

4th Australian Influenza Symposium

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Australian Influenza Symposium 2008

Thursday 9 October
8:30-9:20 Registration

9:20 Welcome: Ian Barr & Gary Grohmann
Opening Comments
9:30 **Plenary Session 1.** Chair: Anne Kelso
9:30 Heiman Wertheim. Re-emergence of cases of human H5N1 in Vietnam.
10:20 John Spika. Influenza pandemic preparation in Canada

11:00 Morning tea

11:30 **Plenary session 2. Influenza antivirals.** Chair: Robert Booy
11:30 Elena Govorkova. Combination Therapy for Control of H5N1 Virus Infection: Preclinical Data
12:00 Aeron Hurt. Antiviral resistance in seasonal influenza viruses
12:30 David Reddy. Roche. An Update on Tamiflu

1:00 Lunch

1:45 **Advances in vaccines for seasonal and pandemic influenza**
(seasonal and pandemic)
Chair: Gary Grohmann
1:45 Victor Carey, Sanofi Pasteur - Intradermal Influenza Vaccination
2:05 Otfried Kistner, Baxter, Cell cultured influenza vaccines
2:25 David Ryan, CSL Ltd, Pandemic influenza vaccine development
2:45 Manon Cox, Protein Sciences, FluBlok, an update on the clinical development and regulatory status in the US
3:05 Robert Booy, Control of influenza at the extremes of life

3:30 Afternoon tea

Please note there will be a memorial service for Graeme Laver in the Common room at University House at ANU from 3:30-4:30pm.

4:00 **Workshop 1.** (Selected from abstracts 7x15min)
Chairs: David Anderson & Tuckweng Kok
4:00 Mark Kendall, Nanopatches for targeted delivery of influenza to the skin
4:15 Euan Tovey. Respiratory viruses including influenza are aerosolized
4:30 Karen Wong, The fidelity of PB1 subunit of influenza A ...
4:45 Rebecca Attwood, A mutation at a non-conserved residue Y155H ...
5:00 Mohammed Alsharifi, Intranasal flu vaccine protective against seasonal.
5:15 Jeff Butler, A pathogenic comparison of 2 phylogenetically distinct H5N1
5:30 Anna Axell, Antigenic mapping of Indonesian HPAI isolates at AAHL
5:45 Frank Wong. OFFLU study of current H5N1 HPAI isolates in Indonesia.

6:00 Day one concludes

7:30 Symposium Dinner - free for all registrants

Influenza Vaccination in Australia – Yesterday, Today and Tomorrow.

Alan W. Hampson

¹Chairman Influenza Specialist Group
²Honorary Senior Research Fellow, School of Applied Sciences and Engineering, Monash University.

The use of influenza virus vaccine in Australia predated that in most other countries, commencing in 1944, initially for the military and then progressively for civilian use. However, apart from the pandemic years of 1957 (1.6 million doses) and 1969 (4.8 million doses) vaccine use remained low (around 0.5 million doses/year) through into the 1980s. In 1990 media predictions of a coming epidemic drove up demand and resulted in vaccine shortages. The resulting recriminations heaped on government, vaccine companies and the health care industry at large prompted a review which resulted in promotion of vaccination by some state departments of health and the evolution of a group, now known as the Influenza Specialist Group (ISG), that has been a major force in promoting vaccine according to the NH&MRC guidelines. During the following decade, per capita influenza vaccine use in Australia increased over five-fold and vaccination rates in a key target group, those aged 65 and over, increased from less than 30% to 74% coverage. In 2001 the Commonwealth Government introduced free influenza vaccine for those aged 65 and over and vaccination rates are now approaching 80% coverage. However, surveys showed little increase in vaccination of a second major risk group, those people under the age of 65 with pre-disposing risk conditions, where only 42% were vaccinated. The ISG has been active in assessing the reasons for poor uptake, directed awareness activities and in lobbying for increased vaccine availability for this group. A recent recommendation by the PBAC to extend the provision of free vaccine to these high-risk individuals may be introduced shortly by the Australian Government. Monitoring of the proposed approach for success will be essential and may help guide future program changes. While targeting those at greatest risk of severe illness or death from influenza can ameliorate some of the impact of the disease, the introduction of a universal free vaccine program as in Ontario Canada, or targeting infants and children as in the USA may eventually offer a greater level of seasonal influenza control and needs to be kept under consideration in the Australian context.

Seasonal influenza programs in Canada – Can we reach a consensus?

John Spika

Acting Director General, Centre for Immunization and Respiratory Infectious Diseases, Public Health Agency of Canada, Canada.

Friday 10 October.

8:30 Plenary session 3.

Chair: Jenean Spencer

8:30 Jodie McVernon Modeling strategic use of the national antiviral stockpile during the CONTAIN and SUSTAIN phases of an Australian pandemic influenza response.

8:55 Paul van Buynder. WA Influenza Vaccine Effectiveness Study in children (WAIVE): Recruitment, Conduct & Preliminary outcomes

9:20 Peter Kirkland. Lessons for a human pandemic from the Australian 2007 equine influenza outbreak

9:45 David Fedson. Generic agents for pandemic treatment

10:00 Morning Tea

10:30 Workshop 2 (Selected from abstracts - 8x15 min)

Chairs: Jodie McVernon & Paul Van Buynder

10:30 Heath Kelly, Estimation of influenza vaccine effectiveness from routine ..

10:45 Craig Dalton, Flutracking: A weekly Australian online community survey

11:00 Suzanne Schindeler, Syndromic surveillance of respiratory illness...

11:15 Sam Beckett, AUSFLU spatial simulation and social contact networks ...

11:30 Joel Kelso, A small community model for the transmission of infectious..

11:45 Jan Gralton, Protecting healthcare workers from pandemic influenza ...

12:00 Sheena Adamson, Diagnostic influenza serology; seasonal,avian,pand.,

12:15 Paul Selleck, Neutralizing antibodies against avian H5N1 influenza

12:30 Lunch

1:15 Open forum. Moderators: Anne Kelso & Heath Kelly

Panel: John Spika, Elena Govorkova, Jodie McVernon, David Fedson, Heiman Wertheim, Peter Kirkland.

