significant for influenza A but not influenza B (Table 5), and for both DD and SD (Table 6). This was in the context of no significant relationship between influenza strain and patient age. AEs were not significantly different by recruitment site (general practice 13/35, 37.1% versus The Children’s Hospital 4/17, 23.5%).

Other results

Overall, there were no significant differences in the distribution of influenza strains between either treatment groups, the site of recruitment or age band (data available on request). In total, 49 cases were initially recruited based on positive POCT and 3 by positive rapid NAT; all had a subsequent ‘in-house’ real-time (RT)-PCR performed. Four cases, two in the DD group and two in the SD group, were POCT positive, but both RT-PCR and cell culture negative. Of these, two were influenza B positive by POCT, but the influenza type was not recorded for the other two. Influenza A peaked earlier than influenza B (Figure 4).

Discussion

This study examined the impact of SD versus DD oseltamivir on the selection of OsR virus, safety and effectiveness of therapy. Oseltamivir treatment was associated with a significant increase in overall AEs, particularly GI. The study did not support the hypothesis that DD reduced oseltamivir-resistance, however, it was underpowered. DD oseltamivir was not shown to be more clinically or virologically efficacious. Of those with influenza A, more adults than children reported AE; age-related differences in AE were not shown for influenza B. Thus, influenza type may influence manifestation of GI symptoms. Whilst more adults than children reported AE, there was no statistically significant difference in median age between the two dosing regimens, thus age did not confound the influence of dosing on AEs. The age differential may reflect children’s AEs being recorded by parent/guardian while persons aged \( \geq 15 \) years recorded their own AEs; however, neither of the two 14-year-old children reported AEs, although numbers are too small to draw conclusions. In contrast to our study, a study of 273 pupils and 53 staff at a junior school reported a high frequency of minor AEs to oseltamivir, but did not find a difference in rate of AEs between staff and pupils [35].

The large number of cases of influenza A(H1N1)pdm09 and the lack of A/H3N2 patients recruited amongst the Children’s Hospital recruits reflected national Australian surveillance data for the 2011 influenza season, where most hospitalized cases were predominantly influenza A(H1N1)pdm09 with far fewer influenza A/H3N2 infections [36]. Analysis by age and site of recruitment revealed no statistical difference in distribution of influenza types. All three cases with OsR influenza A(H1N1)pdm09 experienced an illness with similar severity to other subjects, however the study was underpowered to draw conclusions about the effect of resistance on illness severity. All resistant viruses were similar antigenically to that contained in the 2011 influenza vaccine, highlighting the importance of immunization as a measure to reduce circulating OsR influenza.

| Table 4. Cases with oseltamivir-resistant influenza A(H1N1)pdm09 virus |
|---------------------|---------------------|---------------------|
| Gender/age          | Case 1              | Case 2              | Case 3              |
| NAT visit 1         | M/5 years           | M/5 years           | M/11 years          |
| Viral culture (visit 1) | pH1N109             | pH1N109             | pH1N109             |
| OseLtamivir sensitivity (visit 1) | Influenza A | Influenza A | Influenza A |
| Dose                | S                  | S                  | S                  |
| NAT visit 2         | A(H1N1)pdm09       | A(H1N1)pdm09       | A(H1N1)pdm09       |
| Viral culture (visit 2) | Negative           | Influenza A        | Negative           |
| OseLtamivir sensitivity (visit 2) | Resistant (H275Y)  | Mix of sensitive and resistant (H275Y) | Resistant (H275Y) |
| Comorbidities       | Nil                | Nil                | Nil                |
| Compliance          | Yes                | Yes                | Yes                |

Three influenza A(H1N1)pdm09 viruses were oseltamivir-resistant, one pre-treatment and two post-treatment, all carrying the H275Y mutation. D, double; M, male; NAT, nucleic acid test; S, standard.
Co-circulation of different influenza strains/types has characterized the recent 2010, 2010/2011, 2011, 2011/2012 influenza seasons in Northern and Southern hemispheres [37–43]. The reported low prevalence of OsR in currently circulating influenza viruses is consistent with our result of 1/51 cases with pre-treatment OsR (2%) [9–11]. However 2/52 (4%) of the cases developed OsR on treatment, both influenza A(H1N1)pdm09. In Stephenson et al.’s study [23] OsR development occurred in 27% (3/11) post-treatment of pre-pandemic influenza A (H1N1) virus amongst children. In contrast, two large studies with 106 cases with influenza A(H1N1)pdm09, one of which recruited predominantly children, did not yield any OsR-influenza A(H1N1)pdm09 strains post-treatment [44,45]. An increasing number of neuraminidase-resistant influenza strains demonstrate mutations associated with reduced susceptibility other than due to H275Y [46–51]. The WHO recommends continued monitoring of OsR prevalence [10,52].

Globally, the WHO has reported that of those from whom OsR influenza was isolated, of the immunocompetent subgroup, 37% had not received prior oseltamivir, 

<table>
<thead>
<tr>
<th>Table 5. Adverse events by age and influenza type</th>
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<tbody>
<tr>
<td>Age group</td>
</tr>
<tr>
<td>Adults (n=20)</td>
</tr>
<tr>
<td>Children (n=30)</td>
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<tr>
<td>P-value</td>
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Data are n/total n (%) unless otherwise indicated. This age-based difference was significant for influenza A but not influenza B. Influenza type was unknown for two children. Neither reported adverse events.

<table>
<thead>
<tr>
<th>Table 6. Adverse events by age and dose regimen</th>
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<tbody>
<tr>
<td>Age group</td>
</tr>
<tr>
<td>Adults ≥15 years (n=19)</td>
</tr>
<tr>
<td>Children &lt;15 years (n=32)</td>
</tr>
<tr>
<td>P-value</td>
</tr>
</tbody>
</table>

Data are n/total n (%) unless otherwise indicated. P=0.049. DD, double dose; SD, standard dose.

**Figure 4. Influenza A and B seasonal distribution**

Influenza A peaked earlier than influenza B.
demonstrating community OsR virus circulation [9]. Our case of pre-treatment OsR-influenza A(H1N1)pdm09 was included in the ‘Newcastle cluster’, to date the largest reported community cluster of OsR-influenza A(H1N1)pdm09 infection [10,41]. Whilst this was one of two cases in the cluster found up to 380 km from Newcastle, it appears significant spread of this virus has not developed [32,53]. A review reported several clusters and cases of OsR influenza A(H1N1)pdm09, including untreated cases, and a cluster of six cases of community-acquired OsR influenza A(H1N1)pdm09 was reported from Japan, recently [43,54]. A smaller proportion of OsR influenza A(H1N1)pdm09 cases in the USA received pre-treatment with oseltamivir in the 2010–2011 season (26%) compared to the 2009–2010 (89%) season, suggesting community transmission of the resistant strain [55,56].

A recent randomized controlled trial by Farrar et al. [44] of over 300 hospitalized South East Asian patients with influenza, mostly children, demonstrated that there were no improvements in virological or clinical outcomes with DD over SD oseltamivir. Interestingly, there was also no difference in the AE rate between the two arms of the study (approximately 16% in each arm), a finding which differed from our own; this difference may be partially because our study recruited a lower proportion of children (61% <15 years in our study versus 75% <13.5 years by Farrar et al. [44]), and, in our study, adults experienced more AEs for DD, SD and overall. Farrar et al. [44] did not analyse AE by age. There was no significant difference in mean age between the two dosing regimens in our study, so age did not confound AE incidence between doses. No OsR developed in the 72 patients with influenza A(H1N1)pdm09, half of whom received DD. The study was conducted in hospitalized cases in Asia, conferring potential case severity, ethnic and other differences. Our study is unique in addressing developed world patients with mild (non-hospitalized) influenza and finding significant differences in AEs based on age and dose regimen.

Another recent study from Hong Kong comparing SD versus DD oseltamivir also focused on hospitalized patients, again demonstrating no difference in clinical outcomes or virological clearance, but did not find emergence of OsR. A greater incidence of GI AEs was reported in the DD group when analysed by dose regimen, but no significant difference in AE was reported when intention-to-treat arms were compared [45]. Interestingly, subgroup analysis demonstrated a trend towards increased rate of viral NAT clearance for influenza B with DD oseltamivir, whereas in both our study and Farrar’s et al.’s study [44], there was no difference in clearance of influenza B by day 5. Unlike our study, the Hong Kong study included neither children nor community cases; the average patient was aged in their 60s and no OsR emerged post-treatment.

Two older studies demonstrated that higher dose oseltamivir did not reduce influenza viral load more than lower dose oseltamivir [57,58], Treanor et al. [57] revealed no difference in symptom resolution between doses but Hayden et al. [58] did not compare between dose regimens. Neither study compared safety outcomes between doses [57,58]. Another study by Hayden et al. [59] reported a greater frequency of GI AEs in higher dose oseltamivir compared with lower dose but a significance level was not reported. No significant difference in viral clearance was elicited and clinical comparisons were not made [59].

Limitations of our study include the small sample size and the exclusion of both those aged <5 years and immunocompromised patients. Despite our best efforts, we were unable to fulfil our aim of recruiting 125 cases in an attempt to demonstrate a significant difference in rates of development of oseltamivir resistance. In conclusion, this study demonstrated no difference in development of antiviral resistance and no difference in efficacy of different oseltamivir doses, but increased GI adverse effects were elicited with DD oseltamivir.

Acknowledgements
Aeron Hurt, WHO Collaborating Centre for Reference and Research on Influenza, North Melbourne, Victoria, Australia for conducting phenotypic NAI susceptibility and genotypic sequencing analysis. The trial was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR), registration number/ACTR number: ACTRN1261000004066.

Disclosure statement
Roche Pharmaceuticals funded this study in an unrestricted manner. RB has received financial support from CSL, Sanoﬁ, GSK, Roche, Novartis and Wyeth to conduct research and present at scientiﬁc meetings. LH has performed consultancy work for Novartis for which payment was made to the National Centre for Immunisation Research and Surveillance. He has had travel expenses covered by GSK and has conducted sponsored research and investigator-driven research with funding from GSK, Wyeth, Merck, CSL, Roche and Sanoﬁ Pasteur. GK was an investigator in studies supported by Roche Products Pty Ltd and currently funded by National Health and Medical Research Council (NHMRC). DD has received financial report from Roche, Crucell and Biota to conduct inﬂuenza research. The remaining authors have no competing interests. Roche Products Pty Ltd, manufacturers of oseltamivir (Tamiflu), funded the study, but did not collect or review data.

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3.3 CHAPTER 2: Synopsis

A Randomized Study of Standard versus Double Dose Oseltamivir for Treating Influenza in the Community

Our study did not support any clinical advantage to doubling the dose of oseltamivir in at least the healthy population, and adds to the literature examining such a strategy amongst different population groups. There was not enough power in the study to demonstrate a difference in emergence of oseltamivir resistance on treatment. However, there was a trend towards more rapid virological clearance, particularly in those with influenza A, in the double dose group (p = 0.16), suggesting that conditions for oseltamivir resistance, prolonged viral shedding, may be mitigated by double dose regimens.

Recent studies on high dose influenza vaccine showed, promisingly, that immunogenicity improved in cardiac patients, cancer sufferers and elderly recipients. However, other specific antivirals have not yet been developed. Could there be a place for resurrecting old strategies, such as polyclonal antibodies, currently used in, for example, rabies infection and arachnid venom toxicity? This is the focus of my next paper.
4.1 CHAPTER 3: Preamble
Benefits of Using Heterologous Polyclonal Antibodies and Potential Applications to New and Undertreated Infectious Pathogens

Neuraminidase inhibitors (NAI) are the only anti-influenza antivirals currently registered in Australia, leaving the general population vulnerable to epidemics and pandemics were antiviral resistance to develop. This risk of untreatable infection is potentiated in those most at risk of influenza, in whom vaccine efficacy is reduced, such as those with immune deficits, chronic diseases and those at extremes of age. Additionally, the global population is at risk from novel strains of influenza such as ‘avian flu’, arising from antigenic shift, to which there is low background immunity and no effective vaccine. Were they to develop the ability to efficiently transmit between humans, a pandemic could ensue. Epidemiological data indicate that the elderly and those with chronic conditions are most at risk of clinical avian influenza, as well as poorer poultry farmers in Asian countries. Here, I present an extensive review of an alternative strategy for management of resistant influenza, avian influenza (and other infections) – heterologous polyclonal antibody therapy. Currently, heterologous polyclonal antibodies are used against snake venom toxin and rabies virus infection. However, they have had historical applications to a range of viruses and bacteria in the pre-antibiotic era, and the World Health Organisation recognise their potential in the fight against pandemic influenza in its ‘Position paper on collection and use of convalescent plasma or serum as an element in pandemic influenza planning; July 2009’. I examine their past use, how safety limitations in their applicability have been overcome, and what current studies have demonstrated about their effectiveness against avian influenza.
Review

Benefits of using heterologous polyclonal antibodies and potential applications to new and undertreated infectious pathogens

Rashmi Dixit a,*, Jenny Herz b, Richard Dalton c, Robert Booy a

a The Children's Hospital, Westmead, Sydney, Australia
b Biointelect, Sydney, Australia
c University of Southampton, Southampton, United Kingdom

A B S T R A C T

Background: Passive immunotherapy using polyclonal antibodies (immunoglobulins) has been used for over a century in the treatment and post-exposure prophylaxis of various infections and toxins. Heterologous polyclonal antibodies are obtained from animals hyperimmunised with a pathogen or toxin.

Aims: The aims of this review are to examine the history of animal polyclonal antibody therapy use, their development into safe and effective products and the potential application to humans for emerging and neglected infectious diseases.

Methods: A literature search of OVID Medline and OVID Embase databases was undertaken to identify articles on the safety, efficacy and ongoing development of polyclonal antibodies. The search contained database-specific MeSH and EMTREE terms in combination with pertinent text-words: polyclonal antibodies and rare/neglected diseases, antivenins, immunoglobulins, serum sickness, anaphylaxis, drug safety, post marketing surveillance, rabies, human influenza, Dengue, West Nile, Nipah, Hendra, Marburg, MERS, Hemorrhagic Fever Virus, and Crimean-Congo. No language limits were applied. The final search was completed on 20.06.2015. Of 1960 articles, title searches excluded many irrelevant articles, yielding 303 articles read in full. Of these, 179 are referenced in this study.

Results: Serum therapy was first used in the 1890s against diphtheria. Early preparation techniques yielded products contaminated with reactogenic animal proteins. The introduction of enzymatic digestion, and purification techniques substantially improved their safety profile. The removal of the Fc fragment of antibodies further reduces hypersensitivity reactions. Clinical studies have demonstrated the efficacy of polyclonal antibodies against various infections, toxins and venoms. Products are being developed against infections for which prophylactic and therapeutic options are currently limited, such as avian influenza, Ebola and other zoonotic viruses.

Conclusions: Polyclonal antibodies have been successfully applied to rabies, envenomation and intoxication. Polyclonal production provides an exciting opportunity to revolutionise the prognosis of both longstanding neglected tropical diseases as well as emerging infectious threats to humans.

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1. Introduction and methods

The aims of this review are to examine the history of animal polyclonal antibody therapy use, its development into a safe and effective product and its potential future application to humans for emerging viruses and neglected tropical diseases.

A literature search was undertaken by an experienced medical librarian to identify articles on both the safety of polyclonal antibodies and the use for the treatment of rare or neglected diseases. The databases searched included OVID Medline and OVID Embase. The searches contained database-specific MeSH and EMTREE terms in combination with pertinent text-words: polyclonal antibodies and rare/neglected diseases, antivenins, immunoglobulins, serum sickness, anaphylaxis, drug safety, post marketing surveillance, rabies, human influenza, Dengue, West Nile, Nipah, Hendra, Marburg, MERS, Hemorrhagic Fever Virus, and Crimean-Congo. To minimise bias, no language limits were applied. The final search was completed on 20.06.15. These searches yielded 1960 articles. Title searches excluded many irrelevant articles, yielding 303 that were then read in full. Of these, 179 are referenced in this study.
In 1890, von Behring and Kitasato discovered that sera from rabbits immunised against diphtheria or tetanus were able to protect exposed mice [1]. By 1894, animal-derived anti-diphtheria serum was used in humans during a European epidemic [2]; concurrently, Phisalex and Bertrand demonstrated that the blood of horses immunised with Viper aspis (the European viper) had antivenin properties [3]. Sera from both humans and animals were subsequently used for management of illness caused by viruses such as measles, varicella and the pandemic Spanish influenza of 1918 [4,5]. Anti-rabies polyclonal antibody preparations from hyper-immunised horses were developed in the early 20th century [6]. In this pre-antibiotic era, serum therapy was also applied to bacterial infections such as pneumococcal pneumonia, meningococcal meningitis and streptococcal scarlet fever [7,8].

The early, crude preparations of serum were often contaminated with animal proteins; as a result, serum sickness and anaphylaxis limited their safe application. Antibiotics against bacterial infections largely superseded serum therapy in the late 1930s and 1940s [7]. During the 1960s, however, there were improvements in enzymatic digestion and purification of equine immunoglobulins, enabling safer polyclonal antibody therapies to be developed for envenomation, rabbit exposure and viral infections such as hepatitis A and B, varicella–zoster virus and respiratory syncytial virus (RSV) [8].

The development of monoclonal antibodies (mAbs) in the 1970s enabled large quantities of specific antibody to be produced [8]. The humanisation of mAbs allowed their use in the treatment of chronic disease by repeated administration. However, polyclonal antibodies still proved more effective than monoclonal antibodies in the treatment of many infectious diseases. To date, most monoclonal antibody therapies are produced for either autoimmune conditions (such as systemic lupus erythematosus or Crohn’s disease) or neoplastic conditions. The only licenced monoclonal antibody to an infectious target is palivizumab, for RSV (such as systemic lupus erythematosus or Crohn’s disease) or neoplastic conditions. The only licenced monoclonal antibody to an infectious target is palivizumab, for RSV [9]. In contrast to monoclonal antibodies, polyclonal therapy, by targeting multiple epitopes, can protect against epitope mutation [4]. Additionally, more may be needed of a low avidity monoclonal antibody preparation, compared with a polyclonal preparation, to neutralise a given amount of toxin or pathogen, as is generally the case for antibiotics to snake envenomation [10].

Animal derived polyclonal antibody therapy has been successfully and safely applied to (i) medication overdoses such as colchicine and digoxin, (ii) poisoning by snake, arachnid, marine and plant toxins, and (iii) post-exposure prophylaxis against the rabies virus [11–15]. Polyclonal antibody products can be made in large quantities and cost-effectively to respond to the great endemic demands in Asia and Africa as well as potential pandemic situations, globally.

2. Modern processing dramatically reduces adverse reactions

Adverse reactions in humans to animal-derived polyclonal antibodies are usually due to the presence of highly immunogenic animal proteins. Type 1 hypersensitivity reactions, including anaphylaxis, begin within minutes. Type 3 hypersensitivity, or serum-sickness, results from deposition of immune complexes on small vessels of the skin, joints and kidneys; this can develop at any point over the three weeks it takes to clear the injected antibodies [3]. Antibodies consist of the antigen binding (Fab) fractions, linked by disulphide bonds as F(ab′)2, and the crystallisable fraction (Fc). The reagentogenic Fc fragment can induce complement-mediated urticaria, angioedema, lymphadenopathy, arthralgia, and nephropathy [3]. Fc removal with retention of the immunogenic F(ab′)/F(ab′)2 components was first applied to digoxin antiserum in 1976 [16]. The F(ab′)F(ab′)2 fragments were isolated initially through salt precipitation and subsequently, since the mid-1990s, via chromatographic purification [17]. Complement mediated anaphylactoid reactions, fever and hypersensitivity have been reduced by careful elimination from the final product of bacterial endotoxins (pyrogens) as well as protein and cell aggregates, through pasteurisation, ultrafiltration and additional chromatography [18]. With this combination of purity and safety advancements, serious adverse events are now rare, even very rare. With equine rabies immunoglobulin (ERIG), anaphylactic shock is reported in less than 1/45,000 treatments, serum sickness in <0.5% and all grade adverse events in <5% [3,15].

Ammonium sulphate precipitation of the F(ab′)2 was the main method of purification until the mid-1990s when chromatography was introduced [19]. Examples of current production techniques are for that of the rabies equine immunoglobulin produced by QMSI (Queen Saovabha Memorial Institute, Bangkok) and Favirab (Sanofi-Pasteur) as well as equine snake antivenins [19,20]. Briefly, the techniques involve hyper-immunisation of the source animal daily for several days and collection of sera 2–4 weeks after the last injection, which enables antibody affinity maturation, resulting in a highly avid, concentrated product, which reduces the co-administered load of contaminant animal proteins [21].

The purification process often starts with an anion-exchange chromatography step which isolates the immunogenic horse IgG subclass, and removes other immunoglobulins, proteins, cell aggregates and contaminants [19]. Enzymatic cleavage or digestion by low pH peptisin of IgG to Fc and active F(ab′) or F(ab′)2 regions reduces adverse reactions [17]. A second anion-exchange chromatography excludes the Fc fragments, protein and cell aggregates which result from low pH peptisin digestion, as well as bacterial pyrogens [22]. European guidelines require animal immunosera that are test-negative for pyrogens [23]. A final pasteurisation step involves heat-treatment for 10 h at 60 °C, which destroys viruses, and thermocoagulates excess proteins [20]. High performance liquid chromatography is used to control purity of the final product; usually 90% of the content is covalent F(ab′)2, 5% F(ab′) fragments, and <0.5% are polymers/aggregates [18]. Importantly, immunoreactivity remains intact after these steps [19]. The final antiviral antibody titre is determined for example by ELISA or seroneutralisation assays for antiviral immunoglobulins. Variations in the production process include application of potassium sulphate for F(ab′)2–Fc separation, and precipitation of non-immunoglobulin protein/cell reaggregation and precipitation to purify whole IgG (resulting in near total exclusion of albumin, less activation of complement as well as lower total protein/protein aggregates compared to ammonium sulphate precipitation), and centrifugation to eliminate cellular elements and proteins [3,24–27]. Preservatives are added to the final products to prevent bacterial and fungal contamination [28]. Refrigeration and avoidance of prolonged storage avoids protein/cell reaggregation and precipitation [28].

Apart from pasteurisation, steps that neutralise pathogens include low-pH pepstin hydrolysis and high-temperature caprylic acid precipitation, both of which lipolyse enveloped viruses such as Herpes, Sindbis and West Nile viruses; and ultra/nano filtration e.g. using 0.22 μm gauge filters, for virus and bacterial removal [4,29,30]. Subculturing can confirm bacterial sterility [4]. The WHO, in recognition of some inconsistency in antivenin production quality, has released the ‘WHO Guidelines for the Production, Control and Regulation of Snake Antivenom’ [31].

Large relative molecular mass (Mr) bivalent antibodies (IgG and F(ab′)2 fragments) have a smaller volume of distribution and a longer half-life in the human body than the lower Mr F(ab′) fragments [32]. Both IgG and F(ab′)2 fragment elimination occurs mainly by formation and elimination of immune complexes (catabolism), whereas F(ab′) is cleared renally [33].
The longer half-life means that antivenins made from IgG and F(ab′)2 persist, which reduces rebound symptoms of envenomation associated with F(ab′)2 antitoxins [34]. Additionally, the preservation of large Mr antivenins in the vascular compartment draws venin out of tissues into the bloodstream to form antivenin-venom complexes, promoting venom clearance [29]. However, recent human pharmacokinetic data suggests that F(ab′)2 also extravasates into tissues [36,37]. This tissue penetration may assist with neutralizing viruses infecting various organs.

Cleaving of the Fc fragment, whilst reducing adverse events, possibly reduces its potentiating effect on generation of natural immunity towards a pathogen or toxin. Fc interaction with antigen-presenting may stimulate and promote development of active immunity towards the exposure [38,39].

These features may have implications for the selection of molecule type in the production of polyclonal immunotherapy, particularly for post-exposure prophylaxis against viruses such as Mers-CoV and Ebola. Dosing intervals are longer and therefore dosing regimens more pragmatic with IgG and F(ab′)2 and IgG also may promote development of host immunity. However, this needs to be balanced against the increased risk of hypersensitivity reactions from the Fc molecule.

Half-time of elimination (t1/2) of the product in the plasma compartment was analyzed after intravenous (IV) administration of F(ab′)2 against avian influenza A H5N1 in 3 healthy volunteers receiving 1 dose and in 10 healthy volunteers receiving 5 doses [40]. The plasmatic elimination of F(ab′)2 after one IV infusion had a mean t1/2 of 16.77 h and after 5 infusions 24 h apart, a mean of 10.89 h. These results indicated the persistence of equine F(ab′)2 in the plasma for the duration of the therapeutic protocol with evidence of a slight accumulation between day 1 and day 5. After the fifth infusion, equine F(ab′)2 remained detectable by ELISA in plasma, (above > 1 μg/mL) for between 3 to 14 days. These results were consistent with another human study of intravenous equine F(ab′)2, with a plasma t1/2 of 14.2 h and a later catabolism of F(ab′)2 with a t1/2 of around 7 days [37]. Thus, the protection induced may continue several days after the end of the treatment protocol, up until the patient’s immunity generates host antibodies.