Potential Topics

- Pandemic preparation (antivirals, vaccines, who, when, why??)
- Improving vaccination rates, Immunising kids, 50-64 adults
- Improving outbreak detection/investigation
- Clinical trials for influenza/pandemic matters in Australia/NZ
- Cell cultured influenza vaccine
- Antivirals, usage and resistance
- Surveillance issues. use of rapid tests for influenza
- Vet issues:. controlling H5, equine influenza
- Research questions

2:15 Plenary session 4.

Chair Ian Barr

2:15 John Spika. Seasonal influenza programs in Canada - can we reach a consensus?

2:50 Alan Hampson. Influenza vaccination in Australia – Yesterday, Today and Tomorrow

3:10 Closing comments

3:15 Workshop concludes.

Re-emergence of cases of human H5N1 in Vietnam.

Heiman Wertheim,

Clinical Microbiologist, Oxford University Clinical Research Unit, Hanoi, Vietnam.

Neutralizing Antibodies against Avian H5N1 Influenza in a Normal 'Exposure-Naïve' Australian Blood Donor Population

Paul W. Selleck³, Garry W. Lynch^{*1,2}, Anna-Maree Axell³, Teena Downton¹, Natalie M. Kapitza¹, Ingrid Boehm², Wayne Dyer^{1,2}, Ying-Fan Yvonne Wang¹, Sacha Stelzer⁴, William Rawlinson⁴ and John S Sullivan^{1,2}

¹The Transfusion Immunobiology and Biosafety Group, Transfusion Medicine and Immunogenetics Research, The University of Sydney, Camperdown, Sydney and ²Research & Business Development, The Australian Red Cross Blood Service, 153 Clarence St, Sydney, NSW 2000, Australia; ³The Australian Animal Health Laboratories, CSIRO, Geelong, Victoria, Australia; and the ⁴Virology Division, Department of Microbiology, South Eastern Area Laboratory Service, Prince of Wales Hospital, Randwick, NSW 2031, Australia.

The H5N1 highly pathogenic avian influenza virus presently circulating in birds across Asia, The Middle East and Africa represents the greatest potential threat for the next human influenza pandemic. In order to better understand the influence of "herd immunity" to influenza virus infections in a naive human population we tested the ability of Australian intravenous immunoglobulins (IVIGs) and individual sera to react with a range of influenza A viruses. IVIG's are highly purified antibody therapeutics derived from large pools of plasmas from thousands of normal blood donations. In this study we have identified antibodies in IVIGs and individual sera that bind to both H5N1 surface envelope and internal viral proteins. As this reactivity is removed by adsorption with purified H3N2 and H1N1 strains, anti-H5N1 cross-reacting heterotypic antibodies are implicated. Most important is the finding that these antibodies neutralize H5N1 avian influenza clade I, clade II and human-derived H5N1 isolates in vitro. Our findings suggest that some individuals do contain low levels of specific and neutralizing anti-H5N1 antibodies. The age-dependent neutralization of H5N1 viruses observed may explain the age-biased case fatality rate seen in human H5N1 infections in Asia.

Diagnostic influenza serology: seasonal, avian, pandemic

Adamson S, Armstrong P, Rawlinson W.

Direct testing of respiratory specimens for influenza virus is the method of choice for diagnosis of acute influenza infection. Indirect testing by serology may be useful when respiratory specimens have not been collected, are unsuitable, or clinical presentation is too late for detection of influenza virus, for epidemiological surveys and for determining the rate of subclinical infection. Such tests could also be adapted to quantitate anti-influenza antibody. The serological test must be fit-for-purpose, and may be type, sub-type or strain specific. Selection of the method will depend on the purpose, and factors such as the required sensitivity and specificity for a screening or confirmatory assay, turnaround time, number of specimens per assay, assay complexity and performance mode (manual, semi or fully automated), and availability of appropriate reagents. Methods available include complement fixation (CF), enzyme immunoassay (EIA), haemagglutination inhibition (HI) and neutralisation (NT). In the animal sector, type or sub-type specific influenza serology is used to detect an immune response due to infection or vaccination in a naïve population, and may also differentiate those naturally infected from vaccinees. In the EIA used in the recent equine influenza outbreak, influenza A nucleoprotein was used as antigen and so only immune responses due to natural infection, but not those due to vaccination with recombinant haemagglutinin, were detected.

Type-specific serology is used for diagnosis of seasonal influenza infection in humans, but there are problems which include (i) reinfection due to antigenic drift of the influenza virus, requiring differentiation of an immune response to recent infection from prior vaccination or infection, (ii) technical difficulties with EIAs, the results of which often do not correlate well with clinical history eg due to use of split virion antigen, rather than nucleoprotein, rendering them susceptible to changes in circulating virus, (iii) currently available CF assays use nucleoprotein antigen, and are complex, subjective and have low sensitivity. However, CF remains the method of choice in Australia as results correlate with clinical history, presumably due to detection of complement fixing antibodies in an acute immune response, particularly IgM and IgG3. EIAs for detection of acute immunoglobulin isotypes (specific IgM, IgA, IgG3) are preferable as these assays are simple, objective, more sensitive and can be automated. The potential roles of serology in an influenza pandemic have not been agreed, and will depend upon the stage of the pandemic. During the early phases of 'delay' and 'contain', diagnosis of pandemic influenza will be by direct testing. Serology specific for the pandemic strain could have a role later in the pandemic in determining those who have been infected, or (depending on the nature of the pandemic vaccine used) could be used to differentiate immune response due to vaccination from natural infection for epidemiological studies. It has been suggested that demonstration of specific antibody, whether due to natural infection or vaccination, could be used to screen healthcare workers prior to caring for infected patients, or to determine if antiviral prophylaxis may be discontinued. However, a detectable antibody response may not correlate with protection against infection or disease. Once the primary role of serology is decided, appropriate methods can be developed for the purpose eg limited HI for small scale testing or large scale EIA for mass screening or sero-epidemiology.

In conclusion, diagnostic serology for seasonal influenza using complement fixation would be better replaced by automated EIA, but results from current EIAs are not clinically useful. The role of serology in an influenza pandemic needs to be decided so that planning for deployment of appropriate assays can occur.

Influenza pandemic preparation in Canada.

John Spika

Acting Director General, Centre for Immunization and Respiratory Infectious Diseases, Public Health Agency of Canada, Canada.

Combination Therapy for Control of H5N1 Virus Infection: Preclinical Data

Ilyushina NA, McClaren JL, Boon ACM, Webster RG, and Govorkova EA

St. Jude Children's Research Hospital, Memphis, TN 38105-2794, USA

Influenza A (H5N1) viruses are currently of the greatest public health concern. Antiviral drugs will be an important intervention strategy at early stages of influenza pandemic when strain-specific vaccines are unavailable. Because the disease caused by highly pathogenic H5N1 influenza viruses can be very severe, current treatment strategies approved for seasonal influenza may be not optimal.

We assessed the effectiveness of two drug combinations against highly pathogenic H5N1 influenza viruses: (1) neuraminidase (NA) inhibitor (oseltamivir) and M2-ion channel blocker (amantadine); (2) oseltamivir and an inhibitor of influenza virus polymerases (ribavirin). *In vivo*, combinations of oseltamivir and amantadine showed markedly better protection from lethal infection than does monotherapy ($P < 0.05$), completely inhibited virus replication in the lungs of mice infected with rg-A/Vietnam/1203/04_{sens} and inhibited virus spread to the brain. *In vitro*, after five sequential passages in MDCK cells, the M2 protein of H5N1 virus cultivated with amantadine alone mutated at positions V27A and S31N/I. Virus cultivated with oseltamivir carboxylate ($\geq 0.001 \mu\text{M}$) possessed mutations in the hemagglutinin (HA) protein. Importantly, no mutations in the HA, NA, and M2 proteins were detected when the drugs were used in combination.

The combinations of ribavirin at 37.5 mg/kg/day and 1 or 10 mg/kg/day of oseltamivir were synergistic against A/Vietnam/1203/04 and A/Turkey/15/06 virus infection in mice, respectively. These optimal oseltamivir-ribavirin combinations significantly inhibited virus replication in mouse organs, prevented the spread of H5N1 viruses beyond the respiratory tract, and abrogated the cytokine response ($P < 0.01$). We observed clear differences between the efficacy of drug combinations against two H5N1 viruses: higher doses were required for the protection of mice against A/Turkey/15/06 virus than for A/Vietnam/1203/04 virus.

We conclude that understanding of the optimal strategies of antiviral therapy for infection with highly pathogenic influenza viruses and clinical significance of drug-resistant variants have important implications for public health.

This study was supported by the NIAID, NIH and HHS under Contract No. HHSN266200700005C and by the ALSAC.

Protecting healthcare workers from pandemic influenza: P2 or surgical masks?

J Gralton, M-L McLaws, W Rawlinson

Background

The successful management of influenza pandemic planning is reliant on the expertise of healthcare workers (HCWs). Yet, paradoxically HCWs will be at high risk for occupationally-acquired influenza. Recommended masks include surgical masks that protect against droplet-spread respiratory transmissible infections, such as meningococcal infections, and P2 masks for aerosol-spread infections, such as chicken pox. Contention exists about the possibility of aerosol transmission of influenza based on rare findings of unique outbreak situations, such as the Alaskan Airline outbreak. The interim *Australian Management Plan for Pandemic Influenza* does not yet specify the type of mask to be worn. A literature review was undertaken to determine whether one mask type would provide significantly superior protective value for HCWs.

Methods

Four scientific search engines using 12 search sequences identified publications for review with further exclusions made where studies populations were not healthcare workers or of comparable age groups. Twenty-five publications were critically assessed in accordance with NHMRC for study design, study bias and potential confounders.

Results

With the exception of one RCT study all used medium or lower evidence level designs. Important confounders included "bundling" of other infection control measures, mask compliance and improper use. Outbreaks, such as SARS, were opportunistically used to suggest the protective nature of the different mask types. However, none tested both mask types comparatively. Laboratory studies identified P2 masks as theoretically providing superior protection against influenza yet these findings are not easily generalisable to healthcare settings.

Conclusion:

The World Health Organization recommends surgical masks for all patient care with the exception of P2 for aerosol generating procedures, such as intubation and suctioning. Because of the paucity of good quality studies the current guidelines cannot be disputed on evidence-based grounds.

A Small Community Model for the Transmission of Infectious Diseases: Comparison of School Closure as an Intervention in Individual-based Models of an Influenza Pandemic

George J Milne¹, Joel K Kelso^{1*}, Heath A Kelly^{2,3}, Simon T Huband¹, Jodie McVernon³

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² Epidemiology Unit, Victorian Infectious Diseases Reference Laboratory, Carlton, Victoria, Australia

³ School of Population Health, University of Melbourne, Carlton, Victoria, Australia

* Presenter and Corresponding Author

Background. In the absence of other evidence, modelling has been used extensively to help policy makers plan for a potential future influenza pandemic.

Methods / Results. We have constructed an individual based model of a small community in the developed world with detail down to exact household structure obtained from census collection datasets and precise simulation of household demographics, movement within the community and individual contact patterns. We modelled the spread of pandemic influenza in this community and the effect on daily and final attack rates of four social distancing measures: school closure, increased case isolation, workplace non-attendance and community contact reduction. We compared the modelled results of final attack rates in the absence of any interventions and the effect of school closure as a single intervention with other published individual based models of pandemic influenza in the developed world.

We showed that published individual based models estimate similar final attack rates over a range of values for R_0 in a pandemic where no interventions have been implemented; that multiple social distancing measures applied early and continuously can be very effective in interrupting transmission of the pandemic virus for R_0 values up to 2; and that different conclusions reached on the simulated benefit of school closure in published models appear to result from differences in assumptions about the timing and duration of school closure and flow-on effects on other social contacts resulting from school closure.

Conclusion. Models of the spread and control of pandemic influenza have the potential to assist policy makers with decisions about control strategies but attention needs to be given by policy makers to the assumptions about control strategies in these models.