### 3. Management of rare secondary effects

A meta-analysis of seven studies and a Cochrane review each concluded that adrenaline premedication, but not other agents, significantly reduced early adverse reactions [41]. Additionally, early use of adrenaline for anaphylactic reactions post antivenin is effective [42].

Ovine (sheep-derived) products may be somewhat less reagentogenic than equine products, but the latter are more economical to produce given the larger amounts of sera available, and have a longer half-life, reducing requirement of re-administration [43,44]. Equine antivenin may also have superior antitoxin effects compared to ovine [45].

There are no recorded cases of viral or prion transmission from equine-derived antitoxins and current processes aim to preserve this safety record [29]. Both initial measures (e.g. donor selection, epidemiological exclusion, quarantine, health status of the animal) and processing of final product reduce the risk of a contaminating virus. Animals are contained within closed flocks or maintained in areas free of insect vectors of certain arboviruses. For example, rattlesnake, viper and dog skin antitoxins are manufactured in South Australia, a region free of prions and many viral pathogens [46]. Source animals may also be vaccinated against local pathogens such as rabies, anthrax and viral equine encephalitides [31]. Molecular diagnostic screening of animals for viruses may be performed [29,31]. Record-keeping and

regular stock inspections contribute to quality control [4]. The rigor of the application of such processes varies by resource availability e.g. in resource-challenged countries where much of the world’s antivenin/antitoxin is both required and produced, not all safety measures may be employed [15,47]. Polyclonal (source animal immunised with more than one venin) polyclonal antivenins have higher rates of adverse reactions than monovalent (source animal immunised to just one venin) polyclonal antivenins, e.g. 24% vs. 9% for Australian produced CSL snake antivenins, due in part to the larger volumes required to be administered for treatment with the polyclonal rather than monovalent polyclonal antibodies [48].

### 4. Efficacy and safety of modern polyclonal immunoglobulin products

Clinical studies have demonstrated the safety and efficacy of equine immunoglobulin. These therapies can even be given to pregnant women as no passage of F(ab′)2 across the placenta is expected and thus no teratogenicity anticipated [49].

Of 7660 Filipino recipients of F(ab′)2 equine rabies immunoglobulin (ERIG), (Favirab, Sanofi Pasteur, Lyon, France) only two developed rashes; neither had received post-exposure prophylaxis (PEP) strictly as per the WHO guidelines [50,51]. Of the 151 subjects in this cohort who sustained bites from laboratory-confirmed rabid animals, there were no reported cases of rashes. Of 193 persons bitten by rabid dogs in the Philippines, there was just a single recorded PEP failure; due to location of the bite (on the lip) local ERIG infiltration was difficult, and, against official protocol, she received a mix of intradermal and intramuscular rabbies vaccine [52]. These results emphasise the importance of adherence to WHO rabies guidelines in administering PEP.

The safety of current ERIG products has also been demonstrated in clinical trials and post marketing surveillance. Of over 12,000 ERIG recipients in the Philippines, Thailand and India, 0.3 and 1% of recipients developed local reactions and 0.03–3% had systemic reactions [15,52–54]. Some of these reactions may have been due to co-administered tetanus toxoid.

A retrospective review of over 70,000 patients in Thailand who received either human rabies immunoglobulin (HRIG) (59.6%) or ERIG (40.4%) demonstrated that 1.83% of ERIG recipients had an adverse event, versus 0.03% of HRIG recipients; however the broad date ranges included ERIG produced both before and after the introduction of modern purification techniques [55]. Serum sickness was reported in 0.72% of ERIG recipients vs. 0.007% of HRIG recipients, and no deaths were reported.

Antivenins are also effective. While there are few randomised prospective controlled human studies, mice studies, retrospective human studies and human case reports provide evidence for effective and safe treatment of life-threatening envenomations and coagulopathy [56,57].

Several human reports confirm efficacy of various snake antivenins (Tables 1 and 2) [33,58–62]. Efficacy of rattlesnake antivenin has been studied, particularly in the USA, where rattlesnakes predominate; introduction of rattlesnake whole IgG polyclonal crotalid antivenin (ACP) reduced mortality from over 25% to <0.5% when delivered in a health care facility in the United States [63]. A 10-year retrospective chart review revealed the fractionated Crotalidae polyclonal immune F(ab′)2 (CroFab®) to be more effective at avoiding fasciotomies than the whole IgG product ACP [64]. A 12 year review of CroFab® revealed a response rate of 77% amongst 24 cases of severe envenomation, including neurotoxicity, whilst in another series of 28 severe envenomations, all responded to CroFab® [65,66]. A literature review of controlled and observational studies confirmed the
efficacy and safety of CroFab® [67]. Crofab® has also been demonstrated to be effective in paediatric patients [68,69]. However, a South American study demonstrated a locally produced whole IgG antivenin was more effective than two F(ab′)2 preparations but with more anaphylactoid reactions [70]. Amongst the fractionated products, a comparison of F(ab′) and F(ab′)2 rattlesnake antivenin demonstrated a significant reduction in late coagulopathy in those who received F(ab′)2 compared to F(ab′) (29.7% vs. 10.3%) due to the longer half-life of the davalent over the monovalent product [34]. This advantage of F(ab′)2 over F(ab′) has been demonstrated for other antivenins [71,72].

Epidemiological data from Europe demonstrates that the intro­duction of antivenin has resulted in a several-fold decrease in snakebite mortality [73]. Australian snake antivenins have been considered more effective at neutralising the neurotoxic effects than the pro-coagulant effects, and plasma transfusions are recommended along with antivenin [46,74,75]. This limitation of current Australian antivenins has been disputed, however [76].

Safety of snake antivenins has improved with antibody fractionation and modern processing. Whole IgG rattlesnake antivenin (e.g. Antivenin Crotalidae Polyvalent ACP) was associated with a 18–50% frequency of immediate and delayed reactions [77–83]. This was more than halved after the removal of Fc and implantation of modern purification methods (e.g. Crofab®) [63,67,84–93,65,94].

A meta-analysis of CroFab® revealed an immediate hypersensitivity rate of 8% and serum sickness of 13% [95], with even lower rates reported in a subsequent study of 340 cases: <2% immediate hypersensitivity reactions and 5% serum sickness [96]. Likewise, the adverse event rate of bothrops antivenin improved from 25 to 82% for ammonium sulphate precipitated whole IgG preparations to 11–28% for caprylic acid precipitated whole IgG and 12–36% for F(ab′)2 antivenin [26,28].

A meta-analysis was conducted by us, the authors, of 30 observational and controlled studies of various snake antivenins, excluding rattlesnake antivenin for which a meta-analysis has been reported above [27,33,43,48,59,60,62,70–72,97–116] (Table 2). A simple linear weighting by the sample size of each study was applied. The weighted mean and standard deviation for each set of studies were compared using a formula to isolate the effect of the independent variable (the anti-venin) given to an otherwise similar population distribution. We determined an estimate of the percentage difference with $z = 1.96$ to give 95% confidence intervals, which are reported below. This analysis demonstrates that F(ab′) and F(ab′)2 are safer than whole IgG and that there was a reduction in adverse events after introduction of purification techniques in the mid-1990s, with the exception of no reduction in anaphylaxis pre and post 1995 for all types of antivenins, combined.
There are approximately 1.5 million scorpion envenomations annually resulting in about 2600 deaths, from autonomic over-stimulation and/or an overwhelming inflammatory response. A randomised controlled trial (RCT) of anti-scorpion F(ab')_2 in Arizona revealed a significant difference in the following: rapid symptom resolution, midazolam requirement and plasma venom levels, compared to placebo [117]. A meta-analysis of 4 RCTs and 5 observational studies demonstrated effectiveness in new world (American) scorpion envenomations but not in the old world[118]. Adverse events were higher in non-randomised trials, e.g. serum sickness occurred in 57% of those treated in one study, but lasted only 3 days, and antivenin was highly effective, reducing symptom duration from 22h to 31 min [119]. Adverse events were infrequent in the RCTs (0–2%). However, this meta-analysis included a 2011 Indian (‘old world’) controlled trial where scorpion F(ab')_2 antivenin reversed clinical envenomation more effectively than no antivenin [120]. Three other old-world studies not included in the meta-analysis have demonstrated efficacy. An uncontrolled Indian study of scorpion F(ab')_2 antivenin where 41/48 (85.4%) responded, with one mild reaction [121]; a prospective case-control series from India of 62 patients under the age of 18 years which demonstrated that dopamine and dobutamine requirement was reduced and recovery was faster in those who received scorpion antivenin [122] and a controlled trial from Saudi Arabia that reported a reduction in mortality from 4–8% to <0.5%, with mild reactions only (13.9%) [123]. In a review of 10 studies, only this Indian uncontrolled trial of scorpion F(ab')_2 antivenin revealed efficacy, as opposed to whole IgG in all the other trials [124]. However, withdrawal of IgG scorpion antiserum in the USA in 2002 with no alternative substituted, resulted in a 5-fold increase in ICU admissions for stings [125]. Saudi Arabian data demonstrated a reduction in deaths from 1.7% to nil, in pulmonary oedema from 11.1% to 1.2% and in cardiac arrest from 7.4% to 0.4% after introduction of scorpion antivenin in 1991 [126].

Spider antivenin is also effective and safe. A review of whole IgG funnel-web spider antivenin use in Australia reported a complete response in 97% of 75 recipients, with only one local reaction, one case of anaphylaxis and one case of serum sickness [127]. Whole IgG black widow spider (lactodectus) antivenin reduced durations of symptom and hospitalisation in moderate to severe envenomation in a US review [128,129]. Intravenous (as compared to intramuscular) administration was associated with high rates of anaphylaxis (e.g. 5% in 1989) and serum sickness (33%) prior to reducing the speed of administration, which reduced the total adverse event rate to less than 3% [130]. Equine derived F(ab')_2 black widow antivenins have been developed and are being tested [131]. An Australian study of 95 cases of redback spider antivenin F(ab')_2 yielded 4 cases of anaphylaxis (4.2%) and a serum sickness rate of 9.3% [132]. Overall, polyclonal antibodies used as antidotes are also reportedly safe. A study of 717 patients who received anti-digoxin F(ab') revealed an allergy rate of 0.8% [133].

5. Polyclonal antibodies for emerging and neglected viral diseases

Polyclonal serum therapy is emerging as potentially applicable to a range of viruses for which there are limited therapeutic options. Highly pathogenic avian influenza (HPAI) viruses such as H5N1 and H7N9 are new targets for polyclonal F(ab')_2 immunoglobulin therapy. Stockpiles of effective product for prophylaxis and treatment are required in anticipation of epidemics. The neuraminidase inhibitor oseltamivir remains the mainstay of treatment with an overall reduction of mortality risk reported of 49% [134]. Reports of oseltamivir resistance in HPAI H5N1 as well as in seasonal human influenza strains suggests complementary treatment options are necessary [135,136].

The World Health Organisation (WHO) recognises the potential role of serum therapy for influenza pandemic planning, given its historical success in infectious outbreaks [137]. Use of convalescent plasma during the 1918 Spanish influenza epidemic of 1918 apparently reduced mortality by 50% [5,138]. Convalescent plasma has been used in two cases of HPAI H5N1, both of whom survived [139,140]. Since the first human case of HPAI H5N1 in 1997, the WHO has recorded 718 human infections with 413 deaths as of 26 January 2015, a case fatality rate of 57.5% [141]. All cases have been in Africa, Asia or the Middle East, or travellers returning [142]. Survivors produce demonstrable neutralising antibodies [143]. Whilst there is currently only limited person-to-person transmission, antigenic shift (via reassortment with circulating human viruses) could confer this, setting the stage for a devastating pandemic [144].

In vivo proof-of-concept studies of equine polyclonal F(ab')_2 to HPAI H5N1 have been conducted in mice [145]. The product was able to prevent infection after an intranasal HPAI H5N1 challenge. Sero-neutralising assays and haemagglutination inhibition tests confirmed the potent neutralising abilities of equine polyclonal anti-HPAI H5N1 F(ab')_2 preparations in mice [145]. In another study, four H5N1 avian influenza equine F(ab')_2 preparations demonstrated cytopathic effect against cultured Madin–Darby canine kidney (MDCK) cells infected with H5N1 and protected mice against lethal challenge, both given prior (prophylaxis) or post (therapeutic) exposure [146]. These studies concur with earlier mouse studies of polyclonal antibodies to seasonal influenza A. In one such study, mice were immunised with the M2 antigen of influenza A, anti-influenza IgG was obtained and intravenously injected into other exposed mice leading to 100% survival with high dose (320 micrograms) IgG [147].

A phase 1 study in 16 healthy young human males aged 21–40 years who received intramuscular polyclonal F(ab')_2 to HPAI H5N1 yielded no serious adverse events, no changes in blood or urinary parameters, and only one febrile reaction, likely related to the product; there was also evidence of clinical benefit as assessed by seroneutralising and haemagglutination inhibition testing of the subjects’ plasma samples [40]. As ethically no placebo-controlled efficacy studies in humans can be performed, further effectiveness data will have to be gathered from compassionate case-based use.

Other fatal avian influenza viruses are emerging in people: in March 2013 the H7N9 avian influenza virus emerged in China and spread to Hong Kong, Taiwan and Malaysia; 718 cases and 413 deaths have been reported to date [148]. Both HPAI H5N1 and H7N9 have demonstrated rare person-to-person transmission [148–151]. In May 2013, the first case of avian influenza A H6N1 was reported in Taiwan [152]. Three human cases with avian influenza H10N8 were reported in China between December 2013 and February 2014 [153–165]. Both of these are low pathogenic strains currently, but the potential remains for acquisition of mutations that may increase pathogenicity. Several studies have suggested the potential role of respiratory tract administration of polyclonals to prevent and treat seasonal influenza infection, suggesting a potential for these to be applied to avian influenza strains [156–158].

As well as the threat of new avian influenza viruses, the Middle Eastern respiratory syndrome coronavirus (MERS-CoV) that emerged in 2012 has pandemic potential. MERS-CoV causes severe respiratory illness and, sometimes, renal injury [133,159]. Whilst most cases have occurred in several Middle Eastern countries, particularly Saudi Arabia, cases have been reported from Korea, Europe and North Africa [160,161] Of the 1368 reported cases of MERS-CoV to WHO by 7 July 2015, 487 have died (35.6%); but a large proportion of cases may go undiagnosed [162]. There appear to be a mix of zoonotic (bat and/or camel) and human sources for transmission; person-to-person transmission has been particularly documented in healthcare settings [162–164]. There is
neither a specific treatment nor a licenced vaccine. In a recent study, serum of Egyptian dromedary camels who were seropositive for Mers-CoV was administered to mice infected with Mers-CoV, with Australian camel sero-negative sera serving as a control [165]. Mers-CoV seropositive camel serum given both pre- and post-exposure protected infected mice from weight loss, diminished lung histological changes and accelerated virus clearance. Polyclonal antibodies could provide post-exposure prophylaxis for close contacts as well as a therapeutic strategy for those severely affected by MERS-CoV. One potential setting for the development of an epidemic of either H5N1 or MERS-CoV is the annual Hajj pilgrimage in Saudi Arabia, where preparations for a potential MERS outbreak are being refined [166].

Equine polyclonal antibody therapies could also be developed for other widespread and severe neglected tropical diseases e.g. the viral haemorrhagic fevers Crimean-Congo Haemorrhagic Fever, Dengue, Ebola and Marburg; bat-transmitted viruses such as Nipah and Hendra, as well as Lassa virus, West Nile Virus (WNV) and severe acute respiratory syndrome-associated coronavirus (SARS). The WHO has reported 27,705 cases of Ebola with over 11,269 reported deaths in the 2014–2015 West African epidemic [167]. A stockpile of polyclonal antibodies may form part of epidemic preparation.

Animal studies exist demonstrating efficacy of polyclonal antibody therapy for various neglected tropical viral diseases. Unimmunised hamsters that received WNV-immune hamster antisera one hour before and 24 h after a WNV challenge were protected from lethal WNV infection [168]. Duck egg polyclonal F(ab')2 neutralised Andes virus (the primary agent for pulmonary haantavirus syndrome in South America) in vitro; hamsters given the polyclonal F(ab')2 post-exposure had improved survival compared to controls [169]. Polyclonal antibodies against the Marburg and Ebola filoviruses were acquired from non-human primates (NHPs) that survived filovirus challenge and, when given post-exposure, prevented disease and death in virus-challenged NHPs compared to controls [170]. Similar results were obtained from injecting Ebola infected mice with polyclonal sera from E. bola immunised mice [171]. Other studies of mice, monkeys and guinea pigs have demonstrated purified equine whole IgG has prolonged survival against Ebola [172–176]. Equine F(ab')2 has been verified to protect both mice and hamsters from development of infection with SARS-CoV infection when given prophylactically and reduced lung viral titres when given therapeutically, compared to controls [177–179].

6. Conclusions

In summary, clinical studies have demonstrated the efficacy of animal-derived polyclonal antibody therapies against various infections, toxins and venoms. The safety of current products has been demonstrated in clinical trials and post marketing surveillance. When WHO guidelines are followed in administering rabies equine polyclonal antibodies, post-exposure prophylaxis is highly effective in averting rabies. Antivenins are also safe and effective for snake and arachnid bites, with available data suggesting a positive effect against scorpion bites. Antidotes are also reportedly safe and effective. Polyclonal antibodies are being developed against viruses with epidemic and pandemic potential for which prophylactic and therapeutic options are currently limited, such as avian influenza and other zoonotic viruses. Preclinical studies as well as phase 1 safety studies against avian H5N1 influenza, are promising and the technology exists to rapidly apply the methods of polyclonal production against a wide range of pathogenic antigens, providing an exciting opportunity to revolutionise the prognosis of both longstanding neglected tropical diseases as well as emerging viral threats to humans.

Conflict of interest statement

The authors have no conflict to declare.

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CécileHerbreteau-Delah (Fab'entench).

References


CORRIGENDUM

Corrigendum to Benefits of using heterologous polyclonal antibodies and potential applications to new and undertreated infectious pathogens.

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Dixit R1, Herz J2, Dalton R3, Booy R4.

1. The Children's Hospital, Westmead, Sydney, Australia
2. Biointelect, Sydney, Australia
3. University of Southampton, Southampton, United Kingdom

The authors regret that under section 5: "Polyclonal antibodies for emerging and neglected viral diseases", the line reads: ‘In another study, four H5N1 avian influenza F(ab')2 preparations demonstrated cytopathic effect against cultured Madin–Darby canine kidney (MDCK) cells infected with H5N1 and protected mice against lethal challenge, both given prior (prophylaxis) or post (therapeutic) exposure’ [146]

However, the line should read: "In another study, four H5N1 avian influenza equine F(ab')2 preparations prevented cytopathic effects of H5N1-infection of cultured Madin–Darby canine kidney (MDCK) cells, and protected mice against lethal challenge, both given prior (prophylaxis) or post (therapeutic) exposure".

Likewise… the isolated “1” after reference 167 should not be there,

Likewise, the authors regret that under section 5: “Polyclonal antibodies for emerging and neglected viral diseases”, the line reads: “E. Bola on page 1157 reads : ‘Similar results were obtained from injecting Ebola infected mice with polyclonal sera from E. bola immunised mice [171]’.

However, the line should read: ‘Similar results were obtained from injecting Ebola infected mice with polyclonal sera from Ebola immunised mice [171]’.

The authors would like to apologise for any inconvenience caused.


Dr Rashmi Dixit
rushmi7@gmail.com
4.3 Chapter 3 Synopsis

Benefits of Using Heterologous Polyclonal Antibodies and Potential Applications to New and Undertreated Infectious Pathogens – Synopsis

Heterologous Polyclonal Antibodies have been used for over a century in the pre-antibiotic age to treat influenza and other infectious pathogens, and were subsequently developed for application to neutralise rabies virus, treat toxicities and as antivenin against snake, scorpion and other animal bites. Initial limitations of their use to hypersensitivity reactions to animal proteins have been overcome with modern purification techniques. They are cheaper and more rapidly generated to a range of infectious illnesses than monoclonal antibodies. There has been resurgence in their interest in application to neglected and emerging viral diseases, such as avian influenza, E-bola and Zika virus. For the purposes of this thesis, they may provide an alternative to neuraminidase inhibitors in the event of the predominance of a resistant seasonal strain predominating, or pandemic strains that may be inherently resistant. Proof of concept studies, in cell cultures and in mice, have been promising, and Phase 1 safety studies have been successful. Further development for clinical application is awaited.

These three chapters concluded my examination of the risk posed by oseltamivir-resistance to the general populous, but particularly to those vulnerable to influenza infection. In the subsequent three chapters, I examine three subgroups vulnerable to influenza infection and severity –the elderly, infants and colonised populations – specifically Indigenous Australians.
5.1 Chapter 4 Preamble

A Protocol for a Randomised Controlled Trial of Oseltamivir Treatment and Prophylaxis During Influenza Outbreaks in Aged Care Facilities in the Context of Optimal Influenza Vaccination and Infection Control

Elderly citizens, particularly of aged care facilities (ACFs), are at a heightened risk of acquiring influenza and dying from it. Moreover, communal living arrangements, and sharing of both staff and amenities, promote influenza outbreaks. Aged care facilities are also a potential source of community outbreaks.

The Australian Communicable Diseases Network of Australia guidelines on influenza outbreak management in resident-care facilities advise ‘consideration’, only, of oseltamivir for treatment of cases plus treatment of contacts (prophylaxis) during outbreaks, as opposed to no prophylaxis of contacts. The lack of firm recommendations reflects the paucity of evidence.

In this study, we proposed a cluster-randomised, unblinded, controlled trial of residents and staff of 70-100 ACFs with partnership between various stakeholders: aged care facilities, clinical researchers, general practitioners, government public health bodies, and relevant industry partners. There will be a programme of information sessions held on preventing and detecting possible influenza, and institution of active computerised surveillance. Point-of-care testing will be used to diagnose cases, following screening for influenza-like illness. ACFs will be randomised to receive either oseltamivir treatment of cases, or treatment of cases plus treatment of contacts (prophylaxis). Primary outcome is information on the attack rate of influenza in treatment (T) vs. treatment and prophylaxis (T & P) groups, and hospital admission incidence. Secondary outcome measures will be rates of case fatality, pneumonia rates, adverse events, and influenza outbreak duration.
5.2 Chapter 4

A Protocol for a Randomised Controlled Trial of Oseltamivir Treatment and Prophylaxis During Influenza Outbreaks in Aged Care Facilities in the Context of Optimal Influenza Vaccination and Infection Control


A National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, The Children’s Hospital at Westmead; B Sydney Medical School, University of Sydney; C Health Protection NSW, North Sydney, Australia; D Centre for Population Health at Western Sydney Local Health District; E Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, NSW, Australia; F Marie Bashir Institute for Infectious Diseases and Biosecurity, School of Biological Sciences and Sydney Medical School, University of Sydney, Australia; G Sydney Medical School, University of Sydney; H The George Institute for Global Health; I Western Sydney Local Health District; J South Western Sydney Local Health District.

*Address correspondence to this author at the National Centre for Immunisation, Research and Surveillance, The Children’s Hospital at Westmead, Locked Bag 4001, Westmead, NSW 2145, Australia; Tel: +61 435 752 969; Fax: +61 2 9845 1418; E-mail: rashmid@uni.sydney.edu.au
5.2.1 Abstract:

Background

Influenza outbreaks in aged care facilities (ACFs) affect up to 40% of elderly residents, and involve residents and staff as well as foster influenza transmission to the community. They are a significant cause of hospital admissions and deaths each influenza season. Surveillance for respiratory infections is recommended by various national and international public health bodies and would inform outbreak management. Co-ordinated surveillance activity is sparse. Currently, there are scant data about the role of anti-viral therapy in influenza outbreaks in ACFs on which to base national recommendations. This study has two main purposes: firstly, to examine the impact of oseltamivir, as treatment only, versus treatment and prophylaxis, for residents and staff of ACFs during influenza outbreaks with respect to morbidity, mortality and outbreak size; secondly, to develop and test an enhanced surveillance system, utilising bedside rapid point-of-care tests with laboratory confirmation, for respiratory infection outbreaks in ACFs; both in the context of promoting influenza vaccine uptake and infection control measures.