Antiviral resistance in seasonal influenza viruses

Aeron C Hurt^{1,2}

¹ WHO Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia

² Monash University, Churchill, Victoria, Australia

There are currently two classes of influenza antiviral drugs available for the prophylaxis or treatment of influenza: the adamantanes, or M2 ion channel inhibitors, and the neuraminidase inhibitors (oseltamivir, Tamiflu™ or zanamivir, Relenza™). Analysis of seasonal influenza strains circulating in Australasia, South East Asia and the South Pacific has revealed alarmingly high frequencies of adamantane resistance in A(H3N2) and A(H1N1) viruses since 2005, whilst a high frequency of oseltamivir resistance has been observed in A(H1N1) viruses since mid-2008. Although adamantane resistance is reasonably common in treated patients, levels of resistance in circulating strains (untreated population) was low prior to 2003 (<1%). However a significant increase in the frequency of adamantane resistance in A(H3N2) viruses was observed in Asia during 2003 and in the USA in 2004, after which large numbers of adamantane resistant A(H3N2) viruses circulated globally. Increases in adamantane resistance have also been observed in A(H1N1) viruses in recent years. The frequency of adamantane resistance in Australian viruses remains high (x% for A(H3N2) and y% for A(H1N1) viruses). The level of resistance to oseltamivir, the most widely used neuraminidase inhibitor, in circulating viruses prior to 2007 was low (<1%). However reports from Europe and USA in late 2007 revealed increased oseltamivir resistance in viruses from untreated patients, with frequencies as high as 60% in some countries. Since the 2008 southern hemisphere influenza season, high frequencies of oseltamivir resistant strains (up to 100%) have circulated in the untreated community in South Africa, Australia, the Philippines and New Caledonia, however resistance remains low in other countries such as Thailand, Taiwan, Malaysia and Macau. The observed resistance is associated with a H274Y mutation in the neuraminidase, which although impacts on oseltamivir binding, has no effect on zanamivir binding, meaning the viruses remain fully sensitive to this antiviral drug. The currently high levels of adamantane and oseltamivir resistance seen in circulating strains in the absence of drug pressure is of significant concern and may suggest the need for the future development of new classes of influenza antiviral drugs.

An Update on Tamiflu

David Reedy,

F.Hoffmann-La Roche Ltd., Pharmaceuticals Division, Basel, Switzerland.

AUSFLU spatial simulation and social contact networks modelling environment for pandemic influenza

Dr Sam Beckett, Dr Graeme Garner, Prof Pip Pattison, A/Prof Garry Robins, Dr Moira McKinnon, Dr Rochelle Watkins, [Prof Aileen Plant]

AUSFLU is a simulation modelling environment for influenza that encompasses a range of models and modelling philosophies. These range from simple models with few assumptions, to very detailed spatial simulation models and social contact networks models. AUSFLU simulations can be customised to enable public health policy analysts to examine a wide range of questions relating to the epidemiology of influenza or its control. The more complex models include transmission pathways for households, schools, workplaces, churches, hospitals, general practices and medical centres and a range of opportunities for social and cultural mixing. Each of these can be activated, de-activated or otherwise configured to examine its influence on the development of an epidemic. AUSFLU also contains a wide range of non-pharmaceutical and pharmaceutical intervention strategies, including measures for personal biosecurity, movement restrictions, school closures, fever clinics, antivirals and vaccination. Each of these can be activated, de-activated or otherwise configured to particular subsets of the population, and the model thus used to examine the effectiveness or efficiency of intervention strategies.

AUSFLU is an interactive Windows-based modelling environment, and requires no programming nor specialist technical skills to operate. It was written in MapBasic and runs within MapInfo Professional GIS. This provides access to spatial functionality and mapping; all of which can be accessed through interactive outputs menu items. AUSFLU is supported by extensive data collection studies from the rural township of Tamworth in north-western NSW. The model includes, however, a wizard to guide users through the process of compiling and importing other town datasets and can thus be used to model any township. AUSFLU is a collaborative project funded by the Australian Biosecurity CRC for Emerging Infectious Disease.

Syndromic surveillance of respiratory illness and incidence of RSV and influenza

Schindeler S¹, Muscatello D¹, Ferson M², Rogers K¹, Grant P¹, Churches T¹.

¹Centre for Epidemiology and Research, NSW Department of Health

² South Eastern Sydney and Illawarra Public Health Unit

Introduction: Influenza and respiratory syncytial (RSV) viruses contribute substantially to the burden of seasonal respiratory illness. This study aims to describe the time-based (temporal) relationship between the incidence of influenza and RSV infection and of respiratory syndromes presenting to Emergency Departments (EDs). This could help interpret syndromic surveillance.

Methods: Semi-parametric generalized additive models (GAM) were applied to ED data spanning 1/6/2001-1/12/2006. Poisson distributions were assumed. Respiratory syndromes included ED diagnoses of 1. pneumonia, 2. influenza 3. bronchiolitis. Each model included as predictors laboratory counts of RSV and influenza, and a spline to control for season and trend. Lagged ED visit counts were used to find the time at which laboratory counts and ED visits were most strongly associated.

Results: Lags with strongest associations are reported. For each unit increase in RSV laboratory counts, bronchiolitis ED visits 1 week prior increased by 3.3% (95%CI: 2.9%-3.7%), pneumonia visits 4 weeks in the future increased by 1.6% (95%CI: 1.3%-1.9%) and influenza visits 2 weeks in the future increased by 1.7% (95%CI: 1.0%-2.4%).

For each unit increase in influenza laboratory counts, bronchiolitis visits 4 weeks prior increased by 1.0% (95%CI: 0.6%-1.3%), pneumonia visits 1 week prior increased by 1.1% (95%CI: 0.9%-1.4%), and influenza visits 1 week prior increased by 4.8% (95%CI: 4.3%-5.3%).

Conclusions: Large increases in bronchiolitis syndrome and large increases in influenza syndrome may be early and proxy indicators of changing incidence of RSV and influenza infection, respectively. This can help distinguish between RSV and influenza as causes of respiratory syndromic surveillance signals.

Intradermal influenza vaccination

Victor Carey.

Sanofi-Pasteur, Sydney, Australia.

Cell cultured influenza vaccines

Otfired Kistner

Baxter, Baxter AG, Donau, Austria

Flutracking: A weekly Australian online community survey of influenza-like illness, 2006-2008.

Craig Dalton*, Sandra Fairbairn, David Durrheim, John Fejsa, Lynn Francis, Edouard Tursan d'Espaignet.

Background: Surveillance for influenza is an important public health function as it allows: 1) early detection of epidemics to enable the implementation of public health measures such as the vaccination of high risk groups, outbreak control campaigns, enhanced laboratory testing and enhanced infection control measures to protect the vulnerable in nursing homes and paediatric settings; 2) description of the size and severity of an influenza epidemic; and 3) determination of the impact of an influenza epidemic and effectiveness of public health measures. Flutracking is a weekly online survey of influenza-like illness (ILI) completed by community members. Flutracking has been trialled in the 2006, 2007, and the current 2008 winter influenza seasons.

Methods: The online survey allowed participants to record their past and current influenza immunisation status with a weekly email prompt to answer questions on the previous week's experience of cough, fever and time absent from normal activities. The weekly survey took participants less than 15 seconds to complete. Recruitment occurred through organisation's email systems and media releases. Symptom rates of Flutracking participants were compared by influenza vaccination status to estimate the incidence and severity of influenza and the field effectiveness of influenza vaccine.

Results: Participation rates increased from 400 in 2006 to 900 in 2007 and in 2008 there were over 3,500 participants from around Australia responding to the weekly email survey. In mid-September, responses came from 2,581 people who completed the survey for themselves and on behalf of 1,166 other household members. These 3,747 respondents were from the following jurisdictions: 56.5% NSW, 25.0% Tasmania, 8.2% VIC, 3.6% ACT, 3.2% QLD, 2.4% WA, 1.1% SA. Eighty percent of respondents replied within 24 hours of the survey being sent. In jurisdictions with high participation rates (NSW, TAS), higher weekly point prevalence of ILI among unvaccinated compared with vaccinated participants correlated with influenza activity peaks in laboratory and emergency department surveillance systems. The 2008 influenza season appeared milder than 2007 with the peak rate of cough and fever among all unvaccinated participants at 15% in 2007 compared with only 7% in 2008. The peak week of influenza activity detected by Flutracking in 2008 was the week ending 31st August.

Conclusion: Participation in the weekly online survey of ILI continues to grow and appears sustainable, however, more balanced recruitment across jurisdictions is required to provide a national perspective. Flutracking signals appear to correlate well with other influenza surveillance systems and provide complementary information, however, it will take several influenza seasons to refine the interpretation of the data provided by this system.

Estimation of Influenza Vaccine Effectiveness from Routine Surveillance Data

Heath Kelly¹, Kylie Carville¹, Kristina Grant¹, Peter Jacoby², Thomas Tran¹, Ian Barr³

1. Epidemiology Unit and Virus Identification Laboratory, Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia
2. Telethon Institute for Child Health Research, Centre for Child Health Research, the University of Western Australia, Perth, Australia
3. World Health Organization Collaborating Centre for Reference and Research on Influenza, Melbourne, Australia

Background

Influenza vaccines are reviewed each year, and often changed, in an effort to maintain their effectiveness against drifted influenza viruses. There is however no regular review of influenza vaccine effectiveness during, or at the end of, Australian influenza seasons. It is possible to use a case control method to estimate vaccine effectiveness from surveillance data when all patients in a surveillance system are tested for influenza and their vaccination status is known.

Methodology/Principal Findings

Influenza-like illness (ILI) surveillance is conducted during the influenza season in sentinel general practices scattered throughout Victoria, Australia. Over five seasons 2003-7, data on age, sex and vaccination status were collected and nose and throat swabs were offered to patients presenting within three days of the onset of their symptoms. Swabs were tested using a reverse transcriptase polymerase chain reaction (RT-PCR) test. Those positive for influenza were sent to the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza where influenza virus culture and strain identification was attempted. We used a retrospective case control design in five consecutive influenza seasons, and estimated influenza vaccine effectiveness (VE) for patients of all ages to be 53% (95% CI 38- 64), but 41% (95% CI 19-57) adjusted for age group and year. Comparison of VE estimates with vaccine and circulating strain matches across the years did not reveal any significant differences.

Conclusions/Significance

These estimates support other field studies of influenza vaccine effectiveness, given that these values should underestimate true effectiveness. Incomplete recording of vaccination status and under-representation of children in patients from whom a swab was collected limit the data. Improvements have been implemented for prospective studies.

Clinical development of a prototype adjuvanted split-virion H5N1 pandemic influenza vaccine

David Ryan

CSL Limited, Parkville, Victoria.

The preliminary objective was to evaluate the safety and immunogenicity of a prototype inactivated Clade 1 split virion H₅N₁ (avian) influenza vaccine. Secondary objectives were to assess cross reactivity of immune responses to a Clade 2 H₅N₁ virus and to determine antibody persistence.

Four clinical studies were completed. Two studies were conducted in healthy adults, 7.5 µg or 15 µg HA +/- AIPO₄ adjuvant (N=400) and 30 µg or 45 µg HA + AIPO₄ adjuvant (N=400). Other studies were conducted in elderly subjects (N=201; 65-75 years old, 30 µg HA and 45 µg HA +AIPO₄) and children (> 6 months to < 3 years, N=74 or >3 years to < 9 years N=75).

All studies were randomised, dose comparison, parallel assignment, multicentred trials conducted in Australia. Two doses (IM) were given 21 days apart with bleeds at 0, 21, 42 and 180 days (for trials 2-4). Responses were assessed by HA inhibition (HI) and virus microneutralisation (MN) and by frequency and nature of adverse events (AE). All formulations were well tolerated; no serious adverse events were reported. Two doses of 30 µg or 45 µg adjuvanted vaccine elicited the highest response with considerable MN antibody persistence up to 6 months post vaccination. The pandemic study showed children to give particularly high immune responses, an encouraging finding given their perceived role in dissemination of the disease. The prototype H₅N₁ vaccine also elicited modest levels of cross protective MN antibodies against a variant Clade 2 H₅N₁ strain.

The data was used to achieve Australian regulatory approval for a Prototype Pandemic Vaccine.

FluBlok: A Next Generation Influenza Vaccine Manufactured in Insect Cells

Manon Cox

Protein Sciences Corp. Meriden, CT, USA,

FluBlok is a recombinant HA vaccine produced in cell culture using the baculovirus vectors system. FluBlok provides an attractive alternative to the current egg-based influenza vaccine (TIV) manufacturing process and presents the possibility for safe and expeditious vaccine production. The high purity of the antigen enables administration at higher doses without a significant increase in side-effects in human subjects.

The HA genes from the annual World Health Organization recommended strains are cloned, expressed and purified using a general purification process. An overview of the expression technology used to make the annual adjustments will be provided. The insect cell - baculovirus production technology is a modern solution for rapid viral or parasitic antigen production and that this technology is particularly suitable for influenza where annual adjustment of the vaccine is required.

The insect cells –baculovirus system is generally considered a safe production system, with limited growth potential for adventitious agents. Still questions from regulators regarding the safety of this novel cell substrate were challenging as FluBlok advances towards product approval. The speaker will discuss the *expresSF* cell line substrate and the baculovirus used for the manufacturing of FluBlok.