Methods

This protocol proposes a cluster-randomised, unblinded, controlled trial of residents and staff of 70-100 ACFs with partnership between various stakeholders: aged care facilities, clinical researchers, general practitioners, government public health bodies, and relevant industry partners. Over one influenza season, screening of symptoms to identify influenza-like illness will be followed up by rapid point-of-care tests to diagnose influenza. ACFs will be randomised to receive either oseltamivir treatment in cases or oseltamivir treatment in cases plus prophylaxis in contacts.

Results

The primary outcome measures will be a comparison between the two groups of the attack rate of influenza in treatment (T) vs. treatment and prophylaxis (T & P) groups, and hospital admission incidence within four weeks from onset of Influenza symptoms. Secondary outcome measures will be case fatality rate within 4 weeks from the onset of an influenza outbreak, pneumonia in subjects within 4 weeks from outset of influenza symptoms, adverse events in subjects within 4 weeks of commencing oseltamivir and outbreak duration: date of the onset of the first symptomatic resident in a confirmed influenza outbreak to date of onset of last case.

Conclusions

Our proposed study aims to inform policy decision makers in formulating recommendations for the use of oseltamivir in influenza outbreaks in ACFs. This study presents an opportunity to develop and test active surveillance systems for respiratory and other infections. The overall aim is to prevent and better manage influenza outbreaks in aged care facilities.

Trial Registration: ACTRN12615000638538
5.2.2 BACKGROUND

In developed countries, the highest rates of influenza-related hospitalisation and death occur in people aged more than 65 years who either have comorbidities and/or reside in aged care facilities (1,2). Attack rates of 20-40% have occurred in aged care facility (ACF) influenza outbreaks (3–5).

The proportion of the Australian population aged over 65 years is projected to rise from 14% now to 25% by 2045 [6,7]. The proportion of the population aged over 85 years is projected to increase four-fold from 1.8% in 2011 (approximately 420,000) to 5.0% (approximately 1.7 million) by 2045 (6–9). Chance of residing in an ACF rises with age; currently <1% of those aged 65 years and 15% in those aged 85 years or older reside in ACFs (9). Thus, the health needs of this demographic will require far greater attention and research.

Oseltamivir, a neuraminidase inhibitor that acts to prevent release of viral progeny from host cells and thus avert subsequent infection of uninfected cells, is the antiviral agent of choice against seasonal influenza (10). It is registered for treatment and prophylaxis of influenza, but its efficacy and role are topics of controversy. The most recent Australian CDNA guidelines on influenza outbreak management in resident-care facilities were published in 2009 and updated in 2017 (11,12). Both advise consideration, only, of oseltamivir for treatment and prophylaxis during outbreaks, due to unavailability of evidence on which to base firmer recommendations. The 2017 guidelines defer the decision to individual general practitioners. The 2014 European “Fluresp” guidelines deem antiviral treatment alone as more cost-effective than application of antiviral prophylaxis during influenza epidemics, but did not specify applicability to institutional outbreaks, and this recommendation was within the context of mass population influenza vaccination (13).

A 2014 Cochrane review of published and unpublished randomised, controlled trials concluded that oseltamivir reduced symptoms of influenza by 17 hours in adults and, when used as household prophylaxis, reduced the risk of developing symptomatic influenza, but whether there was a reduction in pneumonia was unresolved, and they found no reduction in bronchitis, sinusitis or otitis media or serious complications (14). They reported an increase in gastrointestinal and psychiatric side effects. The review did not address the role of oseltamivir in institutional outbreaks. The review was criticised for excluding observational data, which, due to ethical constraints, was the main data type collected during the 2009 pandemic, and its findings were controversial.

The UK based Multiparty Group for Advice on Science (MUGAS), presented findings of controlled trials and observational data to the 2014 European Scientific Working group on Influenza, in Riga Latvia. Roche, who manufacture oseltamivir, and who provided some funding for this review, were reportedly given no access to findings prior to presentation. They reported that in adults with confirmed influenza, oseltamivir reduced development of otitis media and pneumonia, reduced hospitalisation and lead to an 18% reduction in mortality. There was an increase in gastro-intestinal adverse events but not in neuropsychiatric events. The role of oseltamivir in residential facilities was not addressed. The same group later published a meta-analysis of published and unpublished
randomised controlled trials in 2015 (15). Their findings conflicted with Cochrane and concurred with their earlier review of both observational and controlled trials. There were fewer lower respiratory tract (LRT) complications (risk ratio RR = 0.56, 44% risk reduction) and hospitalisations (RR = 0.37, 64% risk reduction) in those with laboratory-confirmed influenza who received oseltamivir treatment, and no neuropsychiatric side effects. In those ≥ 65 years, oseltamivir did not reduce symptom duration but did reduce LRT complications. A 2014 meta-analysis by the same group of the observational, individualised data of 30,000 hospitalised patients demonstrated a reduction in mortality in those who received oseltamivir within 48H compared to no oseltamivir in those with laboratory-proven influenza (OR 0.50); oseltamivir started after 48H reduced mortality in those admitted to critical care (OR 0.65) (16). They also performed a 2013 meta-analysis of 25 comparative observational (case series, case-control and cohort studies) and randomized controlled studies, which demonstrated a reduction in mortality (0.35) if NAIs are given within 48H (17). In 2016, Hurt and Kelly, freshly reviewed the literature and concluded that oseltamivir reduces symptoms by up to 1 day, and possibly reduces mortality in severely ill patients; both when given within 48 hours (18).

Booy et al. conducted a cluster-randomised study on oseltamivir for the control of influenza outbreaks in ACFs (3). In 16 ACFs, oseltamivir significantly reduced acute influenza attack rates and outbreak duration when used for treatment of cases and also prophylaxis of contacts, as compared to treatment of cases, alone. The study also demonstrated a trend towards lower staff infection rates, fewer hospitalisations and deaths and less pneumonia when oseltamivir was used as prophylaxis. In contrast, a recent study of 42 nursing homes in which 17 outbreaks were recorded over 2009-2013 demonstrated that oseltamivir prophylaxis was ineffective in preventing influenza or influenza-like illness, but was underpowered with fewer outbreaks than anticipated (19). A retrospective comparison of three different prophylaxis approaches in three separate ACFs during one influenza season showed that prophylaxis did not reduce attack rate (26.4% prophylaxis to all residents vs. 38.3% to direct contacts vs. 18.9% no prophylaxis) (20). Mortality was lower for universal or selective prophylaxis (1.8% all, 1.6% direct contacts) compared to no prophylaxis (9.7%). Hospital admissions were lower in universal prophylaxis (3.6 %) compared to contact (7.8%) or no prophylaxis (16.1%), as was outbreak duration (8 days for universal vs. 14 days for direct contacts and 12 days for no prophylaxis). This was not a randomised study and only one ACF was in each cohort. Given the genuine uncertainty over whether oseltamivir prophylaxis is effective, it is arguable that we have clinical equipoise.

An expanded study of 70-100 ACFs would provide the power needed to confirm benefits of antiviral prophylaxis and more accurately estimate their effects.

Due to their relative immunosuppression, the elderly can shed the influenza virus for longer and at higher levels than younger adults, providing a potential source of infection to staff, visitors and fellow residents (21). Annual influenza vaccination remains the primary method of influenza prevention in the elderly (22). However, the immune response to vaccination is lower in the elderly compared to younger adults and does not always protect against transmission (23,24). Immunisation of ACF staff can provide protection to them and to residents via herd immunity, and reduces transmission of
influenza between ACFs and the community. Data from 2009-2012 demonstrated that in hospital settings in California, for every 15 health care providers vaccinated against influenza, one fewer person in the community contracted an influenza-like illness (25).

The Australian Aged Care Quality Agency require ACFs to incorporate infection surveillance within their infection control programmes, but no specific directives are provided as to the nature of such surveillance systems (26). There is international recognition of the role of influenza surveillance, with the 2014 European “Fluresp” guidelines recommending electronic surveillance systems for early recognition and intervention in influenza epidemics (13). There are currently no systematic surveillance systems for infectious diseases across ACFs in Australia, although the majority of ACFs have indicated that some internal surveillance is carried out (27). Improved surveillance of infectious diseases in this setting can enable earlier outbreak identification and institution of infection control processes plus treatment +/- prophylaxis to limit morbidity, mortality, hospitalisations and health care costs (27). An influenza A outbreak in six ACFs with 324 residents in the Hunter region during 2004 resulted in a 41% attack rate and a mortality rate of 4% among those infected; late recognition and notification of the outbreaks were considered modifiable risk factors (5). The recent study in Sydney ACFs with enhanced surveillance found an annual influenza outbreak incidence of nearly 20%, more than ten-fold greater than routine reporting of outbreaks in NSW ACFs suggested (3). This has been brought into high relief by the occurrence of over 100 separate ACF influenza outbreaks during the 2017 season (28). Improved surveillance data can inform infection control programmes and enable better planning of aged care health services.

This study has dual aims: to investigate the role of antiviral treatment and prophylaxis in ACFs during influenza outbreaks, and to implement a systematic surveillance system with rapid point-of-care testing to identify and intervene during influenza outbreaks. These will be on a background of optimised immunisation and infection control measures.

Funding is proposed to come from a National Health and Medical Research Council partnership grant, with partnership with NSW Health, local aged care facilities and possibly industry as potential partners. This study protocol proposes an ambitious but worthwhile undertaking to clarify the utility of oseltamivir in influenza outbreaks in aged care facilities and to trial a systematic surveillance system for influenza-like illness, which may then be applied to other infectious diseases.

5.2.2.1 Aims

5.2.2.1.1 Primary

- To assess the value of the anti-influenza medicine, oseltamivir, as treatment only, versus treatment and prophylaxis, for residents and staff of ACFs during influenza outbreaks, against a background of best practice of outbreak management
- To develop and test an enhanced surveillance system for outbreaks of respiratory infection in ACFs
5.2.2.1.2 Secondary

- To optimise immunisation uptake and infection control training and practices to reduce incidence and duration of influenza outbreaks
- To collect data about the emergence of drug resistance to oseltamivir

5.2.3. METHODS

5.2.3.1 Study Design

A cluster-randomised trial of oseltamivir use in aged care facilities during influenza outbreaks.

All aged care facilities in the Western Sydney Local Health District and South Western Sydney Local Health District will be approached to participate in the trial. A member of the study team will visit each facility whose management agree to participate, to assess the structural characteristics and the capacity of the facility to engage in the. Where the site is deemed suitable information will be provided to staff, residents and GPs. GPs will be encouraged to maximise staff and resident vaccination. The study will fund staff vaccination in facilities where cost is a barrier to uptake.

5.2.3.2 Participants

- Any person residing in a study ACF in New South Wales (NSW)
- Any staff working in a study ACF in NSW: clinical or non-clinical.

5.2.3.2.1 Inclusion Criteria

- Any resident or staff member from a participating ACF, aged over 18 years, who has been diagnosed with influenza
- Any resident of staff member, aged over 18 years, from a participating ACF who has been in contact with another ACF resident or staff member diagnosed with influenza

5.2.3.2.2 Exclusion Criteria:

- Subjects who have a known hypersensitivity to oseltamivir or any of its components (see Appendix)
- ACFs who do not agree to participate in the study
- Residents or staff who have had symptoms suggestive of influenza for longer than 72 hours, as from then, there is an incremental reduction in the efficacy of oseltamivir
- Staff who are pregnant or currently breastfeeding
- Residents of staff who are known to have acute or chronic renal insufficiency

5.2.3.2.3 Consent

The current standard of care for influenza in ACFs (outlined in the 2009 guidelines) is either to treat only cases with oseltamivir or to treat both cases and contacts to control outbreaks and prevent influenza transmission; the great majority of public health physicians more often only provide
treatment for cases (11). Thus, to scientifically address this question and provide an answer that will inform policy makers one way or the other, a large number of ACFs will be randomised to either treatment of cases only or treatment of both cases and contacts.

Each ACF is a cluster/group, which will be randomised to a particular approach for all its residents and staff, understanding that there will be some patients and their health care practitioners who may decline to be involved.

Agreement to participate occurs at the level of the ACF in cooperation with General Practitioners serving the facility and Nursing Directors of ACFs. Information about the study protocol will be distributed. An information letter to the residents and their next of kin constructed in English and other main language groups in Australia will be distributed to participating residents informing them of the study protocol and how residents and staff in the ACF will be managed in the event of an influenza outbreak.

The conduct of this study does not alter the right of any resident or their health care practitioner to, where applicable, receive, prescribe or dispense oseltamivir. General Practitioners and Public Health Specialists retain full rights to implement, cease or alter any aspect of influenza case or outbreak management.

5.2.3.3 Interventions

This study is designed to test institution of a surveillance system for influenza-like illnesses with application of a specific rapid diagnostic test for influenza (and potentially RSV) infections, as well as the most effective way to utilize oseltamivir in an influenza outbreak: as either T or T & P.

5.2.3.3.1 Surveillance

Active surveillance for an influenza-like illness (ILI) will be carried out, as described below.

ILI definition:

- Acute onset of fever of \( \geq 37.8^\circ C \) (or patient feels hot to touch) OR an acute deterioration in cognitive or physical health or abilities, PLUS
- Acute cough or any other respiratory sign or symptom in a resident or staff member OR worsening of chronic respiratory symptoms.

Elderly patients may not mount a fever in the context of a viral illness and symptoms may be less specific than in younger patients (29). This is taken into account in the ILI definition.

Research staff will visit participating ACFs to explain the study. At each ACF, selected ACF staff members will be designated as ILI surveillance officers for that facility and be trained in active ILI surveillance and in the rapid point-of-care testing (POCT; e.g. SOFIA / QUICKVUE Influenza A+B Test; Quidel Corp., San Diego, CA., USA; or BinaxNOW® Influenza A&B; Alere Inc., Florida, USA see below: “Collection and Testing of Samples”). A recent evaluation of the Sofia Influenza A+B fluorescent immunoassay of 209 adult respiratory samples during the 2013 Southern Hemisphere
influenza season demonstrated that the assay performed well (30). Compared to RT-PCR, the sensitivity and specificity of the Sofia Influenza A+B FIA for detection of influenza A was 72.4% and 98.3%, respectively.

Research nurses will email or text-message each nursing home twice a week leading up to the influenza season and three times a week during the influenza season to ask about any residents exhibiting symptoms consistent with ILI. If an ILI is identified, the ACF will be contacted daily until either an influenza outbreak is identified or until eight days after the last ILI case (five days for duration of infectiousness plus three days for incubation period). When an ILI is identified, POCT will be performed; if this is positive, either a research doctor or a GP will collect confirmatory swabs and prescribe oseltamivir treatment. If an outbreak is identified, the GP and/or PHU staff will collect confirmatory swabs for NAT testing and prescribe oseltamivir treatment +/- prophylaxis. An outbreak management team (OMT) consisting of ACF staff and GPs will be assigned within each ACF experiencing an influenza outbreak, to supervise and coordinate the response. They should meet daily during the outbreak to discuss, delegate and review the infection control and therapeutic activities being undertaken, ensure records are up to date and co-ordinate communication with all agencies. This is as recommended by the 2009 CDNA Australian guidelines regarding influenza outbreak management in resident-care facilities (11).

An attempt will be made to identify the ‘first case’ in each ILI outbreak by establishing the apparent sequence of transmission. The dates of symptom onset for all residents and staff who have an ILI within an ACF will be determined and temporally sequenced. An apparent sequence of transmission is accepted when there were no more than three days between the onset dates of a probable influenza case and the next probable influenza case.

5.2.3.2 Collection and testing of medicine

Nose and throat swabs will be collected from each participating resident and staff member during screening by ACF staff using flocked swabs for rapid point-of-care testing (SOFIA / QUICKVUE Influenza A+B Test; Quidel Corp., San Diego, CA., USA or BinaxNOW® Influenza A&B; Alere Inc., Florida, USA).

Where feasible, a second nasal swab from a sample of patients affected by the outbreak will be collected for nucleic acid testing for influenza virus resistance testing; study staff may assist with this. These will be transported to the laboratory at 4°C in viral transport medium.

Paired serum samples will not be collected to determine rise in influenza antibody titres, given the large number of study subjects.

Laboratory methods

Where feasible, a second nasal swab from a sample of patients affected by the outbreak will be collected for oseltamivir resistance testing; study staff may assist with this. These will be transported to the laboratory at 4°C in viral transport medium.
Nasopharyngeal (NP) swabs (or a throat swab if NP swab were not obtained) will be collected from cases with an ILI by ACF staff using Copan Nylon® Flocked swabs placed in viral transport medium (Universal Transport Medium, UTM™ [Copan Italia, Brescia, Italy]). Swabs are frozen at -80°C within the same day of collection, prior to transport to the laboratory for testing.

Nucleic acid testing will be performed at the Institute for Clinical Pathology and Medical Research (ICPMR) viral laboratory at Westmead Hospital.

Nucleic acid extraction will be performed using the QiagenbioROBOT EZ instrument (Qiagen, Valencia, CA), and amplification carried out using the Roche LC 480 (Roche Diagnostics GmbH, Mannheim, Germany) real-time instrument.

Influenza virus isolation (culture) will be undertaken on MDCK cells. After four days incubation (35°C) the cells are to be stained with fluorescent influenza A & B monoclonal antibodies (SimulFluor FluA/FluB MoAb, Light Diagnostics, Temecula, CA, USA). The WHO Collaborating Centre will perform virus subtyping for Reference and Research on Influenza (Melbourne, Victoria, Australia) on a sub-set of isolates.

Resistance testing

- A rolling-circle amplification method confirmed the presence of the H275Y resistance mutation on selected samples nasal swabs:
  - from start of an influenza outbreak
  - in those whom ILI is diagnosed beyond 1 week after the first case
  - of those receiving prophylaxis who have break through ILI.

5.2.3.3.3 Study medicine

Oseltamivir is currently registered in Australia for treatment and prophylaxis of influenza. NSW Health uses it routinely to manage outbreaks. Roche, Australia may supply the medicine or we will purchase it.

Study staff will prompt ACFs to brief GPs / PHUs of outbreaks, as above, and request that a standing order to dispense oseltamivir for treatment or prophylaxis be enacted.

Treatment of influenza cases by GPs will proceed, as normal. Ethics approval will be sought to request agreement from ACF GPs and Nursing Directors to participate in the study to prescribe and dispense oseltamivir to contacts of cases in those ACFs assigned to the T & P group, as consistent with current guidelines for influenza outbreaks in ACFs (11).

5.2.3.3.4 Influenza Case Definition

- Definite: Positive nasopharyngeal swab for influenza as tested by POCT, direct immunofluorescence, nucleic acid testing or viral culture
- Probable:
ILI epidemiologically linked to confirmed case if either NOT TESTED or NEGATIVE RESULT

Epidemiological linkage is ACF-specific and is determined by the opportunity for contact amongst residents or between staff and residents. For example, an ACF where all residents frequently share facilities such as lounge rooms or outdoor decks may be linked epidemiologically, versus ACFs in which there are distinct geographical wings with no mixing of staff or residents between wings. PHUs will, as per the 2009 CDNA guidelines, assist ACFs in determining and managing an outbreak (11).

5.2.3.3.5 Influenza Outbreak Definition

The definition of an influenza outbreak is derived from the 2009 CDNA guidelines for managing influenza outbreaks in aged care facilities (11).

“Potential influenza outbreak alert:

- Three or more cases of ILI in residents or staff of the facility within a period of 72 hours.

Influenza outbreak:

- Three or more epidemiologically linked cases of ILI in residents or staff of the facility within a period of 72 hours PLUS:
  - at least one case having a positive laboratory test OR
  - at least two having a positive point-of-care test.”

If an ILI is identified, then the interventions are instituted and infection control processes reiterated and documented.

There will be no restrictions on relief medications or other drugs prescribed by clinical staff. Limited data are available regarding drug interactions and oseltamivir (31). The manufacturers of Tamiflu (oseltamivir), Roche®, state that drug interactions are unlikely and that no interactions have been observed with co-administration of paracetamol (32). Booy et al. demonstrated good tolerability of oseltamivir in ACF residents, with no cessation of either treatment or prophylaxis courses due to adverse events (3).

Standing orders for oseltamivir treatment and prophylaxis will be enacted, and ongoing courses prescribed, as follows. If there are no standing orders the OMT coordinator will liaise with GPs covering the ACFs to arrange oseltamivir prescriptions and generate standing orders for execution in the event of an influenza outbreak.

Arm 1: Treatment only Arm

- Oseltamivir for treatment of residents and staff with confirmed Influenza
  - 75mg p.o. b.d. oseltamivir for five days diagnosed within 48 hours of symptoms by POCT or laboratory testing
  - Medication to be given with a snack or at bedtime.
Arm 2: Treatment and Prophylaxis Arm

- Treatment as above
- Prophylaxis of resident and staff in an ACF when an influenza outbreak is identified
  - 75mg p.o. daily oseltamivir for ten days or until outbreak is declared over, whichever is longer
  - Medication to be given with a snack or at bedtime.

Renal impairment and dosage adjustment:

No dose adjustment is necessary for calculated creatinine clearance of > 30 mL/min.

For those with calculated creatinine clearance of 10-30 mL/minute, treatment dose of oseltamivir is 75 mg once daily for five days, and prophylaxis dose is 75 mg every other day for 10 days or until outbreak is over. For those receiving renal dialysis, study medical staff can guide dosing, which may differ depending on type of dialysis (high-dose continuous renal replacement, continuous veno-venous haemodialysis, intermittent haemodialysis).

Residents whose renal function is unknown or in whom a normal serum creatinine is determined receive standard dose of oseltamivir (11).

If respiratory symptoms develop in a resident receiving prophylaxis in the trial, the dose should be changed to treatment dose and the following should be tested for and documented for the case:

- POCT for influenza
- Nucleic acid testing of nasopharyngeal swab for respiratory viruses ('respiratory panel')
- Oseltamivir resistance.

If the local PHU determines that the outbreak has not been contained in a reasonable time frame, then the treatment only arm should be changed to treatment and prophylaxis, and subsequent new cases may be tested for:

- POCT for influenza
- Nucleic acid testing of nasopharyngeal swab for respiratory viruses ('respiratory panel')
- Oseltamivir resistance pre-treatment.

5.2.3.4 Outcomes of Interest

5.2.3.4.1 Primary

- Attack rate of Influenza in treatment (T) vs. treatment and prophylaxis (T & P) facilities
- Hospital admission incidence within four weeks (4/52) from onset of Influenza symptoms.

5.2.3.4.1 Secondary

- Case fatality rate within 4/52 from onset of an outbreak
- Pneumonia in subjects within 4/52 from outset of influenza symptoms
- Adverse events in subjects within 4/52 of commencing oseltamivir
- Outbreak duration: date of the onset of the first symptomatic resident in a confirmed influenza outbreak to date of onset of last case.

5.2.3.5 Sample size calculations

A sample size of 37 ACF per treatment arm would give 90% power at a two-sided alpha of 0.05 to detect a reduction in attack rate from 10% to 3%, assuming an average of 45 residents per ACF and intra-cluster correlation coefficient (ICC) of 0.1.

5.2.3.6 Randomisation Procedure

The unit of randomisation will be at the ACF level; each facility randomised to either arm 1 or arm 2.

There are two types of ACF, stratified based on architecture type, which influence risk of infection transmission (see below). Within each stratum, participating ACFs are randomised to one of the two arms of the trial.

Type A: dormitory style ACFs with shared toilets and bathrooms (these facilities tend to be ‘more crowded’)

Type B: ACFs with mostly single rooms: some shared facilities, staff and resident movements.

Cluster-randomisation will be in a 1:1 ratio to each arm. Computer-generated random numbers will be elicited by a research partner who is not involved in the recruitment or assessment of participants.

5.2.3.7 Blinding

Neither ACF nor study-staff are blinded once to study group allocation. There will be no placebo used.

5.2.3.8 Data collection

Data for each ACF will be collected and recorded in Excel.

- Treatment arm
- Category of ACF
- Bed numbers / assisted living unit numbers.

Frequency of contact with ACFs is described above (see “Surveillance”).

Surveillance phone calls/emails to each ACF will document the date, contact person, phone call number (1st, 2nd etc.), incidence and total numbers of any staff / resident with ILI, date of onset, and results of rapid POCT.

Data will be collected on a study form from any recruited resident and staff and with a daily follow up form for each participant, staff or resident. Both initial data and follow up data will be recorded on Excel for each resident receiving treatment or prophylaxis.
Information about serious adverse events (SAE) will be collected throughout the trial by the study team members and assessed by the chief investigators and reported using the SAE initial and follow-up forms in a timely fashion e.g. on the day of reporting of the SAE. These will be updated with outcome, medical history, results of investigations, copy of hospital reports and discharge summaries. The chief investigators will assess any causal relationship between the SAE and oseltamivir use.