In addition, the safety, immunogenicity and efficacy results from four recent clinical studies conducted in different populations (ages 18 - 49; 49- 64 and 65 and older) will be presented. The highly purified protein vaccine, administered at three times higher antigen content than TIV is well tolerated and results in stronger immunogenicity particularly in older adults ($\geq 75y$), a long lasting immune response and provides cross protection against drift influenza viruses.

Let's be silly and do something intelligent: confronting the next influenza pandemic with inexpensive generic agents

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Ludwig Wittgenstein once wrote, "If people did not do silly things, nothing intelligent would ever get done."

Avian influenza A/H5N1 could cause a pandemic. Reliance on adjuvanted inactivated vaccines won't succeed because today all vaccine companies could produce in 6 months enough doses to vaccinate only 750 million people, a number less than the combined populations of the nine major influenza vaccine-producing countries. Resistance to oseltamivir among seasonal H1N1 viruses suggests resistance could develop in a pandemic virus. In Indonesia, everyone infected with a clade 2 H5N1 virus who received no antiviral treatment died. Current government stockpiles of oseltamivir would treat only 1% of the world's people who live in countries that don't produce influenza vaccines. If the Indonesian H5N1 virus leads to a human pandemic, we could see a global population die off.

Confronting a global pandemic demands effective measures, including generic agents that target the host immune response or the pandemic virus. Most influenza scientists doubt these agents would be effective and advocate antivirals instead. Yet inactivated H5N1 virus alone unquestionably causes severe acute lung injury. Statins reduce mortality in patients with bacteremia and pneumonia and might be effective against influenza. A recent observational study of 130,000 pneumonia patients showed that in-hospital statin treatment reduced all-cause mortality by 15-25%, and a randomized controlled trial in ICU pneumonia patients showed statins reduced hospital mortality by 50%. Gemfibrozil, a PPAR α agonist, reduced mortality in H2N2 influenza virus-infected mice by 54%. Studies of acute lung injury suggest PPAR γ agonists (glitazones) might be useful. Chloroquine impairs influenza virus release into the cytosol. Resveratrol, a polyphenol found in red wine, reduces influenza mortality in mice. These and several other generic agents are inexpensive, are produced in developing countries, could be stockpiled and would be available in all countries on the first pandemic day.

Given the lack of realistic alternatives, we must do something silly and undertake the research needed to determine whether one or more generic agents could mitigate the effects of the next pandemic. This task is too important to be left to influenza scientists alone. It must involve others who understand the host response to inflammation, acute lung injury and sepsis and the maintenances of energy homeostasis at the level of the whole animal. Only in this way will we be able to intelligently prepare for the next pandemic

Lessons for a human pandemic from the Australian 2007 equine influenza outbreak

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Control of influenza at the extremes of life

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Nanopatches for targeted vaccine delivery of influenza to skin

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In the context of a potential influenza pandemic, a particular need is rapid-mass vaccination of the population. However, currently we are technologically ill-equipped to meet this need, with a particular challenge in rapidly producing and deploying sufficient vaccine doses to protect the population. Great gains will be made in addressing this problem with a vaccine delivery system effectively targeting the skin's immunologically sensitive cells – which the needle and syringe does not do – and achieving influenza vaccination protection with a spared dose. Further, the new delivery device must also be easy to distribute and administer. In this presentation, we outline research into physical targeting methods to meet this goal, using arrays of micro-nanoprojections on patches (Nanopatches).

We begin by analysing the skin target, choosing the delivery to Langerhans cells (and associated cells) for immunotherapeutics as a pertinent case study. The location of Langerhans cells are presented together with an analysis of key skin mechanical properties. Briefly, current physical approaches for targeting these cells are introduced, together with a discussion of their effectiveness and limitations as practical delivery devices in the immunotherapy of major disease. As one case-study, we briefly discuss the Gene Gun (PowderJect/PowderMed) the primary technology.

This presentation then focuses on the Nanopatch technology. The of arrays of projections—on a patch—accurately, efficiently and safely deliver biomolecules not just to specific skin cells, but also to organelles within them. Conceptually, the delivery device is a set of needles (of microscale length with nanoscale tips), coated with drug substance and applied to the skin as a small patch. The patch is pain-free and needle-free. By eliminating the cold-chain, it is applicable to developing world vaccinations. We will present Nanopatch configurations, coating approaches and key immunology in the skin generated by delivery. Finally, I will provide an overview of resultant influenza (FluVax 2007) vaccination progress in mice (ELISA and HI data) and significant dose-reduction gains achieved by Nanopatch delivery. The dose reduction achieved, as well as the possibility of self applicability of the influenza vaccine coated Nanopatch may be of great utility in a pandemic situation.

WA Influenza Vaccine Effectiveness Study in children (WAIVE): Recruitment, Conduct and Preliminary Outcomes

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Modeling strategic use of the national antiviral stockpile during the CONTAIN and SUSTAIN phases of an Australian pandemic influenza response.

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We aimed to define optimal utilisation of the national antiviral stockpile during the early phases of the response to an influenza pandemic in Australia. A mathematical model was used to compare strategic uses of antiviral agents to limit transmission until availability of a strain-specific vaccine. Optimal constraint of epidemic growth was achieved by intensive ascertainment of contacts of cases for post-exposure prophylaxis for as long as feasible. While pre-exposure prophylaxis of health care workers utilised a substantial proportion of the stockpile, this did not impede disease control or the ability to treat cases. Absolute delays to outbreak depended on both the intervention strategy and the growth rate of the epidemic. As vaccination was only effective when introduced before explosive growth, this timing was critical to success. In keeping with others we concluded that, in reality, additional non-pharmaceutical control measures are likely to be required to constrain transmission until vaccines can definitively contain the epidemic. Further, model interpretation must be guided by a clear understanding of the limitations of existing data resources upon which to base assumptions regarding epidemic dynamics and the likely effectiveness of public health interventions.

Respiratory Viruses, Including Influenza, Are Aerosolised By Breathing

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Introduction: The relative contributions of contact, fomite and aerosol routes towards the transmission of respiratory viruses within populations are unclear. Respiratory aerosols are commonly considered to be generated by coughing, sneezing and talking. We recently showed that breathing can also aerosolise rhinovirus (RV). To further explore this, we analysed the exhaled aerosols of children generated by 3 types of respiratory events for 9 types of virus.

Methods: 187 children (5-15 years), admitted to respiratory and general hospital wards were sampled for aerosols generated by coughing (10 times), talking (5 mins), and deep breathing (5 mins), with nasal mucous sample used as comparison. Sampling of exhaled aerosols was performed using a novel mask with a removable electret filter. Analysis was performed by multiplexed PCR for RV, Adenovirus, Respiratory Syncytial Virus (RSV), Influenza A & B, Parainfluenza 1, 2 & 3 (Para) and Human Metapneumovirus (HMPV), with virus identity confirmed by labelled oligoprobes. RV was also quantified by q-PCR and viability tested by cell culture. Symptoms were established using the common cold questionnaire (CCQ).

Results: Numbers of children with a positive PCR for each virus type and the method of aerosol generation, plus mucous, are shown in the following table.

	Any Virus	Rhino virus	Adenovirus	RSV	HMPV	Influenza A & B	Parainfluenza 1, 2 & 3
Cough	85	49	7	13	1	4	11
Talk	81	49	7	9	1	3	12
Breathe	77	48	5	8	1	4	11
Mucous	69	27	8	18	1	4	11

Of the 56 children shedding RV, 62% were CCQ Positive and 38% were CCQ Negative; all the latter had asthma. By culture, the RV titre in mucous and breathing samples were similar and approximately 8 times higher than in coughing and talking samples, which were also similar.

Discussion. All these respiratory viruses, including influenza, were aerosolised by breathing alone. In addition to symptomatic individuals, exhaled breath was also a source of viable RV from asthmatic children with no respiratory symptoms. As breathing involves low airflows, the particles size of the resultant aerosols is likely to be small. Together this data suggests that breathing alone by asymptomatic people with RV and perhaps influenza infections may generate droplets of a size which would travel significant distances and transmit infections.

The fidelity of the PB1 subunit of influenza A virus and its relation to evolution.

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Introduction

Influenza virus has been known to have high mutation rates due to the lack of proof-reading exonuclease activity. Having a low fidelity polymerase may aid the virus in rapid evolution, and adaptive changes to the selective pressure will be selected out. However, most mutations having a phenotypic effect will be deleterious. A polymerase with a high fidelity would be advantaged in maintaining the favoured mutations in the population. However, increasing the fidelity incurred by a more stringent replication would come at a cost, including lower replication rate and reduced the viral genome diversity. There is a trade-off between replication efficiency and fidelity.

Since 1968, the evolution of influenza A has been characterised by a period of relative stasis lasting from 3-8 years followed by the rapid introduction of a new virus. PB1 is the major replication unit of the influenza virus responsible for its polymerase activity. In 2002, a new lineage A/Fujian/411/02 (H3N2) emerged in Asia and caused significant outbreak on every continent. It is hypothesised that the PB1 of such emerging dominant virus would possess low fidelity. Less energy would be spent on the replication fidelity, resulting in faster replication, adaptation and domination. However, once the virus has dominated, the fidelity might increase due to a lesser need for adaptation, and a greater need to maintain favourable genetic materials. Overtime, this would lead to the domination of another virus, resulting in the observed punctuated evolution of influenza A.

Method

The fidelity of reassortant viruses carrying PB1, HA and NA of A/Wyoming/3/03, A/California/7/04 and A/Wisconsin/67/05 in a PR8 backbone was examined in a MDCK plaque purification assay using the sequencing method. The NS gene from 94-105 progeny virions and the parent virus was sequenced and the mutation rate was compared. Candidate amino acids which affect the fidelity of the polymerase were also investigated.

Results

Reassortant Strain	No. of progeny	Total nucleotide sequenced	Mutation	Mutation per site
A/Wyoming/3/03	105	90930	4	4.4×10^{-5}
A/California/7/04	94	81404	0	0
A/Wisconsin/67/05	95	82270	1	1.2×10^{-5}

Discussion

Plaque formation was done under standard tissue culture conditions in the absence of any new selection pressure which would have an affect on HA and NA. Whole viruses were not used in the assay so any mutations arose can be traced to PB1, the only variable component in the replication machinery. Preliminary data suggest there is a change in fidelity with time in the period between 2003 and 2005. Further assays would need to be performed on more reassortant strains between 2003 and 2005, and from 2006 onwards, to further validate the observation.

OFFLU study of current H5N1 highly pathogenic avian influenza (HPAI) isolates in Indonesia

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This study forms part of a FAO-coordinated OFFLU project, which aims to characterise virus isolates and gather epidemiological data to determine the current distribution of HPAI strains in Indonesia. HPAI is an on-going concern for Indonesia's chicken production sectors. The country also continues to suffer the greatest impact from human infections of H5N1, with no less than 112 confirmed fatalities to date. Vaccine challenge studies with three separate 2007 field isolates have shown at least one strain to be resistant to vaccination with currently recommended seed strains for avian influenza in Indonesia. Our laboratory has subsequently received more than 265 samples from Indonesia, the majority originating from chickens in 2007. Positive H5N1 isolates were able to be cultured from at least 70% of the samples. Complete haemagglutinin (HA) and neuraminidase (NA) gene sequences were obtained from all isolates. Phylogenetic analysis of the HA sequences showed that the isolates shared 94-100% sequence similarity and all belonged to the Indonesian HA clade 2.1, with the majority (92%) clustering within the 2.1.3 sub-clade lineage. Six isolates were located in an indeterminate 2.1 sub-clade. Our results showed evidence of temporal evolution into HA 2.1 sub-clade lineages. Within sub-clade 2.1.3, there was no distinctive sub-clustering based on geographical origin of the isolates or poultry industry sector, although there is currently insufficient representation from the large commercial producers (Sectors 1 and 2). Six isolates were located in an indeterminate 2.1 sub-clade. Sequences confirmed that all isolates possessed the highly pathogenic polybasic amino acid repeats at the HA cleavage site with 5 different cleavage site motifs detected among isolates. Relative phylogenetic position of the three isolates tested by vaccine challenge correlated with evidence of antigenic drift detected by a preliminary antigenic mapping study using haemagglutination inhibition (HI) data. Mapping has shown that antigenic drift variants are in distribution in the field. However there is still severe under representation of isolates from different region in Indonesia and from the large commercial sector. Further samples continue to be analysed by the current OFFLU project to provide further evidence of relationships between temporal, and genetic and antigenic drift of isolates circulating in Indonesia. Co-analysis of the combined data will contribute to identification of new vaccine seed candidates and OFFLU recommendations for vaccine strains for Indonesia.