Data for each participant will be collected and recorded (Appendix). Information gathered will be identifying details- de-identified by re-identifiable, demographic data, geographical location within ACF, background medical history / comorbidities / immunisation status, current respiratory symptoms and signs, systemic symptoms, point-of-care and laboratory data, isolation date, oseltamivir regimen and any adjustments made, adverse events, outcome (resolution / hospitalisations / complications / mortality). Only data that pertains to the outcomes of interest, and which allows comparison of baseline demographic characteristics between the two arms of the study, will be collected.

5.2.3.9 Statistical methods

To compare study group baseline characteristics of ACFs, two-sample t-test for continuous data with normal distribution will be used.

5.2.4. FUNDING

A partnership project grant from the National Health and Medical Research Council (NHMRC) is proposed to request funding for this study, with Health Protection NSW, local PHUs, GPs serving ACFs, industry collaborators such as Quidel and Roche as proposed partners, if they agree. This study fits the aims of the NHMRC partnership project grant very well, of supporting research that informs health policy or practice and service delivery (use of oseltamivir in influenza outbreaks in ACFs), of improving programmes currently in place (surveillance and outbreak management), of evaluating new approaches (rapid point-of-care testing for influenza outbreak identification), and of studying knowledge exchange (information sessions and electronic surveillance databases) in a community setting (ACFs) (27).

We aim to apply for funding to pay for staff salaries, laboratory services and transport and equipment costs.

5.2.5. DISCUSSION

This study of oseltamivir treatment of cases versus treatment of cases plus prophylaxis of controls for influenza in staff and residents of ACFs will help bridge a gap in the evidence deficit for the role of oseltamivir in these contexts. It poses several challenges, but is an opportunity to inform best practice for prevention of influenza outbreaks in this vulnerable population.

The study requires coordination and cooperation between several different agencies, all of which are stakeholders in the outcome of interest. This is a logistical challenge for the study staff, and will require tight adherence to screening and follow up schedules. The study team are highly experienced
in administering such studies and have already published a similar but smaller study of oseltamivir in ACFs (3).

The lack of blinding introduces a potential source of bias. This is mitigated somewhat by the application of a definition for ILI and deployment of a rapid point-of-care test. The definition itself has been broadened to take into account the non-specific nature of symptoms of ILI in the elderly. For example, those without a fever who have other signs of clinical deterioration will be screened; this relies on ACF staff observations, which can limit data quality. Leading up to the commencement of the study, education sessions will be conducted by study staff to train ACF staff in how to screen patients and conduct rapid POCTs. A selection of cases with rapid POCT will be confirmed by laboratory-based testing, and provide information on the point-of-care test sensitivity and specificity in this context.

Calculating sample size in order to generate enough statistical power relies on some assumptions about influenza outbreak incidence in ACFs. However, the severity of any given influenza season is variable and not always predictable based on outcomes of the preceding influenza season in the other hemisphere. Therefore, there remains a degree of uncertainty in sample size calculations to generate statistical power to draw meaningful conclusions.

5.2.6. CONCLUSION

Our proposed study of the role aims to inform policy decision makers with formulation of recommendations for the role of oseltamivir during influenza outbreaks in ACFs. This study presents an opportunity to develop and test active surveillance systems for respiratory infections, which may in time be applied to other infections e.g. gastrointestinal. Both of these aims will be conducted in the context of optimising immunisation uptake and infection control measures. The overall aim is to prevent and better manage influenza outbreaks in aged care facilities.

CONFLICT OF INTEREST

Leon Heron and Robert Booy have received funding from Baxter, CSL, GSK, Merck, Novartis, Pfizer, Roche, and Sanofi Pasteur for the conduct of sponsored research, travel to present at conferences or consultancy work; all funding received is directed to research accounts at The Children’s Hospital at Westmead. The other authors have declared no conflict of interest in relation to this work.

ACKNOWLEDGMENTS

The authors would like to acknowledge Dr Jen Kok for advice about laboratory management of collected specimens.
5.2.7 References


29. Hayward AC, Watson J. Effectiveness of influenza vaccination of staff on morbidity, and mortality of residents of long term care facilities for the elderly. Vaccine [Internet]. 2011 Mar


5.2.8 Appendix: Data Collection for study participants

- Patient identifier
- Staff or Resident
- DOB
- Gender
- Ethnicity: Aboriginal and Torres Strait Islander Peoples/ Anglo-Celtic/Other European/Asian-Indian Subcontinent/Asian-South or South East Asian/South American/Middle Eastern/African
- Room occupied (Resident) or main work area (Staff)
- Comorbidities/Medications
- Flu Vaccine status last 3 years
- Pneumococcal Vaccine status
- ILI onset date / measured T / respiratory signs and symptoms/ duration (any of the below recorded in space)
  - Sore throat
  - Cough
  - SOB
  - Sneezing
  - Increased RR
  - Chest pain
  - Sputum
  - Blocked or runny nose
  - Systemic signs and symptoms
    1. Fatigue
    2. Myalgia
    3. Headache
    4. Chills
  - Changes in mentation
- POCT result
- Lab data:
  - Date of diagnosis
  - Result of
    1. Quickvue POCT/SOFIA
    2. DIF (from index or sample cases in outbreak)
    3. NAT PCR (from index or sample cases in outbreak)
    4. Viral culture (from index or sample cases in outbreak)
    5. Complement fixing antibodies titres (from index or sample cases in outbreak)
- Date when Resident Isolated OR Last Working Day of Ill Staff Member
- Oseltamivir Dose
• Adverse events
  o Nausea
  o Vomiting
  o Diarrhoea
  o Abdominal pain
  o Headache
  o Light headedness / dizziness
  o Rash
  o Insomnia

• Patient on prophylaxis:
  o date developed respiratory symptoms
  o date changed to treatment dose
  o POCT result

• Hospitalisation:
  o Date of hospitalisation
  o Duration
  o Treatment
  o Outcome

• Pneumonia
  o Diagnosis date
  o Treatment
  o Outcome

• Death
  o Date

• G.P. visits
5.3 Chapter 4 - Synopsis
A Protocol for a Randomised Controlled Trial of Oseltamivir Treatment and Prophylaxis During Influenza Outbreaks in Aged Care Facilities in the Context of Optimal Influenza Vaccination and Infection Control

This cluster-randomised study protocol of the role of antiviral medication in outbreak prevention in aged-care facilities remains unpublished as details of the consent processes are being resolved. A prospective observational study similar to this is being conducted in Aged Care. As I elaborate in the thesis discussion, the difficulty is in obtaining consent from potentially large numbers of patients in order to compare the two treatment arms, and the requirement for such specific consent beyond that obtained routinely by a treating doctor, given that both options are “on protocol”.

Extremes of age both confer vulnerability to seasonal influenza. Influenza causes the highest rates of morbidity and mortality in the elderly, but infants are also vulnerable (3). In my next chapter, I present a pharmacological observational study of the pharmacokinetics and pharmacodynamics of oseltamivir in infants in the intensive care unit.
6.1 Chapter 5 Preamble
Pharmacokinetics of Oseltamivir in Infants Under the Age of One Year

Infants are a particular risk group for influenza, with high rates of morbidity and mortality compared to the general population. Infants admitted with influenza to critical care facilities are often administered oseltamivir. There is a paucity of pharmacokinetic data for oseltamivir use in infants under one year of age. The recommended dose by the Communicable Diseases Committee of the USA has been extrapolated from adult data. This may or may not be appropriate, given differences in developmental physiology of infants affecting parameters such as absorption, volume of distribution and clearance. Before this series, only one data set was published by Kimberlin et al, which endorsed the use of 3.0 to 3.5 mg/kg/dose in infants (2).

This study undertook to confirm these recommendations by analysing pharmacokinetic data from infants who received oseltamivir. We sampled blood at set time points post oseltamivir administration of infants admitted to critical care facilities and reported oseltamivir and it’s metabolite levels and adverse events.
Pharmacokinetics of oseltamivir in infants under the age of 1 year

Rashmi Dixit1,2*, Slade Matthews2, Gulam Khandaker1, Karen Walker1,2, Marino Festa1,2 and Robert Booy1,2

Abstract

Background: Oseltamivir is the only antiviral treatment recommended for influenza in young children over the age of 1 year. There is scant data on oseltamivir pharmacokinetics (PK) in infants <1 year. We set out to perform PK measurements in infants who received oseltamivir.

Methods: This study was a prospective, uncontrolled, open label evaluation of the pharmacokinetics of oseltamivir metabolism, safety of oseltamivir, viral clearance in infants <12 months diagnosed with influenza by nasopharyngeal influenza nucleic acid antigen test (NAAT). Blood levels of the prodrug oseltamivir and its active carboxylate were measured prior to a dose of oseltamivir and at 4 time points afterwards, to calculate C_{max} (ng/mL), T_{max} (h), AUC_{0-t} (ng h/mL) and time for AUC (h).

Results: Four children with influenza A received oral oseltamivir, 2.35–3 mg/kg/dose. This dose range produced a target oseltamivir carboxylate plasma concentration in excess of the proposed 12-h target AUC of 3800 ng h/mL, selected from earlier studies to avert resistance. One patient developed GIT adverse event: dry retching.

Conclusion: Oseltamivir was well tolerated at a dose of 2.35–3 mg/kg/dose twice a day in infants under the age of 1 year. In general agreement with earlier data, these doses produced a target oseltamivir carboxylate plasma exposure in excess of the proposed 12-h target exposure of AUC equal to 3800 ng h/mL in two patients. The limited plasma concentration data in the remaining two patients were not inconsistent with the target exposure being reached.

Keywords: Oseltamivir, Infants, Influenza, Paediatrics

Background

Infants and young children are particularly prone to influenza morbidity [1–3]. Influenza morbidity in young children and infants ranges from school absenteeism to acute respiratory distress requiring hospitalisation, and can result in death from complications [1]. Oseltamivir is currently the only antiviral treatment recommended in young children, usually for those aged 1–5 years [4–6]. It inhibits the envelope protein neuraminidase, blocking release of viral progeny from infected cells, preventing subsequent entry into uninfected cells [7]. If commenced within 48 h of symptom onset, oseltamivir reduces both duration and complications of influenza [8, 9], although some dispute this [10, 11]. In December 2012, the use of oseltamivir for influenza treatment, but not for prophylaxis, was approved by the FDA for infants as young as 2 weeks, previously having temporary approval for use in infancy during the 2009 pandemic, from April 2009 to June 2010 [12, 13]. Routine use of oseltamivir in infants <1 year of age has been limited by both a lack of pharmacokinetic (PK) data and concern about adverse events [14–17].

The ontogeny of pharmacokinetic functions has potential dosing implications in infants [18, 19]. Oo et al. proposed a dose of 2–3 mg/kg in infants 6–12 months of age, given that renal and hepatic clearance of oseltamivir adjusted for body surface area reach adult levels by 6–9/12 of age [20]. The only known published data regarding oseltamivir pharmacokinetics in infants <1 year old is by Kimberlin et al. [21]. They recommended doses of 3.0 mg/kg twice a day (BID) for infants less than 8 months old, and 3.5 mg/kg BID for
Method

Study population
Infants aged <12 months who warranted treatment with oseltamivir for influenza-like illness were included. The Sydney Children’s Hospitals Network Human Research Ethics Committee provided ethics approval (approval number: HREC/10/CHW/61). All patients or caregivers signed informed consent forms.

Study design and end points
This study was a prospective, open label evaluation of the pharmacokinetics of oseltamivir metabolism, safety of oseltamivir, viral clearance. The oseltamivir dose prescribed was at the attending clinician’s discretion.

Pharmacokinetic analysis
Specific recommendations were made for the timing of blood samples to measure levels of oseltamivir and oseltamivir carboxylate. However, to minimize the number of tests and patient discomfort, samples were collected at the same time as clinically required samples, whenever possible. Recommended times of sample collection were within 15 min prior to an oseltamivir dose, 1 h ± 15 min, 2–3 h, 5–7 h and 10–12 h post dose. The blood volume required for plasma level determination was 500 µL. Blood was collected into a sodium fluoride/EDTA collection tube, placed on ice and centrifuged (1500 g at 4 °C for 10 min). Plasma was stored at −70 to −80 °C before despatch to the laboratory (PRA Early Development Services, Inc. Kansas, USA). Oseltamivir and oseltamivir carboxylate concentrations were determined by high-performance liquid chromatography with tandem mass spectrometric detection [18].

We adopted a desirable target exposure value proposed by Kimberlin of an AUC12 of 3800 ng h/mL. For computational purposes, concentrations at t = −15 min were taken as concentration at zero time. Non-compartmental analysis was conducted using PKSolver, a published pharmacokinetic analysis Excel plugin [26] to obtain estimates of exposure including AUC0–t and Cmax. A set of 5 time-points from zero to 10 h in the 6th dose cycle was available for two of the four patients while for the other two patients only two time-points were available each, for one patient in the 7th dose-cycle and for the other patient in the 8th dose-cycle. It can be assumed that the patients were at steady-state by this time hence the sparsely sampled data may still give an impression of the exposure to oseltamivir carboxylate in these patients.

Virological analysis
Each nasopharyngeal swab or aspirate was obtained using a sterile synthetic tip swab, with a plastic or aluminum shaft, and inserted into vials containing sterile viral transport medium. These were collected at treatment initiation and analysed at the Children’s Hospital, Westmead; Sydney, Australia. Influenza was diagnosed and strain type determined using nucleic acid amplification testing (NAAT) by polymerase chain reaction (PCR).

Safety evaluation
An adverse event was defined as any untoward medical occurrence in a patient which may or may not have a causal relationship with the administered oseltamivir. The following biomarkers were assessed during oseltamivir treatment and compared to pre-treatment levels: serum creatinine, electrolytes, liver transaminases (AST, ALT), alkaline phosphatase, total bilirubin level and full blood count.

Results
Four children received oral oseltamivir: three at 3 mg/kg twice a day (bd) and one at 2.35 mg/kg bd.
All four patients were infected with influenza A, patients 1–3 were H1N1 and the strain was not documented for the patient 4.

The following table presents the pharmacokinetic parameters for oseltamivir in these four patients (Table 1).

From the AUC0–t estimates, the first two patients (1 and 2) attained oseltamivir carboxylate plasma concentrations in excess of the proposed 12-h target AUC value for antiviral therapy during the 0 to approximately 10-h period; it can be surmised that a 0–12 h AUC exposure value would also be in excess of the proposed therapeutic target given these AUC0–t values (Fig. 1). The two patients with only two samples per dose cycle were exposed to 1747 and 2156 ng h/mL for periods of 2.3 and 6.5 h, respectively. These levels of exposure are not inconsistent with adequate oseltamivir carboxylate exposure sufficient to provide effective therapy given the proposed target AUC12 of 3800 ng h/mL, but further plasma time points would have allowed for confirmation.
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age months (m) and days (d)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Dose (mg/kg/dose)</th>
<th>Dose cycle</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0–t&lt;/sub&gt; (ng h/mL)</th>
<th>Time for AUC (h)</th>
<th>No. plasma samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 m, 23 d</td>
<td>F</td>
<td>9.05</td>
<td>3</td>
<td>6</td>
<td>772</td>
<td>9.4</td>
<td>6376</td>
<td>0–9.4</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>5 m, 13 d</td>
<td>F</td>
<td>9.30</td>
<td>3</td>
<td>6</td>
<td>1960</td>
<td>5.8</td>
<td>18,816</td>
<td>0–10</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>11 m, 15 d</td>
<td>F</td>
<td>5.69</td>
<td>3</td>
<td>7</td>
<td>443</td>
<td>3.7</td>
<td>1747</td>
<td>3.7–6</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3 m, 13 d</td>
<td>M</td>
<td>6.43</td>
<td>2.35</td>
<td>8</td>
<td>466</td>
<td>11.5</td>
<td>2156</td>
<td>5–11.5</td>
<td>2</td>
</tr>
</tbody>
</table>
All patients recovered from acute influenza during their intensive care unit admissions. One infant suffered an adverse event: self-limited dry retching (Table 2).

**Discussion**

Our results are consistent with the proposition that a dose of 2.35–3 mg/kg produced a target oseltamivir carboxylate plasma concentration in excess of the proposed 12-h target AUC of 3800 ng h/mL. Oseltamivir is well absorbed from an early age. Animal studies demonstrate a rapid increase of the transport protein at birth, and a widespread distribution for oseltamivir including good penetration of lung tissue, the middle ear and the nasal mucosa [27]. It is metabolized to the active metabolite oseltamivir carboxylate by the liver carboxylesterase HCE1 [19, 27]. Production of HCE1 is lower in foetuses than in infants <1 year of age, who in turn have lower gene transcription than children 1–10 years [18]. However, much inter-individual variability exists, particularly in the younger age groups. Young children have greater proportionate extracellular fluid and thus a greater volume of distribution (VD) of oseltamivir carboxylate, resulting in a lower circulating plasma concentration compared to older children and adults [27]. Oseltamivir carboxylate is not extensively protein bound and, thus, immaturity of plasma protein levels does not impact on VD [27]. Animal studies indicate good penetration of oseltamivir carboxylate into respiratory tissues [19]. Oseltamivir has been linked to neuropsychiatric side effects in children and young adults, especially in Japan, although it is unclear whether the encephalopathy was induced by influenza or by its treatment [28, 29]. Both rat and human foetus studies showed certain central nervous system (CNS) efflux pumps to be in low numbers at birth and increase with age, whilst others are present from the second trimester [19]. There was, however, no accumulation of oseltamivir carboxylate in the brains of healthy rats. Oseltamivir is filtered and actively excreted from the renal tubules using OAT transporter proteins [27]. Clearance function of these proteins is low at birth and increases over the first year of life, which may lead to reduced oseltamivir clearance in neonates [27]. Oo et al. demonstrated that oseltamivir carboxylate clearance adjusted for body surface area (BSA) reached adult levels by 6–9 months of age, whilst a higher BSA-to weight ratio in those 1–2 years resulted in higher clearance and consequently lower peak plasma concentration (C\text{max}), time to reach C\text{max} (T\text{max}) and AUC compared to those 3–5 years.

**Table 2 Adverse events (AE) in children receiving oral oseltamivir**

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age months (m) and days (d)</th>
<th>Comorbidities</th>
<th>Clinical AE</th>
<th>Laboratory changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 m, 23 d</td>
<td>Tetralogy of Fallot</td>
<td>D1 Dry-retching</td>
<td>Day 1 bloods: raised creatinine (48 mmol/L) and AST (66 mmol/L) Attributed to cardiac condition Normalised after frusemide dose during course of oseltamivir</td>
</tr>
<tr>
<td>2</td>
<td>5 m, 13 d</td>
<td>Albright's osteodystrophi Hypothyroidism Hypoparathyroidism GORD Severe OSA due to epiglottis dystrophy</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>11 m, 15 d</td>
<td>Streptococcus pneumoniae bacteraemia, pneumonia, meningitis Parainfluenza 3/rhinovirus/enterovirus co-infection Developed HUS</td>
<td>Nil</td>
<td>Baseline bloods normal D2 of oseltamivir: rising creatinine, urea, AST/ALT/GGT All parameters normalised 10 days after first dose Laboratory abnormalities attributed to HUS</td>
</tr>
<tr>
<td>4</td>
<td>3 m, 13 d</td>
<td>Exomphalhmos</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

GORD gastro-oesophageal reflux disease, OSA obstructive sleep apnoea, HUS haemolytic uraemic syndrome
Kimberlin et al. achieved their target AUC with doses of 3 mg/kg in those up to 9 months of age whereas those 9–11 months of age required a higher dose of 3.5 mg/kg, due to greater oseltamivir carboxylate clearance over the first year of life [21]. Thus, oseltamivir clearance may peak around 12 months, and then reduce after 3 years.

There was one GIT side effect (dry-retching) from oseltamivir in our cohort. Laboratory anomalies were attributable to comorbidities. Likewise, a dose of 3–3.5 mg/kg of oseltamivir was well tolerated in 87 infants with no premature drug discontinuation [21]. Of eight adverse events (AE) deemed related to oseltamivir (9.1 %), five were emesis, two developed a rash and one developed a serious AE: cutaneous hypersensitivity. There were no CNS AE. In another trial, 11 infants who received a rather high median dose of 5.5 mg/kg/dose of oseltamivir suffered no serious adverse events, and all completed the course [30]. Two developed a rash, two gastrointestinal side effects and three had transiently raised liver transaminases that normalised within 2 weeks of completing therapy. In a report of 35 patients <1 year of age who received oseltamivir, no AE occurred and no effect on liver function was detected [31]. In a report of 5 premature infants, mean gestational age 31 weeks, who received oseltamivir at 2–3 mg/kg/dose, there were no treatment related AE [32].

Conclusion
Oseltamivir was well tolerated at a dose of 2.35–3 mg/kg/dose twice a day in infants under the age of 1 year. These doses were confirmed to produce a target oseltamivir carboxylate plasma exposure in excess of the proposed 12-h target exposure of AUC 3800 ng h/mL in two patients and the limited plasma concentration data in the remaining two patients were not inconsistent with the target exposure being reached.

Authors’ contributions
RD, GK, and RB contributed to the design of the study. RD, MF, GK and KW contributed to data acquisition. RD and SM performed pharmacokinetic analysis and data interpretation. RD, GB, RB and SM all contributed to writing of final manuscript. All authors read and approved the final manuscript.

Acknowledgements
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Competing interests
The authors declare that they have no competing interests. Authors Booy and Khandaker have worked on unrelated projects funded by Roche in the past but have not received any form of remuneration or in kind support from the company. Author Festa has received funding for Intensive Care Unit (ICU) equipment unrelated to this study.

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6.3 Chapter 5 Synopsis
Pharmacokinetics of Oseltamivir in Infants Under the Age of One Year

This study of oseltamivir pharmacokinetics in infants, only the second published data, revealed results consistent with the recommended doses. It was hampered by a low number of recruits and data points. However given the difficulty of conducting such a study in infants with the requirement to collect blood, barriers in caregiver acceptability and sparseness of available recruits, it is likely it will not be oft repeated, making it an important second source of published data on infant oseltamivir pharmacokinetics. The ontogeny of infant physiological processes that absorb, metabolise and clear medications requires caution with assumptions that adult doses are extrapolatable to infants. Doses of 2.35–3 mg/kg produced a target oseltamivir carboxylate plasma concentration in excess of the proposed 12-h target AUC of 3800 ng h/m. Given the paucity of other treatment modalities, and the lack of influenza vaccine uptake in pregnant women, this data helps to generate clinical confidence in use of oseltamivir in this age group. Whilst numbers were small, the side effect profile was as expected – one infant had gastrointestinal side effects, and no other infants suffered adverse events. All infants recovered from influenza. This data may form part of a meta-analysis, were other data to be collected.

The final chapter looks at another demographic group that is particularly at risk of influenza – Indigenous Australians. We examine the impact of the intersection of chronic conditions and Indigenous status on influenza incidence and severity, and, given the recognised vulnerability of extremes of age, we age-standardised our analysis.
7.1 Chapter 6 Preamble

The Role of Chronic Diseases in Influenza Incidence and Severity Differential Between Indigenous Australians and Non-Indigenous Australian Populations During the 2009 Influenza Pandemic in Australia – Preamble

Along with extremes of age, those with chronic diseases and Indigenous Australians are at risk of influenza disease and severity. This data analysis examined the role played by the higher background prevalence of chronic, non-communicable diseases amongst Indigenous Australians in the greater incidence of severe influenza recorded amongst Indigenous Australians during the 2009 ‘swine flu’ pandemic. The ‘Close the Pap’ Indigenous Health campaign addressed the health and life expectancy gap Indigenous Australians face; this analysis addressed the intersection between higher rates of communicable and non-communicable diseases that Indigenous Australians are vulnerable to.

We conducted the first analysis of national data sets to examine influenza notifications, hospitalisations, intensive care unit admissions and deaths by Indigenous status, and by presence of a one of five common background conditions: chronic lower respiratory conditions, renal disease, cardiac disease, diabetes mellitus and obesity, amongst the four states and territories that we were given data access to: Western Australia, South Australia, Northern Territory and Queensland. We compared influenza rate ratios within the total population and sought to deduce if comparing influenza rates amongst those with a given chronic condition ameliorated the disparities in influenza incidence and severity between Indigenous and non-Indigenous Australians.
7.2 Chapter 6

The Role of Chronic Diseases in Influenza Incidence and Severity Differential Between Indigenous Australians and Non-Indigenous Australian Populations During the 2009 Influenza Pandemic in Australia

Dixit, Rashmi ¹; Webster, Fleur²; McIntyre, Peter ¹; Menzies, Robert ².
¹. University of Sydney - National Centre for Immunisation Research and Surveillance, Children’s Hospital Westmead
². University of New South Wales

7.2.1 ABSTRACT

Introduction
The 2009 H1N1 influenza pandemic (influenza A(H1N1)pdm09) disproportionately impacted Indigenous Australians. Indigenous Australians are also affected by a health gap in chronic diseases, which are associated with influenza severity. We hypothesised that the disparity in influenza severity is accounted for by the chronic disease health gap.

Methods
We analysed influenza data from South Australia, Western Australian, Queensland and the Northern Territory. We calculated population prevalence of chronic diseases in Indigenous and non-Indigenous Australian populations using nationally collected health survey data, reported to the Australian Department of Health. We compared reported influenza case notifications, hospitalisations, intensive care admissions and deaths in the total population of Indigenous and non-Indigenous Australians ≥ 15 years of age. We accessed age-specific influenza data reported to the Australian Department of Health National Incident Room during the 2009 swine flu influenza pandemic, classified by Indigenous status and stratified by presence of one of 5 chronic conditions: chronic lower respiratory conditions, diabetes mellitus, renal disease, cardiac disease and obesity. We calculated age-standardised rate ratios and confidence intervals in those ≥ 15 years.

Results
Chronic diseases are more prevalent in Indigenous Australians. Influenza notification rates were higher in Indigenous Australians and more frequent across all indices of severity. Restricting analysis to those with chronic diseases did not lower the Indigenous : non-Indigenous influenza rate ratios. Severity analysis did not demonstrate a reduction in Indigenous : non-Indigenous rate ratios as influenza became more severe within any of the chronic disease sub-populations.

Conclusions
Greater prevalence of comorbid chronic conditions was not demonstrably responsible for higher rates of influenza in Indigenous Australians compared to non-Indigenous Australians. Data limitations
included lack of comparison to those with no chronic conditions, and those with more than one. Social, cultural and environmental impacts of colonisation may warrant further investigation as causes of the disparity in influenza rates and severity between Indigenous and non-Indigenous Australians.

7.2.2. INTRODUCTION

The 2009 H1N1 influenza pandemic (influenza A(H1N1)pdm09) disproportionately impacted indigenous populations in colonised countries globally, including Australians Aboriginal and Torres Strait Islander peoples (Indigenous Australians) (1–15). Indigenous populations in colonised countries around the world have a greater burden of chronic, non-communicable diseases compared to their non-Indigenous counterparts (3,16–20). Both seasonal and pandemic influenza affect those with chronic diseases more frequently and severely (5,12,21–28). These observations beg the question: could the greater influenza A(H1N1)pdm09 disease burden and severity in indigenous populations be due simply to the larger burden of background chronic diseases? Very few studies have examined the role of chronic, non-communicable diseases with respect to incidence versus severity of infection including influenza in indigenous populations (29).

While the pandemic strain appeared almost 10 years ago, the question is still relevant. Influenza A(H1N1)pdm09 continued to be a predominant circulating strain up until and including the 2017 influenza season (30–32). Moreover, strains with pandemic potential continue to emerge, with the potential to cause greater disease severity amongst minority First Peoples populations (33,34). The nature of interactions between acute influenza, Indigeneity and chronic diseases during the 2009 pandemic are also likely to be applicable to other seasonal influenza strains.

If higher rates of influenza infection in Indigenous versus non-Indigenous people are largely or solely due to the higher prevalence of chronic non-communicable diseases, then this disparity would be largely or completely eliminated by comparing infection rates only amongst those with chronic conditions. Further, if chronic diseases predispose patients to more severe influenza disease, then the increasing disparity between Indigenous and non-Indigenous people with increasing influenza severity would be largely or solely eliminated by comparing influenza disease rates only amongst those with chronic disease.

Acute surveillance conducted nationally in Australia during 2009 has provided us with datasets that enable analysis of this issue. National influenza notification rates reflect the incidence of influenza infection, while hospitalisation, Intensive Care Unit and mortality data reflect the incidence of progressively more severe outcomes, notwithstanding the limitations of these sources. Active follow up provided Indigenous status on individual cases.
The aim of this study was to determine whether the higher incidence of infection and severity of influenza A(H1N1)pdm09 in Indigenous compared to the general Australian population could be reduced or eliminated by adjusting for chronic disease prevalence.

7.2.3 METHODS:

Population prevalence of chronic diseases
Prevalence data for chronic conditions were obtained by analysing the Confidentialised Unit Record Files of health surveys conducted by the Australian Bureau of Statistics (ABS). For Indigenous Australians these were from the 2012-13 National Aboriginal and Torres Strait Islander Health Survey (NATSIHS) (35). This was collected between April 2012 and February 2013 across 5,000 private dwellings across Australia. Prevalence data for non-Indigenous Australians were from the 2011-12 National Health Survey (NHS), conducted between 6 March 2011 and 17 March 2012 from a sample of 15,500 private dwellings across Australia (36). Confidentiality rules set by the ABS meant that the results obtained represented both Indigenous and non-Indigenous peoples. Given that the population of Indigenous Australians is < 3%, NHS chronic disease prevalence data for all Australians was used to approximate the prevalence of chronic conditions in non-Indigenous Australians (37). Only data for those ≥ 15 years was available from each survey, therefore our analysis was limited to this age group. Within the health surveys, all reported long-term medical conditions are coded to a classification developed for use in the ABS Health Surveys based on the 10th revision of the International Classification of Diseases and Health Related Problems (ICD-10) – table 1 (38).

All data were self-reported except obesity, which was taken from measurements. Respondents were asked whether they have been diagnosed with the condition, and whether the condition is current and long-term, and were classified with a chronic disease if they answered positively to both questions.

Percentage prevalence of each condition in both the NetEpi and the health survey data was stratified by the following age groups for each state and territory: 15-24 years, 25-34 years, 35-44 years, 45-54 years, and ≥ 55 years. The survey percentages were already adjusted using weightings for age, sex and state / territory, provided by the ABS to adjust for differences between survey and census populations (37).

Influenza laboratory notifications, hospitalisations, ICU admissions and deaths
We obtained data on influenza cases during the 2009 ‘swine flu’ pandemic from the Communicable Diseases Network of Australia (CDNA), a division of the Department of Health and Aging (DoHA). Influenza surveillance data were actively collected during the 2009 pandemic, under the provisions specified within the National Health Agreement and state and territory public health acts. Cases of laboratory-confirmed Influenza A(H1N1) infections, hospitalisations, ICU admission and deaths were collected by states and territories from general practitioners, hospitals and laboratories and reported to the National Incident Room (NIR) of the Department of Health and Ageing, who entered the data
onto the NetEpi database, a web-based outbreak case reporting system. Data from 15 years of age was requested from the CDNA, in order allow NetEpi data to match the age-range of the people surveyed for chronic conditions. Data from 1 April 2009 to 31 December 2009 were released to us and examined.

Indigenous status was recorded by self-reporting to health professionals. If a patient was unable to answer questions e.g. receiving artificial respiratory ventilation, or if they had died, their next of kin was asked about the Indigenous status. ‘Non-Indigenous Australians’ included those in whom Indigenous status was recorded as ‘not Aboriginal or Torres Strait Islander peoples’. Data in which Indigenous status was not specified was omitted from this analysis.

The exact definitions applied to the chronic conditions were not available from NetEpi, and may have differed from that of the health surveys, specified above. The CDNA NetEpi database was unable to provide data on influenza cases without any background chronic conditions. We could not calculate these by simply subtracting cases with a chronic condition, as a case may have had more than one chronic condition.

7.2.4 STATISTICS:

The analyses that were conducted were limited by data availability. Data on all relevant variables – Indigenous status, chronic disease prevalence, state/territory and influenza - for hospitalisations, ICU admissions and deaths were available only from Western Australia (WA), Queensland (QLD), South Australia (SA) and the Northern Territory (NT). This represents 57.5% of Aboriginal and Torres Strait Islander peoples within Australia. Data on influenza notifications were available only from WA and NT. We combined data from states and territories where equivalent data were available to reduce size of confidence intervals and thus increase statistical significance.

To obtain estimated population numbers for each chronic condition, we multiplied the percentage prevalence of each chronic condition reported within the health surveys for each age group, by the total population numbers, obtained from the 2011 ABS census.

For rates of influenza within the total population and within each chronic disease subset, we used reported total cases of influenza for each state / territory as the numerator and ABS census population data as the denominator. We derived a ratio of the actual number of Indigenous influenza cases observed by the expected number of cases - the latter being derived from the rates observed in the non-Indigenous population.

We calculated rate ratios for influenza notifications, hospitalisations, ICU admissions and deaths. These rates were then used to calculate Indigenous: Non-Indigenous rate ratios (RRs) and 95% confidence intervals. We performed direct age-standardisation of the influenza case data and
calculated 95% confidence intervals using the method described by Armitage and Berry (39). We defined lack of statistical significance as overlapping confidence intervals – a conservative indicator of statistical significance. For rates of influenza amongst those with each chronic condition, we used reported cases of influenza with the comorbid chronic disease within each state / territory as the numerator, and the estimated numbers of people with the chronic disease, as described above, for the denominator. We combined data from different states and territories to try to deal with the small number of events particularly in severe outcomes such as intensive care admissions and death. We also indirectly age-standardised the Indigenous influenza case numbers, using the general non-Indigenous Australian population as the standard population, given the small number of events in more severe outcomes, to reduce instability of aged-standardised rate ratios – see Appendix 9.2. However, indirect standardisation requires replacement of those with background chronic conditions as the denominator with a general reference population (non-Indigenous Australians), thus making our hypothesis untestable; therefore this was not presented as our primary results. Confidence intervals were calculated using the Poisson process described by Liddell, via a statistical calculator (40).

Chronic disease population estimates had 95% confidence intervals derived from the health surveys. Therefore disease rates and rate ratios in chronic disease populations were calculated using both the upper and lower chronic disease population estimates. Confidence intervals for the rates and ratios incorporated the full range generated from the upper and lower population estimates.

Ethics Approval was granted on 17 May 2017 from the Human Research Ethics Committee of the University of Sydney; Project no.: 2017/356.

7.2.5 RESULTS

General

All chronic conditions analysed – chronic lower respiratory conditions and cigarette smoking, diabetes mellitus, obesity, renal disease and cardiac disease – were more prevalent in Indigenous Australians (Table 2).

Table 3 presents the total number of cases of influenza reported to NetEpi by state and severity with the total population from the ABS data, and the crude and age standardised reported number of cases of influenza amongst those with background chronic diseases, by state and severity.

Amongst the total population (all reported cases of influenza), Indigenous status was most reliably available from WA and SA, with data less reliable from WA/SA/QLD/NT and from all of Australia (Table 4). Amongst those with influenza and any of the chronic diseases, Indigenous status was most reliable from WA/SA, with <5% of cases were missing Indigenous classification (Table 5). When the four states were combined, Indigenous status was missing from up to one third of notifications,
hospitalisations, ICU admissions and deaths; unclassified cases were excluded from final analysis. These tables reflect Indigenous status data from age 0 years, whereas table 3 reflects case numbers from 15 years that was supplied, as described above.

Rates of influenza amongst the total population were higher amongst Indigenous Australians compared to non-Indigenous Australians for notifications (WA/SA), hospitalisations (WA/SA and WA/SA/QLD/NT), ICU admissions and death (whole of Australia – not available for individual states and territories for total population) – Table 6.

Indigenous: Non-Indigenous rate ratios for influenza in total versus chronic disease populations

For influenza notifications, the Indigenous: non-Indigenous rate ratios (RR) were statistically significantly higher in all chronic disease populations than in the total population (Table 6). For influenza hospitalisations in WA/SA, Indigenous: non-Indigenous rate ratio point estimates were not statistically different in those with chronic lower respiratory conditions, obesity and renal disease, and higher (confidence intervals did not overlap) amongst those with diabetes mellitus and cardiac disease. However in the larger geographic region of WA/SA/NT/QLD, RR point estimates for influenza hospitalisation were higher in all chronic disease populations compared to the total population except for those with obesity, in whom there was no statistical difference.

For intensive care unit admissions for influenza, Indigenous: non-Indigenous rate ratio point estimates were higher for populations with diabetes mellitus, renal disease and cardiac disease, compared to the total population, and no significant difference was noted amongst those with chronic lower respiratory disease and obesity. For influenza deaths, Indigenous: non-Indigenous RR were higher in those with diabetes mellitus and cardiac disease. There was no statistically significant difference in rate ratios for the other chronic conditions. RR point estimates was 3-fold higher for renal disease, but confidence intervals were wide and encompassed that of the total population.

There was no sub-population of chronic disease patients in which the Indigenous and non-Indigenous rate ratio for influenza of any severity was lower than the total population.

Indigenous: non-Indigenous rate ratios, by severity of influenza disease

In the total population Indigenous: non-Indigenous RRs were significantly higher for influenza hospitalisations compared to notifications. However there was no difference between RRs in ICU admissions and deaths.

In those with chronic disease, the Indigenous: non-Indigenous RR was significantly higher for hospitalisations in SA/WA and SA/WA/NT/QLD, compared to notifications in those with CLRCs. In those with other chronic diseases the notification and hospitalisation RRs were not different for
SA/WA. In WA/SA/QLD/NT, RRs were no different for obesity but were higher for diabetes mellitus, renal and cardiac disease hospitalisations compared to notifications. There was no difference in Indigenous: non-Indigenous rate ratios between hospitalisation, ICU admissions and death from influenza amongst those with any of the chronic diseases. Reduced disparity with increasing severity of influenza – as indicated by significant reduction in Indigenous: non-Indigenous rate ratios for influenza deaths versus ICU admissions versus hospitalisations versus notifications - was not seen with any chronic condition.

Whilst individual results varied, there was no overall change in direction of rate-ratio comparisons between total population and each chronic condition for direct and indirect standardized results – that is there was no evidence of reduction in Indigenous and non-Indigenous influenza rate-ratios when comparing the total population with any of the chronic disease subsets (Appendix 3).

### 7.2.6 DISCUSSION

As expected, we demonstrated higher background rates of all five chronic conditions as well as higher rates of influenza notifications, hospitalisations, ICU admissions and deaths amongst Indigenous Australians compared to non-Indigenous Australians. Higher Indigenous: non-Indigenous prevalence rate ratios for the whole of Australia compared to the WA/SA/NT/Qld for chronic respiratory conditions, diabetes mellitus, renal disease and cardiac conditions may be surprising, considering that our four jurisdictions include the majority of remote areas with poorest socioeconomic indicators. However, these results are consistent with ABS published data, showing higher prevalence for all of these chronic conditions except renal disease amongst Indigenous people in the areas not examined individually in our analysis: NSW, Victoria, ACT and Tasmania, compared to national averages for Indigenous people (35).

We found that the rate ratios in Indigenous compared to non-Indigenous Australian total populations were no lower when analysis was restricted to those with chronic diseases. In fact, they were often statistically significantly higher. This suggests that higher background rates of the chronic diseases we examined do not fully explain the higher rates of influenza amongst Indigenous Australians in the total population. Rate ratios were lower for obesity than other chronic conditions: obese Indigenous patients were still more likely to be admitted, receive intensive care and die than obese non-Indigenous patients with influenza, but the rate ratio was lower than for other those with other chronic conditions. This may suggest that, amongst those with obesity, the impact of obesity on influenza was less disparate between Indigenous and non-Indigenous Australians compared to other chronic diseases. Alternatively, it may suggest that, amongst those with other chronic diseases, unrelated factors were driving differences in severity of influenza, such as lower threshold for hospital admissions, other comorbidities or social factors. The unavailability of 15-24 year old data for obesity is unlikely to have explained Indigenous : non Indigenous influenza case rate ratios lower rate ratios. Most chronic diseases occur in those middle-aged and older, and a re-analysis of SA/WA/QLD/NT
cardiac, renal and diabetes mellitus influenza hospitalisation data excluding the 15-24 year age group resulted in even higher Indigenous : non-Indigenous influenza hospitalisation rate ratios.

The greater disparity in more severe outcomes between Indigenous and non-Indigenous Australians seen in other studies was also seen in our data, with significantly higher RRs for influenza hospitalisations amongst Indigenous patients compared to notifications, when examining the total population. Higher background rates of chronic diseases amongst Indigenous Australians may explain some of the increased disparity in the total population as influenza severity increased: when the analyses were restricted to Australians with chronic diseases, the WA/SA hospitalisation RRs were no longer higher than notification RRs for four of five chronic diseases. CLRCs were the exception, with a higher Indigenous to non-Indigenous hospitalisation rate ratio than notification RR. This suggests higher background rates of CLRCs did not contribute to the higher rate of influenza hospitalisation for Indigenous people in the total population, as the rate ratio jump from notification to hospitalisation mirrored that of the total population in the CLRC sub-analysis. However, when both the total population, and in subgroups with chronic conditions, Indigenous to non-Indigenous influenza rate ratios did not increase further upon comparing hospitalisations, ICU admissions and death, nor did they decrease. Thus, the absence of a decrease in rate ratios with increasing severity in the chronic disease populations does not support our hypothesis that higher rates of background chronic diseases are responsible for higher rates of severe influenza in Indigenous Australians.

There was a jump in rate ratios between 2-state data WA/SA and 4-state data WA/SA/QLD/NT influenza Indigenous: non-Indigenous rate ratios for hospitalisations all of the chronic conditions except obesity. This was due to a large number of influenza hospitalisations in the Northern Territory of Indigenous Australians, e.g. 73/153 (47.7%) of hospitalisations for CLRCs amongst all 4 states occurred within the NT (Table 3, data available upon request). A higher proportion of hospitalisations were Indigenous in 4-states compared to 2-states (Tables 4 and 5), which would include remotely located Indigenous Australians in NT. This may reflect the fact that 72% of the Northern Territory’s Indigenous population live remote from hospitals, requiring hospitalisation admission for observation (41). Obesity was the one chronic condition that did not lead to an increase in the Indigenous : non-Indigenous influenza hospitalisation rate ratios, between the two and four state data. This may be because, if not associated with a diagnosed chronic medical condition, it was not considered a risk factor that required admission for observation.

Thus, overall, the presence of chronic diseases didn’t lower the rate ratios between Indigenous and non-Indigenous Australians with influenza in the total population. There may be other clinical reasons for a higher rate of influenza amongst Indigenous Australians, e.g. chronic disease being more clinically severe in Indigenous Australians leading to more frequent or severe influenza, or a greater burden of multiple chronic comorbidities e.g. concurrence of diabetes mellitus and renal disease (42,43). We did not have this data to perform a multivariate analysis, nor on incidence of influenza in those with no chronic conditions. Certain socioeconomic factors, such as overcrowding, may increase
influenza transmission within the Indigenous community and account for some of the disparity (44). Given that the Indigenous: non-Indigenous rate ratio for influenza for the total population is significantly lower than for each chronic disease, it could even be postulated that Indigenous Australians without chronic diseases are relatively protected against influenza. The ‘composite’ RR of 1.50 for the total population could have been driven down by Indigenous: non-Indigenous RRs < 1 amongst those with no chronic conditions, compared to the high RRs we yielded for those with a chronic disease. However, we do not have the data to verify this possibility.

Our results concurred with other data sources, which reported 8-fold hospitalisation and 6-fold death rates for influenza amongst Indigenous Australians compared to non-Indigenous Australians during the 2009 swine flu pandemic (45). The possibility of ascertainment bias seems unlikely, given that universal testing during the 2009 influenza epidemic within the Top End of Australia encompassing the north part of the Northern Territory, a higher notification rate (RR 5.2) was reported amongst Indigenous Australians (46). A serological survey from the Top End revealed a differential attack rate ratio of 1.85 (47). These non age-standardised results may have overestimated incidence in Indigenous Australians, a younger population that non-Indigenous Australians, given pre-existing immunity in older age groups (48). Our age-standardised rates yielded a lower relative risk for influenza notifications than other data (RR 1.50, 95% CI 1.37 – 1.64).

In contrast to our findings, which indicated that the effect of Indigenous status persisted even when restricting analysis to specific chronic conditions, a systematic review of -4 articles examining risk factors for influenza, with over 600,000 participants, did not find an independent association between Indigenous / First Peoples status and all-cause mortality in pandemic influenza, and in fact the association with chronic disease was low to very low for severe influenza (49). In contrast, Kumar et al/ revealed that 98% of all adult ICU admissions for influenza A(H1N1)pdm09 in Canada had at least one risk factor, such as obesity, lung disease, smoking or hypertension (23) and Aboriginal patients were over-represented at 25% of all ICU admissions. They commented that these conditions were higher in Canadian Aboriginal patients but did not determine whether the former impacted the higher mortality from influenza in this population.

Whilst we were unable to demonstrate that restricting analysis to those with chronic diseases ameliorated the impact of Indigenous status on influenza, other studies have demonstrated opposing findings. Goggin et al from Western Australia demonstrated that Indigenous Australians were three times more likely to be hospitalised with influenza during the 2009 pandemic (50). However, multivariate analysis elicited only age and presence of 2 or more comorbidities as associated with hospitalisation, Indigenous status was not a stand-alone risk factor. Flint et al examined influenza A(H1N1)pdm09 in the Top End of Australia (22). Univariate and multivariate analyses revealed that Aboriginal status per se was not associated with ICU admission, despite Aboriginal people being more likely to be admitted to hospital (RR 5). Likewise, there was an Indigenous : non-Indigenous incident RR of 12 for hospitalisation, but little difference in the prevalence of obesity, respiratory,
hepatic, renal, cardiac, neurological or immune disease amongst those hospitalised between the two groups. This suggests that the higher background rate of these chronic diseases was therefore responsible for overall higher rates of influenza hospitalisations amongst Indigenous Australians. However, smoking and heavy alcohol use remained higher amongst Indigenous Australians hospitalised with influenza. Verrall et al in Wellington, New Zealand observed equivalent rates of pre-existing conditions in those hospitalised with influenza A(H1N1)pdm09 Maori and Pacifica peoples vs. Pakeha (European heritage), in contrast to higher background rates of chronic illness amongst Polynesian peoples in the general community (8). Surveillance data indicated a 3-4 fold increased risk of hospitalisation with influenza A(H1N1)pdm09 and a 4-fold mortality rate of American Indian / Alaskan Native (AI/AN) people compared to non-AI/AN, whereas background rates of comorbid disease were similar amongst those with severe influenza (3). The higher background rates of chronic illnesses therefore likely contributed to greater rates of severe illness in the AI/AN population (51,52). Likewise, data from the USA over the 2009 influenza season revealed a similar rate of a high-risk background conditions in those who perished: 81% of deceased AI/AN versus 77.6% of non-AI/AN (25).

On the other hand, a minority of studies of influenza A(H1N1)pdm09H1N1 in Indigenous populations have concurred with our overall findings, and found disparities in prevalence of chronic disease were unable to explain difference in rates of severe influenza between Indigenous and non-Indigenous people. For example, Zarychanski et al in Canada in 2009 showed that, even after controlling for comorbidity, age, sex, income and rural location, First Nations people still had greater risk requiring acceleration of care to ICU for influenza A(H1N1)pdm09 (5).

Genetic and racial heterogeneity cannot fully explain the difference in susceptibility to chronic conditions between colonising and native populations. Both incidence of severe influenza and prevalence of chronic conditions are higher in unrelated colonised populations around the world, compared to the non-Indigenous populations, and these conditions were not evident pre-colonisation. Thus, there are factors relating to colonisation itself that appear to be partially causal in the gap between Indigenous and non-Indigenous populations (1-15,53-56).

Chronic diseases have been shown to predispose Indigenous Australians to other infections. Heart disease, diabetes and harmful alcohol consumption were independently associated risks in Indigenous Australians for septicaemia (29). Another risk factor that emerged was crowded living conditions in town camps that were dislocated from their homelands. A recent presentation to from the Centre for Disease Control USA indicated that diabetes mellitus was driving an increase in hospitalized infections. Data from approximately 20% of all in-patient hospitalisations from 46 states within USA were analysed, and demonstrated that those with diabetes are around two to seven times more likely to be hospitalised with an infection, particularly urinary tract infections, sepsis and skin & soft tissue infections, than the general population (57). Between 2010 and 2015, infection-related hospitalisations rates increased 52% in those afflicted with diabetes mellitus compared to 17% in those without diabetes.
Physical health is also influenced by the downstream effects of intergenerational psychosocial trauma resulting from colonisation, loss of language and culture, and ongoing systemic, structural and personal racism (19). Chronic diseases that were rare in pre-colonial populations have become prevalent amongst Indigenous people. This results partly from the breakdown of traditional lifestyles and poor access to their modern replacements, such as 24-hour health care access, running water and sanitation facilities. It also results from environmental changes to which human physiology is maladapted e.g. drastic changes in dietary quality and caloric volume (19,58,59). Differences in health care access based on race has been demonstrated: once admitted to hospital for cardiovascular disease, Indigenous Australians received fewer medical interventions and prescriptions, but had poorer outcomes, than non-Indigenous Australians (19). The Australian Human Rights Commission identifies financial poverty, chronic stress, lack of control of health services, lack of ownership of traditional lands and social and political racism as social determinants of poorer health amongst Indigenous Australians (60). A recent report stated that connection to traditional ways of life and living in one’s ancestral homelands, rather than educational attainment and monetary resources, was protective against chronic diseases amongst Aboriginal Australians (61). Those living on their ancestral lands also reported less disability and lower psychological distress. Amongst Canadian First Nations communities prevalence of suicide varied greatly based on presence of protective factors: self government (most protective), successful land claims, community control over health, police, fire and education services, and presence of cultural facilities (62). In New Zealand, Those who were socioeconomically deprived had higher rates of pandemic influenza mortality in NZ (63). Attention to these upstream determinants of health may reduce the stigma of aiming specific policies at Indigenous communities (64).