Antigenic Mapping of Indonesian HPAI Isolates at AAHL

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Indonesia has become the global hot spot for the H5N1 epizootic in poultry and for human H5N1 infections. Due to the endemicity of H5N1 virus in poultry and the frequent contact between poultry and humans, Indonesia is the most likely source of any potential human H5N1 influenza pandemic. Antigenic changes in the haemagglutinin (HA) protein of virus isolates must be continually monitored to identify emerging antigenic variants and to select appropriate vaccine and challenge strains. The technique of antigenic mapping provides a visual representation of the relationships between HA proteins by applying a geometric interpretation of haemagglutination inhibition (HI) titres. In collaboration with Erasmus University Rotterdam, we aim to optimize the methodology for antigenic mapping using chicken sera and generate antigenic maps for H5N1 avian influenza virus isolates circulating in Indonesia. Initially, 15 isolates were chosen for analysis based on their genetic sequence and position in a phylogenetic tree. The isolates were tested by HI against a panel of antisera generated at SEPRL and AAHL, and antigenic maps were constructed based on relative HI titres. To determine the conditions that give the greatest map resolution, the study compared the effect of prime and prime-boosted antisera, as well as prime antisera treated with receptor destroying enzyme (RDE). The maps are currently undergoing further analyses and comparison with genetic data to provide more insight into the relationship between virus isolates from different epidemiological regions in Indonesia.

A mutation at a non-conserved residue, Y155H, in the human influenza N1 neuraminidase affects drug sensitivity and enzyme function

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There are two drugs currently available, which are effective against all strains of influenza. These are known as the neuraminidase inhibitors (NAI), zanamivir (Relenza™) and oseltamivir (Tamiflu™). Resistance has been shown to arise after *in vitro* passaging in both drugs. However, to date while resistance has been seen after treatment of patients with oseltamivir, no resistant virus has been isolated from a previously healthy patient treated with zanamivir. As part of a global influenza surveillance program, coordinated by the Neuraminidase Inhibitor Susceptibility Network, (NISN), influenza isolates have been monitored for emergence and spread of resistant strains both before the introduction of the drugs into clinical practice, (1996-99) and post release (>2000). As part of this program 2 viruses were identified as being outliers to both drugs. The A/Hokkaido/15/2001 and A/New York/24/2001 strains have been obtained and plaque purified. In contrast to initial published data, both viruses appear to have the same mutation in their NA, Y155H. This mutation has not previously been associated with drug induced resistance, is not a conserved residue across all strains, and is located at a distance remote from the active site. We will discuss how this mutation affects the properties of the viruses. In order to understand what impacts this mutation has on enzyme function, and how it could lead to drug resistance, we have cloned the NAs into the recombinant baculovirus expression system. Properties of the influenza virus associated NA and the recombinant NAs have been compared, including susceptibility to zanamivir, oseltamivir, and a third NAI, peramivir, thermal stability and effects of pH on enzyme activity. It is clear that the mutation affects not only drug binding, but also the enzyme function, despite being remote from the active site.

Intranasal flu vaccine protective against seasonal and H5N1 avian influenza infections

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Influenza A virus causes significant morbidity and mortality worldwide, and current subunit or split vaccines require annual updating to protect against the rapidly arising antigenic variations due to antigenic shift and drift. Current flu vaccines rely exclusively on antibody responses for protection and do not induce cytotoxic T (Tc) cell responses. The Tc cell response to influenza, however, is broadly cross-reactive between virus strains and is important in the recovery from primary infections. Furthermore, cross-recognition of avian H5N1 influenza virus by human Tc-lymphocyte populations induced by human influenza A virus has recently been reported. We have previously demonstrated that \square -ray inactivated influenza A virus (\square -Flu) preparations can induce cross-reactive Tc cell responses. Our recent data show that intranasal administration of purified \square -Flu effectively induces heterosubtypic and cross-protective immunity, mediated predominantly by Tc cells. A single intranasal administration of \square -A/PR8[H1N1] protects mice against lethal H5N1 and other heterosubtypic infections. Given the importance of obtaining cross-protective immunity, our novel vaccination concept represents a unique approach for a universal influenza vaccine protective against both seasonal as well as possible future pandemic influenza A virus infections.

A pathogenic comparison of two phylogenetically distinct H5N1 avian influenza viruses in ferrets

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The ability of H5N1 avian influenza viruses to cause fatal human infection following direct transmission from avian species and the continuing circulation of H5N1 viruses in Asia, Europe and Africa are of grave concern. Since its emergence in 1997, the geographical spread of H5N1 has led to a phylogenetic diversification resulting in multiple regional sublineages. Epidemiological investigations of human H5N1 infection have identified regional differences in the human mortality rate. Whilst these differences in the pathogenicity of H5N1 virus infection in humans may be a result of host genetic differences in susceptibility, they may also be a result of differences in the pathogenicity of the viruses themselves.

In 2005 a novel H5N1 virus sublineage emerged in China's Fujian province. In the 3 years since, viruses from the "Fujian" sublineage, now known as subclade 2.3.4 have become the predominant H5N1 virus sublineage circulating in several South East Asian countries. Hence the continuing threat to human and animal health posed by H5N1 influenza viruses from subclade 2.3.4 warrants further characterization to gain a better understanding of the variable pathogenicity and evolution of influenza viruses.

Domestic ferrets (*Mustella putorius furo*) are the most appropriate small animal model of human influenza infection. Here, ferrets were used to assess the likelihood of a difference in pathogenicity to humans, between a subclade 2.3.4 virus (A/Chicken/Laos/Xaythiani-26/2006) and that of the previously predominant H5N1 virus lineage, clade 1 (A/Viet Nam/1203/2004).

Groups of ferrets were intranasally infected with 10^6 EID₅₀ of each virus and periodically sampled across 7 days post infection. Virus was isolated from nasal washes and a fever response was observed in ferrets from both treatment groups. However, virus titration and histopathological analyses of infected host tissues demonstrated significant differences in the extent of host infection and the profile of host cells susceptible to infection with each virus. We found the severity and onset of clinical disease symptoms was significantly reduced in ferrets infected with A/Chicken/Laos/Xaythiani-26/2006 compared to animals infected with A/Viet Nam/1203/2004. The reduced pathogenicity of subclade 2.3.4 viruses may allow them to persist for longer periods of time within infected hosts and hence facilitate greater opportunities for geographical spread and adaptation to humans.