Our analysis demonstrated that individual chronic conditions were not necessarily associated with higher rates of influenza infection and severity amongst Indigenous Australians. This suggests that broader models of health, and both traditional and innovative Indigenous approaches to health, and collaboration with and empowerment of community leaders elders, may be more effective than the ‘discrete disease’ or ‘clinical risk factor’ based approach at reducing the gap in severe influenza between Indigenous and non-Indigenous Australians (19, 58, 64). One recent initiative is the integration of traditional Aboriginal healers into tertiary hospitals in South Australia, the Ngangkari healers (65). They utilise a mix of bush medicine, ceremonies, and laying on of hands to impart spiritual remedies.

Our study had certain limitations. Tabulated influenza data from NetEpi assigned to various chronic conditions, may not have perfectly matched the ICD10 coded cases from the health surveys on prevalence of chronic conditions. Data were only available for adults ≥ 15 years. There were small numbers of ICU admissions and deaths from influenza, causing substantial sensitivity to small errors or changes in data; for example large confidence intervals as numbers became small for ICU admissions and death - table 6. Differential influenza testing rates and non-clinical reasons to admit to hospital may also have skewed rate ratios. However, Indigenous : non-Indigenous RRs remained high
for escalation to intensive care and for deaths, which would be more objectively determined. Collection of complete Indigenous status data is a recognised challenge. Amongst WA/SA/QLD/NT total population, 31.3% of influenza notifications reported to NetEpi did not record Indigenous status – Graph 1. Age-specific data for the total population was only available for those in whom Indigenous status was specified; we were not supplied age-specific data on those who were not classified by Indigenous status. For those with chronic conditions, age-specific data was provided for those with and without Indigenous status reported. We ran a test-analysis for CLRC hospitalisations in which this was reclassified as Indigenous, and non-Indigenous respectively, and directly and indirectly age-standardised this data. Point estimates became higher when reclassified as Indigenous and lower when reclassified as non-Indigenous, but no Indigenous: non-Indigenous rate ratio point estimates became lower than that of the total population. However, we do not know the status of those unreported, what biases may have been introduced in the statistical analyses. Likewise, the lack of access to data on multiple co-morbidities, and to data from those with no background chronic diseases, also prevents ability to account for multiple morbidities through multivariate analyses.

7.2.7 CONCLUSIONS

We are the first investigators to access and analyse a national database, NetEpi, to analyse the coexistence of influenza and chronic diseases, and relate it to national health survey and Australian population census data. We demonstrated that there is an increased prevalence of major categories of chronic diseases amongst Indigenous Australians compared to non-Indigenous Australians. We also confirmed that there was a higher rate of influenza notifications, hospitalisations, ICU admissions and death amongst Indigenous compared to non-Indigenous Australians. Importantly, once adjustments were made for the higher rates of chronic conditions amongst Indigenous Australians, substantially higher rates of influenza diagnosis, hospitalisation, ICU admission and death, remained amongst Indigenous compared to non-Indigenous Australians. This suggests that there are other factors associated with high rates of influenza amongst Indigenous Australians, which may include comorbidities not examined, presence of multiple morbidities, socioeconomic disadvantage, cultural disruption from colonisation or health care access. Data limitations include a lack of information on multiple or no chronic diseases in individual cases, given that the data were de-identified and aggregated, as well as limitations with reporting of Indigenous status, and potential ascertainment bias. Collecting prospective data with completeness of diagnostic testing, attention to complete recording of Indigenous status, and presence of no, one or multiple comorbidities, would be useful in resolving these uncertainties that result from such data limitations. Synchronising the parameters of health survey data to clinically collected data would enable more aligned numerators and denominators for generation of rate ratios. Further research on broader social and cultural factors which may impact differences between Indigenous and non-Indigenous populations in influenza diseases and severity, may be illuminating. For example, the role that the strength of cultural identification, access to ancestral homelands, economic and educational empowerment, and
provision of traditional and modern models of health care may be explored with qualitative and quantitative research on influenza rates and severity.
7.2.8 References


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### 7.2.9 Tables

**Table 1**

*Diseases included under each condition analysed as per ICD10 WHO coding*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
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<tbody>
<tr>
<td>Chronic Lower Respiratory Conditions</td>
<td>Current or Long Term:</td>
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<td></td>
<td>- bronchitis</td>
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<td></td>
<td>- asthma</td>
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<td>- emphysema</td>
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<td>Diabetes mellitus - excluding gestational</td>
<td>types 1 diabetes mellitus</td>
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<td>diabetes</td>
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<td></td>
<td>- type unknown diabetes mellitus</td>
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<td></td>
<td>- high blood or urine glucose</td>
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<td>- defined by reduction in glomerular filtration rate</td>
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<td>Current or long term:</td>
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<td>- angina</td>
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<td>- heart attack</td>
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<td>- heart failure</td>
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<td>- ‘other heart diseases’</td>
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<td>Chronic Disease</td>
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<td>Chronic Lower Respiratory Conditions</td>
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<td></td>
<td>All Australia</td>
</tr>
<tr>
<td>Cardiac Disease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>WA/SA</td>
</tr>
<tr>
<td></td>
<td>WA/SA/QLD/NT</td>
</tr>
<tr>
<td></td>
<td>All Australia</td>
</tr>
</tbody>
</table>
### Table 3

#### Influenza cases >= 15 years of age for the Total Cohort and for each Chronic Condition

<table>
<thead>
<tr>
<th>Chronic Disease</th>
<th>State/Territory</th>
<th>Category</th>
<th>Indigenous Australians</th>
<th>Age Standardised Indigenous Australians</th>
<th>Other Australians</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cohort</strong></td>
<td></td>
<td>Population</td>
<td>81,700</td>
<td>81,700</td>
<td>3,167,603</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Notifications</td>
<td>517</td>
<td>483</td>
<td>12,516</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hospitalizations</td>
<td>148</td>
<td>172</td>
<td>893</td>
</tr>
<tr>
<td><strong>WA/SA/QLD/NT</strong></td>
<td></td>
<td>Population</td>
<td>246,189</td>
<td>246,189</td>
<td>6,766,712</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hospitalizations</td>
<td>490</td>
<td>569</td>
<td>1,675</td>
</tr>
<tr>
<td><strong>All Australia</strong></td>
<td></td>
<td>Population</td>
<td>429,261</td>
<td>429,261</td>
<td>17,677,150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ICU admissions</td>
<td>72</td>
<td>84</td>
<td>367</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deaths</td>
<td>22</td>
<td>33</td>
<td>157</td>
</tr>
</tbody>
</table>

#### Chronic Lower Respiratory Conditions

<table>
<thead>
<tr>
<th>State/Territory</th>
<th>Background Cases*</th>
<th>Notifications</th>
<th>Hospitalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/SA</td>
<td>14,663 (13,235-16,226)</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>37,322 (34,639-40,178)</td>
<td>153</td>
<td>205</td>
</tr>
</tbody>
</table>

#### Diabetes Mellitus

<table>
<thead>
<tr>
<th>State/Territory</th>
<th>Background Cases*</th>
<th>Notifications</th>
<th>Hospitalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/SA</td>
<td>9,283 (8,113-10,588)</td>
<td>61</td>
<td>58</td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>25.984 (23,708-28,459)</td>
<td>135</td>
<td>201</td>
</tr>
</tbody>
</table>

#### Obesity

*Data only available from >25 years onwards*

<table>
<thead>
<tr>
<th>State/Territory</th>
<th>Background Cases*</th>
<th>Notifications</th>
<th>Hospitalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/SA</td>
<td>4,368 (3,540-5,372)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>12,235 (10,630-14,054)</td>
<td>38</td>
<td>39</td>
</tr>
</tbody>
</table>

#### Renal Disease

<table>
<thead>
<tr>
<th>State/Territory</th>
<th>Background Cases*</th>
<th>Notifications</th>
<th>Hospitalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/SA</td>
<td>1,515 (1,070-2,141)</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>4,332 (3,422-5,465)</td>
<td>72</td>
<td>108</td>
</tr>
</tbody>
</table>

#### Cardiac Disease

<table>
<thead>
<tr>
<th>State/Territory</th>
<th>Background Cases*</th>
<th>Notifications</th>
<th>Hospitalizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/SA</td>
<td>4,978 (4,126-5,997)</td>
<td>40</td>
<td>47</td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>12,202 (10,635-13,984)</td>
<td>82</td>
<td>125</td>
</tr>
</tbody>
</table>

*Number of cases of chronic condition >= 15 years of age calculated from health survey data and ABS census population data (with 95% confidence intervals)
### Table 4
**Indigenous Status Distribution (by State/ Territory) – Total Cohort Notifications**

<table>
<thead>
<tr>
<th>State/ Territory</th>
<th>Indigenous</th>
<th>Non - Indigenous</th>
<th>Indigenous status not specified</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/ SA</td>
<td>6.4%</td>
<td>75.0%</td>
<td>18.6%</td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>13.9%</td>
<td>54.8%</td>
<td>31.3%</td>
</tr>
<tr>
<td>All Australia</td>
<td>10.8%</td>
<td>49.9%</td>
<td>39.3%</td>
</tr>
</tbody>
</table>

### Table 5
**Indigenous Status Distribution (by State/ Territory) – Chronic Diseases**

<table>
<thead>
<tr>
<th>State/ Territory</th>
<th>Indigenous Status</th>
<th>Notifications</th>
<th>Hospitalizations</th>
<th>ICU</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA/ SA</td>
<td>Indigenous</td>
<td>13.7%</td>
<td>18.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non - Indigenous</td>
<td>82.1%</td>
<td>80.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indigenous status not specified</td>
<td>4.3%</td>
<td>1.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WA/SA/QLD/NT</td>
<td>Indigenous</td>
<td>30.1%</td>
<td>26.7%</td>
<td>20.5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non - Indigenous</td>
<td>41.3%</td>
<td>44.1%</td>
<td>44.6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indigenous status not specified</td>
<td>28.6%</td>
<td>29.1%</td>
<td>34.9%</td>
<td></td>
</tr>
<tr>
<td>Chronic Disease</td>
<td>State/ Territory</td>
<td>Notifications</td>
<td></td>
<td>Hospitalisations</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------</td>
<td>---------------</td>
<td>----------</td>
<td>------------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Relative</td>
<td></td>
<td>Relative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Risk</td>
<td>95% CI</td>
<td>Risk</td>
</tr>
<tr>
<td><strong>Total Cohort</strong></td>
<td>WA/SA</td>
<td>1.50</td>
<td>1.37 – 1.64</td>
<td>7.48</td>
<td>6.35 – 8.80</td>
</tr>
<tr>
<td></td>
<td>WA/SA/QLD/NT</td>
<td>9.34</td>
<td>8.50 – 10.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All Australia</td>
<td></td>
<td>9.46</td>
<td>7.47-11.99</td>
<td>8.71</td>
</tr>
<tr>
<td><strong>Chronic Lower Respiratory</strong></td>
<td>WA/SA</td>
<td>3.26</td>
<td>2.60 - 4.09</td>
<td>5.62</td>
<td>4.26 - 7.40</td>
</tr>
<tr>
<td><strong>Diabetes Mellitus</strong></td>
<td>WA/SA</td>
<td>9.37</td>
<td>7.30 - 12.03</td>
<td>12.68</td>
<td>9.17 - 17.54</td>
</tr>
<tr>
<td></td>
<td>WA/SA/QLD/NT</td>
<td>25.39</td>
<td>20.19- 31.94</td>
<td>34.88</td>
<td>20.23 - 60.15</td>
</tr>
<tr>
<td><strong>Obesity</strong></td>
<td>WA/SA</td>
<td>4.14</td>
<td>2.88 - 5.96</td>
<td>5.55</td>
<td>3.49 - 8.63</td>
</tr>
<tr>
<td></td>
<td>WA/SA/QLD/NT</td>
<td>41.51</td>
<td>29.09 - 59.22</td>
<td>32.11</td>
<td>12.42 - 71.53</td>
</tr>
<tr>
<td><strong>Cardiac Disease</strong></td>
<td>WA/SA</td>
<td>15.46</td>
<td>11.85 - 20.64</td>
<td>15.82</td>
<td>11.21 - 22.33</td>
</tr>
<tr>
<td></td>
<td>WA/SA/QLD/NT</td>
<td></td>
<td>29.52</td>
<td>22.93 - 38.01</td>
<td>30.16</td>
</tr>
</tbody>
</table>
7.3 Chapter 6 Synopsis

The Role of Chronic Diseases in Influenza Incidence and Severity Differential Between Indigenous Australians and Non-Indigenous Australian Populations During the 2009 Influenza Pandemic in Australia – Synopsis

This data analysis examined the role played by the higher background prevalence of chronic, non-communicable diseases in the greater incidence of severe influenza recorded amongst Indigenous Australians during the 2009 ‘swine flu’ pandemic.

We confirmed that both chronic conditions and influenza are more common in Indigenous Australians, but we were unable to demonstrate a distinct impact from higher rates of background chronic diseases. These results may have been affected by data collection and access limitations, which I have discussed. This provides an opportunity to reflect on how such national data collection may be best coded and organized to enable minimization of biases and confounders. This suggests other factors associated with colonization are predisposing Indigenous Australians to more frequent and severe influenza – genetic predisposition being unlikely given disparate colonised populations globally suffer from higher influenza morbidity and mortality. These may include multiple comorbidities, which we could not adjust for with the data supplied.

Our results conflicted with other published analyses. Most - but not all - other analyses, both locally and internationally, indicated that the higher prevalence of a range of chronic conditions was responsible for higher rates of severe influenza amongst First Peoples in many colonized nations around the world during the 2009 ‘swine flu’ pandemic. These findings highlight the importance of addressing upstream determinants of communicable diseases in Indigenous populations.

This analysis suggested a role for a prospective data analysis of influenza risk factors, in which data for communicable and non-communicable diseases is better synchronised, and Indigenous status more accurately and completely collected. It also suggests a place for qualitative and quantitative research of broader social, cultural and environmental factors that result from colonization, which may affect differential influenza rates and severity between Indigenous and non-Indigenous Australians.
8. INFLUENZA IN VULNERABLE POPULATIONS - DISCUSSION

My thesis examined different aspects of influenza in vulnerable populations. I started by looking widely at an issue that places whole populations, particularly the immunologically impaired, at risk of influenza morbidity and mortality: the prevalence of oseltamivir-resistant strains and strategies to address them. Next, I examined different aspects of three different demographics that are over-represented in influenza incidence and severity – the very young, the very old, and Indigenous Australians.

I performed an original literature search, which showed that, for current circulating viruses, resistance to oseltamivir remains relatively low, but is increasing. I wrote this review in 2013 (published 2014) in the post-2009 pandemic era, when the role of oseltamivir was being scrutinised heavily with respect to its efficacy, effectiveness and safety. That an oseltamivir-resistant strain of influenza was able to dominate in the 2007–2008 season suggested the acquisition of additional mutations that conferred a selective advantage over wild-type oseltamivir-sensitive strains, despite the mutation compromising replicative fitness. Since 2009, the most common circulating strains, pandemic influenza A/H1N1 2009 and seasonal influenza A/H3N2, are generally oseltamivir-sensitive, although resistance is being increasingly detected. Zanamivir is an option in those over 5 years of age, although dual oseltamivir-zanamivir resistance has been detected in some isolates. I demonstrated that oseltamivir resistance was rising both independently and locally, and spreading globally, amongst pre-pandemic influenza A (H1N1) (1). Immunocompromised patients, a group vulnerable to influenza, were over-represented in those harbouring oseltamivir resistant strains, due to prolonged shedding and receipt of oseltamivir, leading to selective drug pressure. Since publication, levels of oseltamivir resistance remain low in circulating strains, indicating that the data presented in this review remains relevant (2). Stockpiling of oseltamivir is advised by the World Health Organization (3). Constant surveillance is required to detect a change in strain type and oseltamivir sensitivity (4). This was evident during the 2017 season when influenza vaccine effectiveness was noticeably lower at 33% due to vaccine–wild-type strain mismatch, with larger national influenza epidemics as a result, and reliance on oseltamivir treatment in severely affected patients (5, 6). To date, this paper – my first ‘first-author’ publication – has been cited 21 times, reflecting both the need for such a review and the interest in this topic. This paper is a useful summary of oseltamivir resistance to date and presents a starting point for further assessment and synopses of antiviral-resistant strains. It also highlights the vulnerability in our antiviral armament: the dependence upon oseltamivir susceptibility in influenza strains. It begs the question, what can we do to reduce development of resistant strains and develop new antiviral strategies?

One such strategy is different oseltamivir dosing regimens to potentially promote more rapid virological clearance and hopefully reduce emergence of resistant strains. I presented an unblinded, randomised controlled study of a double dose regimen of oseltamivir compared to
the standard dose in the community setting. This distinguished this paper from those that
studied hospitalised cases. If the results of this study demonstrated a clinical advantage
without increased adverse events, it could provide a case for extrapolating such a study to
those with predisposing health conditions. However, we demonstrated no difference in clinical
or virological outcomes between the two dosing groups, but a significant difference in adverse
events, with the double dose experiencing more gastro-intestinal side effects. Our study adds
to the body of research and demonstrates similar findings, but uniquely dealt with many
patients with milder disease, whom could be cared for outside of hospital (7,8). Only one case
developed resistance in each arm of the study. However, we recruited an oseltamivir-resistant
case at enrolment that was genotypically-related to a 29-case cluster from Hunter New
England, New South Wales; our case was able to be included in published reports regarding
this cluster (9, Appendix 9.3). We aimed to recruit 125 patients but recruited only 52. We
recruited at two sites – a Western Sydney general practice by Peter Hay, co-author and
general practitioner, and myself at the Children’s Hospital, Westmead Emergency
Department. Not meeting our recruitment target was likely due to a mix of factors. Influenza is
a self-limiting illness that, in most cases, does not warrant treatment. Therefore, in this
healthy, community-based population that we received ethics approval for, cases that were
recruited participated for altruistic reasons, and potential recruits may be less motivated than
the ill, hospitalised patients in other studies of double dose oseltamivir. However, this gave us
a unique point of distinction from other studies, in that we were studying oseltamivir in
influenza in the community, where influenza is most often diagnosed and managed. In a
paediatric population, in the Children’s Hospital, Westmead, caregivers were consenting on
behalf of a child, which means we came up against the natural barrier of parental protective
instinct. This is overcome more easily in a GP setting, where the doctor has a pre-existing
relationship with potential recruits; thus about two thirds of our recruitment ended up being
from the general practice. On the other hand, inclusion criteria were for symptoms of less
than 48 hours duration. This condition is met infrequently in people who present to a family
doctor, as most usually attend when symptoms don’t rapidly resolve. The study was
conducted fairly close to the 2009 ‘swine flu’ epidemic and some people had firm fixed ideas
about its manifestations, and denied it was a diagnostic possibility in themselves or their child,
and refused point-of-care testing. The test itself was somewhat invasive, requiring a nasal
swab, which can be uncomfortable. Nonetheless, we recruited 52 cases despite these
obstacles. In future such studies, being proactive and advertising the study in the lead up to
the influenza season may encourage people to present earlier. Focusing on general practices
may be helpful due to the pre-existing relationship, but we found it very helpful to include the
somewhat more symptomatic cases that present to an outpatient emergency department.
Performing the study over more than one season may also be warranted.

Our study did not support any advantage to doubling the dose of oseltamivir, at least in the
healthy population. However, there was non-significant finding of more rapid virological
clearance by nucleic acid testing (NAT), particularly in those with influenza A, in the double
dose group (p = 0.16), suggesting conditions for oseltamivir resistance - prolonged viral
shedding - may be mitigated by double dose regimens. This didn’t reach statistical
significance, however, and may be a spurious finding. Two other studies of double dose
oseltamivir examined virological clearance: one study of 326 cases revealed no difference by
NAT testing (p =0.42) whereas the other study of 155 patients claimed ‘a trend towards more
rapid clearance in influenza B’ (p = 0.05) (10, 11). Therefore, there isn’t a clear role for
oseltamivir double dose to hasten virological clearance or reduce emergence of oseltamivir
resistance, but future, perhaps larger, studies may clarify this, further.

An area for further research is to repeat this study amongst those with immune deficiencies
and those under age 5, given that adverse events, whilst higher in the double dose group,
were self-limiting. Oseltamivir prevents release of virus from cells and thus invasion of
uninfected epithelial cells. It is thus most effective in the first 48 hours after onset of influenza
symptoms, before most target cells have become depleted. Oseltamivir pharmacokinetics are
influenced by the interactions between viral load, target cell dynamics, and innate and
adaptive immune responses (12). Thus, the results of this study may not be extrapolatable to
immunocompromised populations who shed virus for longer and have weakened immune
responses. Nonetheless, other studies have focused on these populations, whilst we were the
first to look at double dose oseltamivir treatment of community-based cases. Like the other
studies of hospitalized populations, even in our relatively healthy population, there was no
evident advantage to double dose oseltamivir. Recent studies on high dose influenza vaccine
show, promisingly, that immunogenicity improved in cardiac patients, cancer sufferers and
elderly recipients (13-15). However, other specific antivirals have not yet been developed. An
old strategy, application of heterologous antibodies, currently used in rabies infection and
snake and arachnid venom toxicity, was the focus of my next paper. By targeting multiple
epitopes, they are an effective strategy for overcoming mutations in influenza strains that may
otherwise be treatment-resistance.

The major limitations of heterologous polyclonal antibody therapies are that they are
extracted from the serum of non-human species, which creates the potential for
hypersensitivity reactions to animal proteins. In my review I demonstrated how the
introduction of filtration, pasteurisation and chromatography steps remove animal proteins
and impurities from the final product, which has resulted in very low rates of hypersensitivity
reactions. This treatment modality is being developed for avian influenza strains, to which
those who are elderly, and those with chronic conditions, are particularly susceptible to
clinical disease manifestation (16). Against avian influenza, there are promising early results
in terms in immunogenic and safety studies of efficacy and tolerance. They can also be
rapidly generated to new strains of influenza virus, resulting from antigenic shift, requiring less
technical prowess and lower cost than monoclonal antibodies, and overcoming the
disadvantages of epitope mutations that make monoclonal antibody therapy vulnerable to antigenic drift (17, 18). Polyclonal antibody therapies can also be applied to a range of undertreated tropical illnesses ravaging different parts of the globe: Ebola virus, Zika virus, and Dengue virus, for example. The data from this extensive review was reassuring. Successful in-vivo proof-of-concept studies, and phase 1 safety trials, indicate that heterologous polyclonal antibody therapy may be developed to treat and even prevent oseltamivir resistant influenza, avian influenza strains, other ‘drifted’, re-assorted strains, and eventually become part of the pandemic planning strategy. Their utility may extend to other undertreated infectious pathogens. This undertaking was a collaboration between myself, medical librarians, Biointelect (a consultancy firm specialising in biological product development), and Fab’entech (a pharmaceutical company currently testing polyclonal antibodies against avian influenza). It was noted by a marker that the phrase ‘equine F(ab’) preparations demonstrated cytopathic effects against cultured Madin–Darby canine kidney (MDCK) cells infected with H5N1’, should perhaps read ‘cytoprotective effects’. I checked the reference, and submitted a correction ‘prevents cytopathic effects’, to the publishers Elsevier, of the journal VACCINE, who have published a Corrigendum on their website, included after the article. A poster summary of this paper was selected be presented verbally to the 2014 conference of the European Scientific Working group on Influenza in Riga, Latvia; (Appendix 2). This literature review can form a basis for ongoing updates in the development, clinical testing, registration and post-marketing surveillance of polyclonal antibody therapies in influenza.

One issue with conducting any type of research in the field of influenza management is ethical considerations in the study design; given how rapidly fatal influenza can be in vulnerable populations. My third offering was a protocol for a cluster-randomised controlled trial on the use of oseltamivir treatment of cases, versus treatment of cases plus contacts (prophylaxis), for the prevention of influenza outbreaks in aged care facilities (ACFs). This is distinct from another earlier study by introduction of information sessions to education influenza, prevention strategies, infection control, and then coach ACF staff on surveillance for influenza-like illness, recorded through active computer surveillance systems, as well as conduct of point -of-care tests. We liaised with Dr Liz Barnes, statistician at National Centre of Immunisation Research and Surveillance, Children’s Hospital, Westmead, who suggested 74 ACFs to give statistically significant findings. On advice, we consulted Professor Christopher Triggs, Head of Department of Statistics at University of Auckland, to obtain a sample size calculation. We used the following assumptions:

AVERAGE SIZE of ACF: mean number of residents in Booy et al: 66 patients with 86 bed capacity; so I think it is safe to say 75 residents.
INFLUENZA SEASON DURATION: June to September: 121 days.
INCUBATION TIME: 1-4 days - average 2. Infectivity time 7 days (without treatment).
CONTACT NUMBER: varies ACF to ACF but Booy et al study was 6-7 contacts per case on average

CHANCE OF ACF resident contacting influenza during a season: Based on Booy et al paper:
‘Confirmed influenza cases in residents were 19.6% (T only) – therefore I extrapolated to 20%.

At the time of this PhD submission, Professor Triggs has not finalised this exercise, and is out of the country. I anticipate liaising with him to obtain the results of his statistical model for sample size. I have also organised a meeting with statistician, Mr Jim Matthews, from the University of Sydney - the organisation of this meeting was delayed due to administrative errors on the part of the university and is now scheduled for 11 November 2018. I will seek input on sample size and appropriate statistical testing. We are also looking forward to the results of an observational study underway by Professor Booy, of oseltamivir treatment versus treatment+- prophylaxis, and will use the findings to guide the statistical inputs of this trial, further.

Immediately, we ran into problems achieving consensus on the issue of informed consent of study participants. The ethical principle that underlies the practice of obtaining informed consent is “respect for individual autonomy” (19). Autonomy is defined as “independence of self determination, freedom from external control” (20). Therefore, to preserve their autonomy, it is usually an ethical requirement to obtain informed consent from each participant in a study of either untested or off guideline treatment or treatment regimens (21). This protocol, however, had several specific features that complicated the issue of obtaining consent. Firstly, oseltamivir is already a registered medication that has been extensively studied for safety and efficacy, albeit with controversial findings (22). Thus, the question of whether consent needed to be obtained arose. Secondly, the two regimens being compared were both outlined as alternative options in the 2017 guidelines for management of influenza outbreaks in residential care facilities, which makes our proposed regimens both ‘on-guideline’, hence the requirement for consent was unclear (23). The reason for not advising one option over the other is due to a paucity of data with which to make firmer recommendations, a matter that this study sought to address. Thirdly, residents of ACFs are often vulnerable, with a high level of dementia as well as hearing and visual deficits, hindering communication of information with which to obtain consent (24,25). This would require identifying next of kin or those with guardianship arrangements for many residents. Fourthly, the huge number of participants, residents and staff of approximately 100 ACFs make obtaining individual consent impractical. Obtaining consent in advance of an influenza outbreak from thousands of potential participants was unrealistic with the resources available to conduct such a study, particularly given that it is likely that the majority of them would not ultimately be involved in an influenza outbreak. Personal discussions with the Booy et al study authors indicated certain pitfalls in the prospective, real time consent process. There is a need to act rapidly
during an influenza outbreak to manage it effectively, with oseltamivir being most effective within the first 48 hours of symptom onset to reduce both individual duration of illness and transmissibility (22). Obtaining consent from the elderly is a time-consuming process. “Catch up” consent during outbreaks reduced the ability to treat in a timely manner. “Time to consent” reduced time to check renal function and added to delays. During serious outbreaks, the risk-benefit assessments of participants or their guardians changed; residents who previously declined consent changed their minds, requiring re-consent. Consent procedure reduced implementation of trial intervention, and led to differential consent rates. This was the main source of criticism by reviewers.

A prolonged process of communication, negotiation, concession and compromise was employed to attempt to obtain a workable consensus. In summary, the proposed consent processes included: (a) obtaining consent from each participant either before, or at time of, an outbreak; (b) obtaining consent to dispense oseltamivir only from contacts of cases, and treating cases without obtaining consent; (c) obtaining consent from ACF managers to be randomised into one of two guideline-recommended treatments; (d) obtaining consent from general practitioners sub-serving both ACFs and individual residents. The first two were unworkable and not seen as consistent with the idea that oseltamivir for treatment and prophylaxis were both on-guideline measures. The last two measures were essentially vetoed, as consent by ACF staff or general practitioners on behalf of residents were not considered appropriate. Ultimately it was decided that, as both regimens were on-guideline and oseltamivir is a registered medication, we would seek from the Ethics Committee a waiver of the requirement for individual consent. Instead, we would seek agreement from the treating general practitioners to institute one or the other of the two arms of the study in the event of an influenza outbreak. The public health bodies that would oversee outbreak management were already included as study co-authors, ensuring their co-operation. Study staff would provide no hindrance to any decisions to change outbreak management at any time, e.g., to withhold treatment from individuals, or to change treatment of cases, only to treatment of cases plus prophylaxis of contacts. We were unable to get agreement from all stakeholders to this decision. In the post-PhD period, we will continue these discussions. One possible solution is to evaluate prospectively collected observational data; funding and staff have been obtained to perform such a study at the Kidney Research Centre at the Children’s Hospital, Westmead and the study is underway.

The other extreme of age, infancy, is also a vulnerable period for influenza with higher rates of hospitalisation, Intensive Care Unit admission, and death (26). This is complicated by lack of universal vaccination, and barriers to vaccinating pregnant women. With vaccine coverage in pregnant women only 20-50% in Australia and the US, infants vulnerability to influenza is compounded by lack of transplacental antibody transfer from mothers who are not recently infected (27, 28). Once infected, infants are treated with oseltamivir, the only registered
treatment for infants, despite a paucity of data in this age group (29). There is only one other trial of oseltamivir in infants, and registration only down to 1 year of age (30). We conducted a pharmacokinetic and pharmacodynamics analysis of infants on oseltamivir in the Intensive Care Unit. Recruitment of cases was limited by several factors including: (a) ethical constraints requiring infants to be cannulated for another purpose to collect blood samples. Given that oseltamivir is administered via nasogastric route or orally, most babies treated with oseltamivir did not require intravenous cannulation; (b) low rates of influenza in infants in one of the seasons over which the study was conducted; (c) inability to easily bleed back from the cannula in one child. Recruitment of cases was complicated by the fact that many babies didn't require cannulation. Some babies were cannulated for only a few hours, too short for our sampling time frame. One of the influenza seasons over which the study was conducted resulted in no suitable cases admitted to the paediatric infective care unit from which we were recruiting. On one occasion we were unable to obtain blood through the cannula. Even so, we were able to obtain a complete data set from two cases and a partial data set from two patients, for a total of four cases. We obtained our drug and metabolite exposure value target from recommendations by Kimberlin et al. (30). Overall, oseltamivir was well tolerated at a dose of 2.35–3 mg/kg/ dose twice a day in infants under the age of 1 year. These doses were confirmed to produce a target oseltamivir carboxylate plasma exposure in excess of the proposed 12-hour target exposure of AUC 3800 ng/h/ml. Ideally, a greater number of recruited cases would have consolidated our findings, but this data contributes to knowledge about oseltamivir in infancy due to a lack of published data to confirm the only other published study, and may be helpful in meta-analyses of oseltamivir pharmacokinetics in infancy. An expanded study over several facilities and several influenza seasons would probably result in more recruits and be useful in improving the reliability of the results, but funding would be a limitation, given the cost of transporting the specimens to the United States and paying for laboratory analysis. Nonetheless, at the time of publication, our data was the only other published analysis of oseltamivir pharmacokinetics in infants, confirming the findings of insulin and providing data for the current guidelines.

Building on the theme of obtaining consent in populations that are vulnerable, obtaining consent for analysing influenza and chronic disease data on Indigenous Australians provided a steep learning curve. An Aboriginal advisor became unavailable for collaboration. Ethics committees rightly wanted evidence of consultation from the outset with key stakeholders within the Australian Indigenous community. My own ignorance and naivety in this regard resulted in missteps, and my possibly offending the Spirits of the Elders, and there were many setbacks. Eventually, we were able to interest Professor Jakelin Troy, Director of Aboriginal and Torres Strait Islander Research and Deputy Vice-Chancellor of Indigenous Strategy and Services, from the University of Sydney. She endorsed my research, commenting that “the publication is an important issue in Indigenous health, I support her desktop research program using extant, publicly available, de-identified data sets and look forward to further discussion about areas of mutual interest in Indigenous health”. An affiliation for
post-doctoral research possibilities was formed between Professor Troy and myself. The Human Research Ethics Committee approved the project in April 2017. Out of all the projects, this may have been the most challenging, most detailed, perhaps most educational, and the one closest to my passions – the role of proximal and modifiable determinants of health in vulnerable populations. This is the first age-standardised analysis of Australian national data sets, in contrast to smaller local analyses, which are usually not age-standardised. We examined a national data set for notifications, hospitalisations, Intensive Care Unit admissions and deaths from influenza by Indigenous status and by presence of one of five common background conditions as coded by the International Classification of Diseases and Health Related Problems (ICD-10) (31). Our results confirmed that chronic conditions are significantly more common in Indigenous Australians, and that influenza of varying degrees of severity was more common in Indigenous Australians, but restricting our analysis to those with each chronic condition reduce the difference in influenza rates between Indigenous and non-Indigenous Australians. Thus, we did not demonstrate that higher rates of background chronic conditions were responsible for more frequent and severe influenza in Indigenous Australians. These findings may be partly explained by data limitations. Attention to Indigenous status, classification of individuals with either no, one or multiple comorbidities, and case identification through consistently applied diagnostic testing would help to reduce data artefacts and biases. This would enable accurate elucidation of the role of chronic diseases in the gap between Indigenous and non-Indigenous Australians with respect to influenza. This study highlighted the importance of rigorous data collection on Indigenous status, and the presence or absence of none, one or multiple comorbid chronic diseases to perform multivariate analyses. But, if our research results were true, this begs the question: what does cause influenza disparities between Indigenous and non-Indigenous Australians? Research of the social, cultural, political and psychological impacts of colonisation has indicated that colonised communities who are able to preserve these ties have better discrete health outcomes. Could attention to broader, less quantifiable factors help resolve this quandary, rather than a modern Western ‘risk-factor’ based approach? Options for further research may include an analysis of prospectively collected data with attention to the above data limitations, such as synchronising health survey and clinical data more closely, and applying influenza testing uniformly. However, there is also a role for researching the impact of broader social and cultural factors that communities and individuals exist within. For example, the impact on influenza rates and severity of strength of cultural ties and identification, markers of economic and educational empowerment, access to ancestral homelands and provision of traditional and modern models of health care could be explored with qualitative and quantitative research. Whilst the timeline of the PhD did not allow for submission for publication to peer review journals, this is the immediate goal, in order to disseminate the results to data collection agencies, the broader community, and particularly to Aboriginal and Torres Strait Islander healthcare providers, elders and communities.
8.1 Conclusion:

There are a number of considerations that affect the impact of influenza and its management strategies in vulnerable populations. These include those particularly susceptible to influenza, such as those in infancy, the elderly, and Indigenous populations in colonised countries. Oseltamivir-resistant strains render immunologically-compromised cases vulnerable in the event that resistant strains predominate, or were highly virulent strains such as avian influenza to develop effective person-to-person transmissibility. I examined strategies to address oseltamivir resistance. Oseltamivir double dose strategies did not show a clinical or virological advantage, nor were we able to show an advantage in terms of avoiding oseltamivir resistance. Resurgence of old strategies such as heterologous polyclonal antibodies look promising – more phase three trials and post-marketing surveillance needs to be undertaken to support their introduction to the anti-influenza artillery. These therapies indicate the need to think outside of the current pharmaceutical models of care for resistant organisms, towards other biologics for therapeutic applications. I then turned my attention to discrete risk groups. The protocol we developed on the role of oseltamivir in averting outbreaks of influenza among the elderly residing in ACFs would support a 3-year study; however, the issue of how to conduct this study ethically requires further discussion amongst the proposed study authors until a consensus is reached. Infants are currently treated with oseltamivir, although it is licensed for those over 1 year of age. Our small case series on pharmacokinetics demonstrated an agreement with the only published data on the appropriateness of current doses given to infants. An expanded study on oseltamivir pharmacokinetics in infants during an active influenza season would consolidate the findings from the current series, were funding available. Lastly, our national, age-standardised data analysis on the role of background non-communicable chronic diseases on higher rates of severe influenza in Indigenous Australians did not show that higher prevalence of chronic conditions influenced influenza rates, and suggests broader socio-political, economic and cultural factors may be worth examining – and highlighted the importance of rigorous, prospective data collection.

Influenza is an ancient, highly contagious viral disease, and continues to evade our best efforts to prevent it and treat it. This thesis examined aspects of its impact on certain circumstances of population susceptibility, and within particular vulnerable populations. It also provided insight into further areas of research and analysis into this globally relevant and elusive disease.
References:


9 Appendices


9.2. Table: Relative Risk of influenza incidence amongst those with the total cohort and chronic diseases – Indirectly Age-standardized

Heterologous Polyclonal Antibodies
Past and Present, with Future Applications to Avian Influenza and Other Neglected Viruses.

Dixit, Rashmi (1); Herbreteau, Cécile Hélène (2); Herz, Jenny (3); Dalton, Richard (4) Booy, Robert (1); Lepine, Bertrand (2)

1 The Children’s Hospital, Westmead, Sydney (AUSTRALIA) 2. Fab’entech, Lyon (FRANCE) 3: Biointellect, Sydney (AUSTRALIA) 4: University of Southampton

Introduction
Passive immunisation with highly purified polyclonal antibodies against toxins or infection, have potential future applications to influenza treatment / prophylaxis. Such therapy is currently applied to:
- medication overdoses e.g. colchicine & digoxin,
- poisoning by snake, arachnid, marine and plant toxins
- post-exposure rabies.

Polyclonal antibodies (immunoglobulins) are obtained from plasma of hyper-immunized animals, e.g. horses or sheep.

Allows affinity maturation and thus high affinity antibodies, enables a more potent formulation, reduces volume administered, minimising allergic reactions from co-administered animal proteins.

In the past, use of animal serum has been associated with high rates of heterotypic allergic and hypersensitivity reactions (1) Modern manufacturing and purification methods have greatly improved safety whilst retaining efficacy (2)

Animal products are cheap and fast to produce, an important consideration for resource-limited settings, and can be produced in large amounts.

Heterotypic Antibodies: Past
Von Behring & Kitasato discovered rabbit sera protected mice from diphtheria & tetanus (3)
- Animal derived anti-diptheria serum used in humans during a European epidemic (4)
- Phisalex & Bertrand demonstrated that blood of horses immunised with Vipers aspicus (European vipers) had antitoxic properties

Human & animal origin sera were used for measles, varicella and 1918 pandemic Spanish influenza
- Pre-antibiotics, serum therapy was applied to bacterial infections such as pneumococcus, meningococcus and streptococcal A (scarlet fever)
- Anti-rabies polyvalent antibodies from hyper-immunised horses was developed in the early 20°C

Fifty years ago:
- 16% treated with equine rabies immunoglobulin (ERIG) for post-exposure prophylaxis developed serum sickness
- 46% >15 years were affected

1980s
- Serum sickness reduced to 0.5-3% by the late 1980s after introduction of enzymatic digestion, ammonium sulphate / caprylic acid precipitation and thermocoeagulation to sterilise and purify serum

Heterotypic Antibodies: Current
New processes have made allergic reactions rarer e.g. ERIG <0.1% serum sickness / anaphylaxis
- Initial anion-exchange chromatography isolates horse IgG subclass, excludes non-protective immunoglobulins, proteins, cell aggregates, contaminants.
- Peptic cleavage obtains F(ab')2, and destroys enveloped viruses.
- A second anion exchange chromatography removes protein aggregates and bacterial endotoxins.
- Final heat pasteurisation destroys non-enveloped viruses; ultrafiltration removes viruses and bacteria.
- High performance liquid chromatography determines purity.
- Preservatives prevent bacterial / fungal contamination
- Animals vaccinated and / or chosen from areas with no arboviruses. Sera routinely screened / subcultured
- Nil documented cases of prior acquisition

ERIG from reliable sources is now effective and safe e.g. in Philippines (5)
- 144 cases given correct Favirab® as per protocol. (Sanofi Pasteur, Lyon, France), no failures
- 193 Favirab® recipients cases with rabid bites, one failure.
- 7680 Favirab® recipients, 0.3% local and 1.2% systemic reactions

Final outcomes. Post marketing surveillance >1 million doses Favirab® > 8 years, 2 cases serum sickness / 1 case anaphylaxis.

Snake antivenoms
- slightly higher rates of AE as greater volume / more protein delivered
- A 1996-2008 review of F(ab')2, rattlesnake antivenin CroFab®: response rate 77% to severe envenomation
- 223 African recipients of equine F(ab')2, snake antivenin IPSER AFRICA (Pasterie Merieux Connaught, Lyon, France)
  - clinical cure rate 96.8%
  - 1 (0.4%) case each anaphylaxis and serum sickness.

Results

Heterotypic Antibodies: Past

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1899</td>
<td>Von Behring &amp; Kitasato discovered rabbit sera protected mice from diphtheria &amp; tetanus</td>
</tr>
<tr>
<td>1994</td>
<td>Animal derived anti-diptheria serum used in humans during a European epidemic</td>
</tr>
<tr>
<td>1918</td>
<td>Phisalex &amp; Bertrand demonstrated that blood of horses immunised with Vipers aspicus</td>
</tr>
</tbody>
</table>

Heterotypic Antibodies: Future application
- Fab’entech propose application of the well known and characterized technology to new pathogens achievable by substituting one vaccine in horses for another; enabling 9 to 12 months from emergence of a new viral threat to release of new antisera.

- Convalescent plasma used in two cases of Highly Pathogenic Avian Influenza (HPAI) H5N1, both of whom survived.

- Administration of polyclonal anti-influenza F(ab')2 to mice 24H post-influenza exposure across several studies, including H5N1.

- Greater viral clearance and less morbidity and mortality compared to controls (6).

- An anti-H5N1 Highly purified F(ab')2 was specifically developed, named Fabenflu®, and propose a 5 consecutive days injection protocol.

- A phase 1 study in 16 healthy young males of Fabenflu®, safe: one minor febrile reaction.

- Unimmunised hamsters who received West Nile Virus- anti-sera one hour before and 24 hours after a challenge were protected from lethal infection (7).

- Ducks egg polyclonal F(ab')2, to Andes virus (pulmonary hantavirus syndrome) given to hamsters post-exposure intranasal or intramuscular had improved survival compared to controls (8).

- Polyclonal antibodies against the Marburg and Ebola filoviruses from nonhuman primates (NHPs) that survived, given post-exposure to other NHP and, prevented disease and death (9).

- Other studies of mice, monkeys & guinea pigs demonstrated purine wealthy IgG prolonged survival against Ebola (10-14).

- Given difficulty in conduction efficacy trials in humans, non-traditional regulatory pathways may be needed to register these products. Provisions to reduce overall review time should include e.g.:
  - utilisation of FDA ‘animal rule where animal data is assessed.
  - rolling submissions where regulatory review can commence whilst data is still being finalised.

- Mock up dose whilst which enable standing approval of manufacturing processes to be applied to development of sera against a new pathogen.

Conclusion
The past precedent and future potential for safe, effective serum prophylaxis and therapeutics in viruses provides hope for pathogens for which there are limited therapeutic options, including for pandemic and resistant influenza strains.

References
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(6) Muehlen C et al Immunotherapy. 6(6):699-708; 2014
(10) Mikhailov VV et al. Vopr Virusol. 39(S2), 82-4;1994
(14) Fab’entech Proprietary. Emerg Infect Dis;8:1392-7;2002
(15) Nicklin I et al. JAMA 1965;193(5):1065
(16) Lepine, Bertrand (2)

Method
- Literature searches were undertaken by an experienced medical librarian
  - To identify items on both the safety of polyclonal antibodies and the use for the treatment of rare or neglected diseases.
  - The databases searched included OVID Medline and OVID Embase.
  - The searches contained database-specific MeSH and Emtree terms in combination with pertinent text-words.
  - To minimise bias, no language limits were applied.
  - The final search was completed on 30.05.14.

Contacts
- The Children’s Hospital
Rasmi Dixit rushmi7@gmail.com
Fab’entech
Cécile Herbreteau-Delale
cecile.herbreteaudelale@fabentech.com

The Sydney Children’s Hospitals Network
www.fabentech.com

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<th>Chronic Disease</th>
<th>State/ Territory</th>
<th>Notifications</th>
<th>Hospitalisations</th>
<th>Intensive Care Admissions</th>
<th>Deaths</th>
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<td>Relative Risk</td>
<td>95% CI</td>
<td>Relative Risk</td>
<td>95% CI</td>
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<td>6.32</td>
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<td>7.25 - 8.67</td>
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<td>4.48 - 10.82</td>
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<td>13.05 - 25.55</td>
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<td>19.60 - 52.27</td>
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</table>
Characteristics of a Widespread Community Cluster of H275Y Oseltamivir-Resistant A (H1N1)pdm09 Influenza in Australia


1WHO Collaborating Centre for Reference and Research on Influenza, North Melbourne, Victoria; 2Hunter New England Population Health, Newcastle, New South Wales; 3Hunter Area Pathology Service, A Division of Pathology North, Newcastle, New South Wales, Australia; 4Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), Singapore; 5Centre for Asthma and Respiratory Disease, University of Newcastle, New South Wales, Australia; 6School of Biological Sciences, Nanyang Technological University (NTU); 7National Public Health Laboratory (NPHL), Ministry of Health, Singapore; 8Centre for Infectious Diseases and Microbiology Laboratory Services, ICPR, Westmead Hospital and University of Sydney, New South Wales; 9PathWest Laboratory Medicine, Nedlands, Western Australia; 10School of Pathology and Laboratory Medicine, University of Western Australia, Nedlands, Western Australia; 11National Centre for Immunisation Research and Surveillance of Vaccine Preventable Disease, Kids Research Institute, The Children’s Hospital at Westmead, New South Wales; 12Sydney Institute for Emerging Infections and Biosecurity (SEBI), University of Sydney, New South Wales, Australia; and 13Academic Unit of Child Health, Queen Mary University of London, London, United Kingdom

(See the editorial commentary by Fry and Gubareva, on pages 145-7.)

Background. Oseltamivir resistance in A(H1N1)pdm09 influenza is rare, particularly in untreated community cases. Sustained community transmission has not previously been reported.

Methods. Influenza specimens from the Asia-Pacific region were collected through sentinel surveillance, hospital, and general practitioner networks. Clinical and epidemiological information was collected on patients infected with oseltamivir-resistant viruses.

Results. Twenty-nine (15%) of 191 A(H1N1)pdm09 viruses collected between May and September 2011 from Hunter New England (HNE), Australia, contained the H275Y neuraminidase substitution responsible for oseltamivir resistance. Only 1 patient had received oseltamivir before specimen collection. The resistant strains were genetically very closely related, suggesting the spread of a single variant. Ninety percent of cases lived within 50 kilometers. Three genetically similar oseltamivir-resistant variants were detected outside of HNE, including 1 strain from Perth, approximately 4000 kilometers away. Computational analysis predicted that neuraminidase substitutions V241I, N369K, and N386S in these viruses may offset the destabilizing effect of the H275Y substitution.

Conclusions. This cluster represents the first widespread community transmission of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza. These cases and data on potential permissive mutations suggest that currently circulating A(H1N1)pdm09 viruses retain viral fitness in the presence of the H275Y mutation and that widespread emergence of oseltamivir-resistant strains may now be more likely.

Oseltamivir (Tamiflu®) is the most commonly used drug for the treatment or prophylaxis of influenza, and has been widely used in Japan, North America, and Europe. Oseltamivir has also been stockpiled by many countries as part of pandemic preparedness and was widely used during the 2009 influenza A(H1N1) pandemic, when it was shown to improve clinical outcomes in infants, adults, and pregnant women [1–6]. Since the emergence of the pandemic H1N1 2009 virus (A[H1N1]pdm09), oseltamivir resistance has been detected at a frequency of approximately 1%, with the majority of resistant viruses being isolated.
from immunocompromised patients undergoing oseltamivir treatment [7–9]. Virtually all of these resistant viruses have contained the histidine (H) to tyrosine (Y) substitution at position 275 of the neuraminidase (NA; N1 numbering—the same substitution is referred to as H274Y based on N2 numbering), which confers highly reduced oseltamivir sensitivity in vitro [10]. This substitution was also present in the oseltamivir-resistant variant of the prepandemic seasonal A(H1N1) subtype that emerged in Europe and then spread globally in less than a year in 2008 [11, 12]. This demonstrated the capacity of an A(H1N1) virus to overcome the inherently detrimental fitness effect of the H275Y substitution [13, 14] and transmit readily between individuals in the absence of drug selection pressure. Clinical data have shown that the effectiveness of oseltamivir was significantly reduced during the treatment of the previously circulating seasonal A(H1N1) H275Y variants [15–18].

Transmission of oseltamivir-resistant H275Y A(H1N1)pdm09 strains to date has been limited or unsustained. Most commonly, transmission has occurred in closed, near-contact settings, such as hospital wards [19–21], or after prolonged close contact in community settings such as a long train journey or at a school camp [22, 23]. Oseltamivir-resistant H275Y variants were detected at a low frequency (<1%) among community cases during the 2010–2011 northern hemisphere influenza season [24, 25] and, based on our testing, this continues to be the case for most countries in the southern hemisphere during the current 2011 influenza season. In contrast, between June and August 2011, in the Hunter New England region around Newcastle, Australia, we detected a significantly increased frequency of H275Y oseltamivir-resistant A(H1N1)pdm09 viruses in community patients, of whom only 1 patient had been treated with oseltamivir [26]. Following our initial brief report [26], here we describe the virological and epidemiological aspects of the cluster of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza and discuss potentially permissive NA substitutions that may have enabled the A(H1N1) pdm09 virus to retain viral fitness in the presence of the H275Y mutation.

MATERIALS AND METHODS

Sample Collection

Newcastle (population 273,805) is a regional coastal city in the state of New South Wales (NSW), and the sixth largest city in Australia, providing tertiary referral specialist services for northern NSW. It is located approximately 120 kilometers north of the NSW capital, Sydney (Figure 1). The Hunter New England (HNE) health district, of which Newcastle is the major city, stretches from Lake Macquarie in the south to the Queensland border. The HNE health district is served by Hunter Area Pathology Services (HAPS), which receive specimens for influenza testing from emergency departments, intensive care units, and general practitioners. Respiratory
specimens (swabs and nasopharyngeal aspirates) that were positive for influenza A or B by nucleic acid testing at HAPS in 2011 were referred weekly to the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza (WHO CCRI) for further virological analysis. A(H1N1)pdm09 viruses from the HNE health district were compared with viruses obtained from WHO National Influenza Centres that form part of the WHO Global Influenza Surveillance and Response System (GISRS), and other laboratories both within and outside of Australia.

To enable the review of oseltamivir-resistant viruses in the HNE health district, the NSW Chief Health Officer granted an ethics waiver and authorized the investigation under the NSW Public Health Act 2010 as an urgent public health investigation. For all patients with oseltamivir-resistant viruses, hospital records were reviewed and treating medical practices contacted to obtain details of clinical symptoms and outcomes, antiviral and concomitant treatment administered during the current and previous treatment episodes, and medical history. Patients infected with oseltamivir-resistant influenza viruses were interviewed using a structured questionnaire to gather data on patient demographics, medical history of immune-compromising conditions, influenza vaccination status in 2011, and antiviral treatment history.

Virological Analysis

Viruses were isolated from original clinical samples and repassed in Madin–Darby canine kidney cells (ATCC CCL-34). Antigenic characterization was performed using a hemagglutination-inhibition assay [27]. All virus isolates were analyzed for oseltamivir, zanamivir, and peramivir sensitivity using a fluorescence-based NA inhibition assay [28], followed by hemagglutinin (HA) and NA sequence analysis of those strains with elevated inhibitory concentration (IC50) values (the concentration of drug required to inhibit the NA activity by 50%). Where virus isolates could not be obtained, original specimens were analyzed for the presence or absence of the H275Y NA substitution using a pyrosequencing assay [29]. The HA and NA genes were fully sequenced using standard techniques on an ABI 3500XL sequencer. Sequences from oseltamivir-resistant strains were uploaded to a publicly available influenza sequence database (GISAID; www.gisaid.org), and given accession numbers EPI134765-334790 and 335634-335637. Maximum likelihood phylogenetic trees were constructed with PhyML (http://www.atgc-montpellier.fr/phyml) using HKY85 as the nucleotides substitution model with bootstrapping (Geneious Pro 5.1.4 software).

Computational Structural Analysis

Computational structural analysis of combinations of candidate permissive mutations in the NA was conducted using FoldX [31], an empirical all-atom force field that allows the calculation of protein stability changes upon mutation by approximating the free energy of unfolding through weighted terms. To validate the calculations, more than 1000 mutations representative of most structural environments were analyzed and achieved a global correlation of 0.8 with experimentally measured thermodynamic data. In this work, the A (H1N1)pdm09 NA crystal structure (PDB:3nss) was minimized with the RepairPDB function, then mutations were introduced separately or as cumulative combinations using FoldX in YASARA [32], where the stochastic side chain minimization procedure is repeated 5 times for each mutant and the averages are taken as final predicted free-energy change.

Results

Frequency of Oseltamivir Resistance

From the HNE health district, 2673 respiratory tract specimens were collected for influenza testing by nucleic acid testing (NAT) at HAPS in the period 20 May 2011 to 28 October 2011 (23 weeks) from a population of 874 644 (0.3%). Of the 749 influenza NAT-positive specimens, 439 (59%) were A(H1N1)pdm09 viruses (Figure 2) and 191 had sufficient volume to enable antiviral susceptibility analysis by the WHO CCRI. Twenty-nine (15%) of these 191 A(H1N1)pdm09 viruses were found to contain the H275Y NA substitution by either pyrosequencing of original specimens (n = 18) or conventional NA sequencing of isolates following an NA inhibition assay (n = 11). H275Y A(H1N1)pdm09 viruses were obtained from patients between 31 May and 19 August 2011 (Figure 3). The frequency of resistance over time was 4/51 (8%) in June, 20/85 (24%) in July, 4/45 (8%) in August, and 0/4 (0%) in September by which time influenza activity had become very low in the region (Figure 2). Twenty-six of the 29 patients infected with oseltamivir-resistant viruses lived in 5 adjacent local municipalities (Cessnock, Lake Macquarie, Maitland, Newcastle, Port Stephens) within 50 kilometers of Newcastle. The other 3 patients lived in rural towns 90, 150, and 490 kilometers away from Newcastle.

In comparison, only 5 oseltamivir-resistant H275Y viruses were detected out of 737 A(H1N1)pdm09 viruses tested (frequency, 0.7%) from the rest of Australia during 2011 (Figure 3). Two of these strains from January and March 2011 were from other Australian states (Queensland and Western Australia) and were isolated from hospitalized immunocompromised patients undergoing oseltamivir treatment. However, 2 oseltamivir-resistant H275Y viruses from elsewhere in NSW and 1 from Western Australia were collected during or after the period of oseltamivir-resistant virus detection in the HNE health district. One of the NSW oseltamivir-resistant viruses was detected in July 2011 from a child in Sydney, NSW, 120 kilometers south of Newcastle, while the other was detected in August 2011 from an infant in Orange, NSW, a town...
approximately 380 kilometers west of Newcastle. The Western Australian oseltamivir-resistant virus was detected in September 2011, 3 weeks after the last detected case in the HNE health district, and in a location near Perth, approximately 4000 kilometers west of Newcastle. All 3 of these resistant viruses were taken from otherwise healthy children who had not been treated with oseltamivir. Neither they nor their family members had traveled recently to Newcastle, and in the Western Australian case, there had been no recent contact with anyone from NSW.

Figure 2. Number of laboratory-confirmed influenza positive specimens detected in the Hunter New England health district during 2011.

Figure 3. Frequency of detection of oseltamivir-resistant H275Y variants in Australia in 2011. A red dot represents the sample date of an individual oseltamivir-resistant A(H1N1)pdm09 H275Y case, and a blue dot indicates the sample date of an individual oseltamivir-sensitive A(H1N1)pdm09 case detected in the states and territories of Australia in 2011.
Patient Details for HNE Health District Oseltamivir-Resistant Cases

Seventeen (59%) of the 29 patients infected with oseltamivir-resistant viruses were female and 3 (10%) were pregnant. Three (10%) identified themselves as Aboriginal. The age range was 4 months to 62 years (median, 31 years) compared with a median age of 29 years for persons infected with oseltamivir-sensitive influenza A(H1N1)pdm09. No cases resided in institutional care or aged care facilities. Cough was reported by 25 (86%) cases and fever by 22 (76%) cases. One patient was asymptomatic, but was swabbed and tested for influenza due to contact with a laboratory-proven A(H1N1)pdm09 case. Six (21%) cases reported a history of asthma; none had a history of chronic obstructive pulmonary disease; 6 (21%) were current smokers with an average pack-year history of 20 (range, 1–62) and none were immunosuppressed. Three (10%) cases had received trivalent-inactivated influenza vaccine in 2011. Importantly, only 1 patient infected with an oseltamivir-resistant virus had received oseltamivir prior to their specimen being collected (in this case, received 4 days prior to specimen being taken). Eleven (38%) were prescribed oseltamivir after specimen collection. Seven (24%) patients required hospital admission with a mean length of stay of 2.3 days (range, 1–7 days). There were no intensive care admissions and no fatal outcomes. The median number of days absent from usual duties was 5. Of the 29 patients infected with an oseltamivir-resistant virus, 5 pairs of cases were epidemiologically linked. Four households had 2 affected cases each, while a fifth pair of cases shared a short car journey. No other links could be identified during interviews with cases. Detailed outcomes were not collected for persons infected with oseltamivir-sensitive influenza.

Virological Analysis of Oseltamivir-Resistant Viruses

For the viruses that were isolated in cell culture, the oseltamivir-resistant H275Y strains (n = 11) had a mean (±1 SD) oseltamivir IC₅₀ of 205.3 ± 23.5 nM that was 513-fold higher than the mean IC₅₀ of oseltamivir-sensitive isolates (n = 3579) (Table 1). The H275Y isolates also demonstrated elevated peramivir IC₅₀ values (16.6 ± 1.5 nM) that were approximately 80-fold higher than sensitive strains, but were fully sensitive to zanamivir (Table 1). The oseltamivir-resistant viruses were also resistant to the adamantane class of influenza antiviral drugs based on M2 sequencing, having the characteristic S31N substitution. These H275Y variants, including those from the 3 patients that received the influenza vaccine in 2011, were antigenically indistinguishable from the oseltamivir-sensitive viruses and were well inhibited by ferret serum samples raised against the current A(H1N1)pdm09 vaccine strain A/California/7/2009 in a hemagglutination-inhibition assay (Supplementary Table 1). Phylogenetic analysis of the hemagglutinin and NA sequences revealed that the oseltamivir-resistant viruses from Table 1.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Zanamivir Mean IC₅₀ ± SD (nM)</th>
<th>Zanamivir Fold Diff*</th>
<th>Oselamivir Carboxylate Mean IC₅₀ ± SD (nM)</th>
<th>Oselamivir Carboxylate Fold Diff*</th>
<th>Peramivir Mean IC₅₀ ± SD (nM)</th>
<th>Peramivir Fold Diff*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean of HNE health district H275Y A(H1N1) pdm09 viruses (n = 11)</td>
<td>0.3 ± 0.02</td>
<td>1</td>
<td>205.3 ± 23.5</td>
<td>513</td>
<td>16.6 ± 1.5</td>
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<td>Mean of HNE health district sensitive A(H1N1) pdm09 viruses (n = 66)</td>
<td>0.4 ± 0.2</td>
<td>1</td>
<td>0.5 ± 0.3</td>
<td>1</td>
<td>0.2 ± 0.1</td>
<td>1</td>
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<tr>
<td>Mean of all sensitive A(H1N1) pdm09 viruses (n = 3579)</td>
<td>0.3 ± 0.2</td>
<td>...</td>
<td>0.4 ± 0.3</td>
<td>...</td>
<td>0.2 ± 0.1b</td>
<td>...</td>
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<tr>
<td>A/Newcastle/2/2011</td>
<td>0.3 ± 0.02</td>
<td>1</td>
<td>191.1 ± 19.4</td>
<td>478</td>
<td>18.6 ± 6.8</td>
<td>93</td>
</tr>
<tr>
<td>A/Newcastle/17/2011</td>
<td>0.3 ± 0.04</td>
<td>1</td>
<td>223.7 ± 14.2</td>
<td>559</td>
<td>18.0 ± 2.3</td>
<td>90</td>
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<tr>
<td>A/Newcastle/37/2011</td>
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<td>16.9 ± 0.8</td>
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<td>A/Newcastle/53/2011</td>
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<td>187.8 ± 30.0</td>
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<td>13.8 ± 1.2</td>
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<td>199.9 ± 19.7</td>
<td>500</td>
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<td>496</td>
<td>15.2 ± 1.5</td>
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<td>226.6 ± 11.4</td>
<td>567</td>
<td>16.7 ± 1.3</td>
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<tr>
<td>A/Newcastle/89/2011</td>
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<td>1</td>
<td>227.8 ± 13.1</td>
<td>569</td>
<td>16.9 ± 1.8</td>
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<td>1</td>
<td>198.1 ± 26.8</td>
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<td>15.8 ± 1.5</td>
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<td>79</td>
</tr>
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<td>165.9 ± 26.1</td>
<td>415</td>
<td>18.8 ± 2.7</td>
<td>94</td>
</tr>
</tbody>
</table>

Abbreviations: Diff, difference; HNE, Hunter New England; IC₅₀, inhibitory concentration.

* Based on comparison with mean IC₅₀ of all sensitive A/H1N1 pdm09 viruses.

b Mean and standard deviation of peramivir IC₅₀ values based on analysis of n = 273 isolates.
the HNE health district, the 2 other NSW viruses detected in Sydney and Orange, and the Western Australian strain from September 2011 were all highly similar (99.9% nucleotide similarity across 22 HA and 18 NA sequences) and formed a distinct subgroup in both the HA and NA phylogenetic trees (Figure 4). Apart from the H275Y NA substitution, the oseltamivir-resistant strains all contained 2 other NA amino acid substitutions, V62I and N386S, which were absent from all but 1 of the local oseltamivir-sensitive viruses (A/Newcastle/84/2011) (Figure 4). In addition to the NA substitutions, whole-genome sequence comparison of 8 oseltamivir-resistant strains and 5 oseltamivir-sensitive strains from the HNE health district revealed 3 further substitutions across 3 other segments that were specific to the resistant strains—E129K in HA, L672F in PA, and S482N in NP. Genetic comparison of the 2 viruses from each of the linked cases showed 100% nucleotide similarity across the HA and NA genes, although sequence data were not available for viruses from 2 of the paired cases. In addition, other oseltamivir-sensitive A(H1N1)pdm09 strains, including A/South Australia/24/2011 and A/Hong Kong/2973/2011, clustered phylogenetically with the resistant strains (Figure 4), sharing a high degree of genetic similarity, except for the H275Y NA substitution. Full phylogenetic trees comparing the HNE health district sequences with publically available 2011 A(H1N1)pdm09 HA and NA sequences are shown in the supplementary data (Supplementary Figure 1A and 1B).

Computational Analysis of Potentially Permissive NA Substitutions

To investigate the role of potentially permissive NA mutations that may be responsible for offsetting the negative effects of the H275Y substitution, a computational analysis of NA protein stability was conducted. Since the emergence of the A(H1N1)pdm09 strain in 2009, a number of NA mutations have been described and, in some cases, have become fixed.

Figure 4. Phylogenetic trees of hemagglutinin (A) and neuraminidase (B) sequences of oseltamivir-resistant H275Y variants and oseltamivir-sensitive wild type A(H1N1)pdm09 viruses. Hunter New England health district and other NSW and Western Australian H275Y variants are shown in red, sporadic H275Y mutants from other regions in green, and Hunter New England health district sensitive viruses in blue. Amino acid mutations common to each clade and bootstrap confidence values >75 are indicated on the trees.
within globally circulating strains (Figure 5). Based on computational analysis, 2 of these mutations, V241I and N369K, both of which emerged in A(H1N1)pdm09 viruses in 2010 and are now present in over 80% of currently circulating strains (Figure 5), could restore approximately 50% of the protein stability that was lost as a result of the H275Y mutation (Figure 6). In addition, the HNE health district and other NSW oseltamivir-resistant viruses contained an N386S NA substitution. This substitution is less common in other A (H1N1)pdm09 viruses, but computational analysis suggested that it would also further stabilize the NA (Figure 6). Another NA substitution that has been on the rise in recent strains and was present in the oseltamivir-resistant viruses described here is N44S. It creates a new potential N-glycosylation site at position 42, changing the motif at positions 42–44 from NQN to NQS. However, this region of the NA is in the stalk and not part of the globular domain and, therefore, is not known how it would affect NA structure and stability.

Discussion

The H275Y variants detected in the HNE health district, other parts of NSW, and in Western Australia represent the largest and most widespread cluster of oseltamivir-resistant A(H1N1)pdm09 influenza identified since the virus first emerged in humans in 2009. The oseltamivir-resistant strains detected from these locations were virtually identical genetically, suggesting emergence from a single source.

The rapid global spread of oseltamivir-resistant seasonal A (H1N1) H275Y viruses during 2007–2008 [11, 12] showed that A(H1N1) influenza viruses with this substitution could retain transmissibility, even though previous in vitro and in vivo studies found the substitution reduced fitness in other A (H1N1) strains [13, 14]. A recent study demonstrated that seasonal A(H1N1) oseltamivir-resistant H275Y viruses exhibited reduced susceptibility to postvaccination antibody inhibition compared to the cocirculating oseltamivir-sensitive viruses.

Figure 5. All human A(H1N1)pdm09 NA sequences containing date information (at least year/month) were downloaded from the National Center for Biotechnology Information influenza virus resource [41] (http://www.ncbi.nlm.nih.gov/genomes/FLU/) and GISAID (http://www.gisaid.org) and merged with the ones reported in this study (keeping only 1 per unique strain identifier). Sequences shorter than 90% of the median length are considered as fragmentary and were removed from the analysis. The resulting 8085 sequences were then aligned with the multiple sequence alignment program MAFFT [42] and mutation frequencies relative to the vaccine reference strain A/California/07/2009 counted with a custom perl script. All mutations with at least 50 occurrences since March 2009 and global frequencies >20% in any month are shown, plus L415M, which is characteristic for an outbreak with H275Y in Japan in January. Data collected in the month of March 2009 were merged with April 2009, while data collected from September 2011 were merged with August 2011 as there were less than 10 sequences in those months.
which may have contributed to the virtual replacement of the oseltamivir-sensitive seasonal A(H1N1) strain within 1 year. Based on antigenic analyses with postinfection ferret serum samples, the oseltamivir-resistant A(H1N1)pdm09 H275Y viruses reported here are antigenically similar to oseltamivir-sensitive A/California/7/2009-like A(H1N1)pdm09 strains circulating in the HNE health district and elsewhere in Australia, although future studies using postvaccination human serum should be performed [30]. The high frequency of resistance in untreated community patients suggests that they are not markedly less fit than sensitive viruses. This situation is similar to the oseltamivir-resistant seasonal A(H1N1) viruses that emerged in Norway in 2007 where, like Australia, the use of oseltamivir was low compared to countries such as Japan and the United States [31].

Recently, it has been shown that certain substitutions in the NA, V234M and R222Q, enabled the seasonal A(H1N1) virus to remain fully functional in the presence of the H275Y substitution [32, 33]. These permissive substitutions are not present in the A(H1N1)pdm09 oseltamivir-resistant H275Y variants reported here. Most A(H1N1)pdm09 sequences (including the strains reported here) have the nonpermissive V at position 234, and the most typical amino acid at position 222 among the A(H1N1)pdm09 NAs is N. However, 3 other NA substitutions, V241I, N369K, and the N386S, which were present in the oseltamivir-resistant strains from the cluster reported here, may offset the destabilizing effect of the H275Y substitution. The V241I and N369K substitutions are present not only in the H275Y variants reported here, but are also in viral sequences of North American and a large number of Japanese oseltamivir-resistant H275Y strains recently deposited on the GISAID sequence database (Supplementary Figure 1A and 1B). Importantly, the N369K substitution, which computationally was predicted to cause the largest change in protein stability, has previously been shown experimentally to increase NA surface expression and activity in combination with H275Y [32].

Infection with oseltamivir-resistant seasonal A(H1N1) viruses significantly reduced the effectiveness of oseltamivir during the 2008–2009 season, particularly in younger children [15–18]. For patients infected with oseltamivir-resistant viruses, fever levels were comparable between oseltamivir-treated and untreated patients, but significantly reduced in zanamivir-treated patients [15]. Mean duration of fever after the start of oseltamivir treatment was also significantly longer in persons infected with oseltamivir-resistant viruses than in those infected with oseltamivir-sensitive viruses [15, 17, 18], while treatment of the H275Y viruses with zanamivir reduced fever duration to normal levels [18]. Although the relationship between in vitro IC50 of a virus and clinical effectiveness is not well understood, it is noteworthy that the mean oseltamivir IC50 of the H275Y A(H1N1)pdm09 viruses is generally lower than that of the H275Y seasonal A(H1N1) variants [12, 34, 35], and is below the steady-state trough plasma concentrations found in children, adolescents, and adults following recommended dosing [36]. As a result, oseltamivir treatment may be more effective for the A(H1N1)pdm09 H275Y variant than has been reported for the H275Y seasonal A(H1N1) variants.

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Computational structural analysis of combinations of candidate permissive mutations in the neuraminidase (NA). Mutations were modeled with FoldX in the A(H1N1)pdm09 NA crystal structure (PDB:3nss) after minimization and using 5 repetitions.
More data regarding the effectiveness of oseltamivir for the treatment of A(H1N1)pdm09 H275Y viruses, and other variants with reduced oseltamivir sensitivity, are needed. Eleven of the oseltamivir-resistant HNE health district cases received oseltamivir after specimen collection but, because the resistant virus was detected after the patient had recovered and treatment had ceased, it was not possible to investigate the effectiveness of oseltamivir. The effectiveness of peramivir is also likely to be reduced for the treatment of H275Y A(H1N1)pdm09 viruses given the 80-fold increase in IC₅₀ [37], as such current WHO guidelines state that intravenous peramivir should only be considered for the treatment of such viruses when intravenous zanamivir is not available [38]. However, recent animal data suggest that 5 days of therapy with intravenous peramivir may overcome the shift in sensitivity to peramivir caused by the H275Y substitution [39]. Given that the H275Y variant is fully susceptible to zanamivir in vitro, this drug remains the recommended alternative antiviral treatment option when oseltamivir resistance is detected [38].

Of the 29 H275Y A(H1N1)pdm09 viruses detected in the HNE health district, none caused severe illness or resulted in patients being admitted to intensive care wards. However, because detailed clinical or epidemiological information was not collected at the same time from a comparison group of patients with oseltamivir-sensitive virus infections, it was not possible to determine whether there were any differences between the types of patients infected or the types of illness caused by oseltamivir-sensitive and -resistant viruses. Another limitation is that a relatively small number of A(H1N1)pdm09 viruses were available for testing (191 from the HNE health district and 737 from the rest of Australia). Therefore, not only may the true prevalence of resistance be different to that reported here, but the spread of the genetically related oseltamivir-resistant virus may also be wider than detected.

Influenza activity in the HNE health district and the rest of Australia has now returned to baseline levels, although the detection of the variant outside the HNE health district is concerning and demonstrates its capacity to spread widely. Oseltamivir-resistant strains that were indistinguishable to those detected in the HNE health district were identified in Sydney, the largest city in Australia and a large international travel hub, and near Perth, Western Australia, on the other side of the country to Newcastle. To date, there is no indication that this oseltamivir-resistant A(H1N1)pdm09 strain has spread into the northern hemisphere, although it will be important for countries to monitor strains throughout the upcoming northern hemisphere influenza season. The most recent A(H1N1)pdm09 sequences strains deposited on GISAID at the time of writing are from the United States (Hawaii, California, Pennsylvania), and although none of them have the H275Y substitution, they all have the NA substitutions V241I and N369K, which may buffer the detrimental effect of the H275Y oseltamivir-resistance mutation. The other non-NA amino acid substitutions in the oseltamivir-resistant viruses from the cluster (HA, PA, and NP) may also play a role in viral fitness. In vitro and in vivo studies are currently in progress to analyze the fitness of the strains from this cluster and investigate the role of these potentially "permissive" mutations. However, this cluster of cases, along with the recent reports of increased detection of H275Y A(H1N1)pdm09 viruses in untreated community patients in the United Kingdom and United States [24, 40] and the large number of recent sequences with the H275Y substitution deposited on GISAID from Japan, suggests that the currently circulating A(H1N1)pdm09 may be becoming more tolerant of the H275Y mutation than it was when the strain first emerged in 2009, and that widespread emergence of oseltamivir-resistant A(H1N1)pdm09 viruses may now be more likely.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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